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Overt increase in lupus anticoagulant level in HIV patients co-infected with pulmonary *Mycobacterium tuberculosis* infection: A prospective cohort study

Obi Simon Osita, Amilo Grace Ifeoma, Ifeanyichukwu Martin Ositadinma, Ejeatulu Obi, Balla Akawu, Ngwu Amauche Martina

ABSTRACT

Aims: Lupus anticoagulant (LA), an antiphospholipid antibody is known to be associated with autoimmune conditions, acute opportunistic infection and viremic challenges. It is now, however, considered as an important risk factor for otherwise inexplicable thrombosis as is seen in HIV infected individual in the recent times. To assess the plasma level of LA in HIV individuals co-infected with pulmonary tuberculosis (PTB). Methods: After obtaining informed consent from the participants and ethical approval from University of Maiduguri Teaching Hospital Ethics committee, two hundred and fifty HIV positive subjects were tested for the presence of LA by dilute Russell's viper venom time (DRVVT). Sputum acid-fast bacilli and *Mycobacterium tuberculosis* IgM antibody were tested by Ziehl–Neelsen and immunochromatographic methods, respectively. Lupus anticoagulant was investigated with reagent kits acquired from Siemens Health Care Diagnostic Products, Germany. Apparently Health, HIV sero-negative subjects (251) served as control. The study was conducted between June 2011 and September 2013. Results: The mean ratio of LA was significantly increased in HIV positive subjects and overtly increased in HIV/PTB subjects compared to the control subjects ($p<0.001$). Conclusion: HIV infection induces the presence of LA; and HIV/PTB co-morbidity worsen this condition. The presence of LA is considered in the recent time as a risk factor for thrombotic events.

Keywords: HIV, Lupus anticoagulant, *Mycobacterium tuberculosis, Pneumocystis carinii*, Pulmonary tuberculosis co-infection, Ziehl–Neelsen stain

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INTRODUCTION

Lupus anticoagulant (LA) is an auto antibody with phospholipid specificity. They are directed against negatively charged phosphor-containing lipids and complexes of phospholipids with either beta-2-glycoproteins or clotting factors such as prothrombin. They are common in various clinical conditions especially autoimmune disease [1, 2], acute infection by opportunistic organisms such as *Pneumocystis carinii* [3] and *Mycobacterium tuberculosis* infection [4]. Lupus anticoagulant is now considered to be a significant risk factor in patients with otherwise unexplained thrombosis [2, 5, 6]. Cohen et al. associated LA with post viral condition postulating that its presence could be in response to viremic challenges [4]. Some studies have shown that LA may be present in 82–92% of patients with HIV/AIDS [7]. Lupus anticoagulant has affinity for phospholipid active in coagulation but paradoxically its presence is not associated with bleeding tendency, but instead increased occurrence of both venous and arterial thrombosis [6, 8–10]. With the emergence of hypercoagulable state in HIV patients in the recent times it is considered worthwhile to study LA presence in HIV subjects found in our environment with pulmonary *Mycobacterium tuberculosis* comorbidity. Pulmonary tuberculosis is a common clinical manifestation in HIV patients in our environment. It has been documented that at least one million adults in Nigeria are co-infected with HIV and *Mycobacterium tuberculosis* [11, 12]. This increase in PTB is believed to relate to expanding HIV epidemics and strong biological association between HIV and *Mycobacterium tuberculosis* [13].

Acute pulmonary embolism (PE) a common sequelae of DVT can be confused clinically with opportunistic pulmonary infection such as PTB or *Pneumocystis carinii* Pneumonia [14, 15]. Confirmation of LA presence with a high degree of suspicion may assist in differential diagnosis of PE and PTB pneumonia in HIV patient seen in our environment.

MATERIALS AND METHODS

Study Site: The study was carried out at University of Maiduguri Teaching Hospital (UMTH) northeast of Nigeria. It was a prospective cohort study and conducted between June 2011 and October 2013 among HIV positive subjects with and without pulmonary tuberculosis PTB comorbidity.

Study Population: These were 250 HIV positive subjects (WHO stage II); comprising 150 (60%) males and 100 (40%) females. They were receiving triple combine antiretroviral therapy; lamivudine (3TC) 150 mg, 2 tablets daily, zidovudine (2DV) 150 mg, twice daily and nevirapine (NVP) 200 mg twice daily. Two hundred and fifty one seronegative apparently health control subjects comprising males 168 (66.9%) and females 83 (33.1%) were also recruited. Fifty-six (22.4%) of the HIV positive subjects were also positive for sputum acid-fast bacilli and *Mycobacterium tuberculosis* IgM antibody, males 36 (14.6%), females 20 (8.05%). The median age was 34.0 years ranging between 17 and 59 years. The age group 17–30 constituted the largest group (56%) for HIV mono-infection and 53.6% for HIV/PTB co-infection. Demographic data were obtained through semi-structured questionnaire and from laboratory records. Informed consent and pre-test counseling were also instituted. The ethical approval was obtained from University of Maiduguri Teaching Hospital Ethics committee. Pregnant subjects, those using contraceptive or hormones or tobacco were excluded from the study. Also all subjects positive for D-dimer screening agglutination test, or had clinical thrombosis were excluded.

Sample Collection

Six milliliters of venous blood was collected from each subject and aliquoted as follows. 2.25 mL was dispensed into plastic bottles containing 0.25 mL of sodium citrate (0.11 molar solutions) to give a final blood citrate ratio of 9:1. Platelet poor plasma was obtained immediately and used for the assay of lupus anticoagulant and activated partial thromboplastin time. Two milliliters (2.0 mL) was dispensed into EDTA container and used for CD4+ T cell count. The remaining 1.75 mL was allowed to clot in a plain container, and serum separated from it was used for HIV screening and confirmation tests, and TB IgM antibody screening test.

Three serial sputum samples were collected from each subject for identification of acid-fast bacilli *Mycobacterium tuberculosis* by Ziehl–Neelsen stain.

Laboratory Analysis

Screening for HIV Antibodies

HIV screening test was carried out using immunochromatographic reagents (Chembo HIV 1 and 2 STAT PAK from Chembio Inc. Melford New York USA) Cat No. HIV 101N. Seropositive samples were further confirmed by Western blotting using Qualicode™ HIV 1 and 2 Kit (Immunetics Inc. Boston USA) Cat No. DK – C152-024.

CD4+ T cell counts was performed using PARTEC Health care immunology CD4 easy count Kit. (Partec GmbH-Am flugplatz Germany) Cat. No 05-8401 and Parteccyflow SL3.

Platelet count was performed by automation using Sysmex KX-21 Hematology analyzer S/N No A8893 (Kobe Japan).

Lupus anticoagulant

This was performed by automation using reagent kits LA1 and LA2 (A dilute Russell's viper venom time

(DRVVT) reagent kit); following the techniques of Thiagarajan et al. [16]; modified and simplified by Exner et al., 1990 [17]. This reagent kit was acquired from Siemens Health Care Diagnostic Product, Germany. Ref. No.OQGP17 EXP 2013. LA1 was a screening reagent and LA2 the confirmation reagent. Automated coagulometer Sysmex CA 560 S/N F1016 from (Sysmex Corporation Kobe Japan) was used.

Activated partial thromboplastin time: This was performed pathromtin SL reagent kit Ref OQGS 34 from Siemens health care diagnostic using products and automated coagulometer as above.

D-DIMER (screening agglutination test): Detection of cross-linked D-dimer fragments was performed with latex agglutination kit acquired from Siemens health care diagnostic products Germany Ref OUVV 15. Agglutination with undiluted plasma is indicative of D-dimer presence in excess of 200 mg/L.

Tuberculosis serology: This was achieved with immunochromatographic test kit for tuberculosis IgM antibody from Clinotech Diagnostic Inc. Canada.

Sputum acid fast bacilli: This staining, Ziehl–Neelsen stain was used to stain heat-fixed –smear of sputum concentrate. Three percent sodium hydroxide solution was to digest and concentrate the sputum samples.

Statistical package SPSS for windows version 20 was use for data analysis.

RESULTS

Socio-demographic data showed that out of the 250 HIV seropositive subjects recruited 150 (60%) were males and 100 (40%) females; and that 56 (22.4%) of the HIV seropositive subject were co-infected with pulmonary tuberculosis (PTB). Among these subjects 36 (14.6%) were males and 20 (8.05%) were females. The median age was 34.0 years, ranging between 17 and 59 years. The age group 17–30 years constituted the largest group (52%) for HIV mono-infection and (53.6%) for HIV/PTB co-infection. In both groups, males were greater than females in number. Approximately, half (50.8%) of the HIV positive subjects were single. This frequency was also reflected in the marital status of HIV/PTB group. The control subjects (251) appeared apparently age and sex matched with the HIV seropositive subjects. The marital status of the control subjects revealed similarly pattern as in the HIV subjects (Table 1). Table 2 gives the CD4+ T cell counts of all subjects. The mean count (cells/μL) of the control subjects 504.12± 213.89 was significantly higher compared to the mean values (cells/μL) of HIV mono-infection 333.11±91.90 and the HIV/PTB subjects 212.000±49.29 ($p<0.001$ in each case). Similarly, there was a significant difference between the mean counts of HIV mono-infected subjects and the HIV/PTB mean count ($p<0.01$). The mean platelet (PLT) count of the controls was also significantly higher than those of the HIV groups ($p<0.001$). Similarly, there was a significant difference in the PLT mean count between HIV mono-infection and HIV/PTB co-infected subjects ($p<0.01$). Table 3 represents mean ratio/range of LA for HIV groups and their controls. There was a significant difference between the mean LA ratio of the controls 0.85±0.05, range (0.83–1.07) compared to the HIV-mono-infection mean values 1.21±0.01, range (0.96–1.35) and the HIV/PTB values 1.86±0.34, range (1.25–2.0) ($p<0.001$). Similarly, the values for the HIV mono-infection and HIV/PTB co-infection revealed a significant difference (p<0.01.). Activated partial thromboplastin time (APTT) mean

Table 1: Demographic characteristics of all subjects

	Control		HIV mono-infection		HIV/PTB co-infection	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
N (%)	168 (66.9)	83 (33.1)	150 (60)	100 (40)	36 (14.4)	20 (8.0)
Total	251 (100)		250 (100)		56 (22.4)	
Age range						
17–30	76 (30.3%)	54 (20.5%)	77 (30.8%)	53 (21.2%)	20 (35.7%)	10 (17.9%)
31–44	44 (17.5%)	25 (10.0%)	35 (14.0%)	33 (13.2%)	8 (14.3%)	2 (3.6%)
45–58	34 (13.5%)	3 (1.30%)	30 (12.0%)	12 (4.0%)	6 (10.7%)	7 (12.5%)
Total	251		250		56	
Marital status						
Single	130 (51.8%)	127 (150.8%)	31 (37.5%)			
Married	97 (38.6%)	98 (39.2%)	13 (23.2%)			
Widow						
Widower	24 (9.6%)		25 (10.0%)		12 (39.3%)	
Total	251		250		56	

value is markedly prolonged in the HIV/PTB subjects compared to the controls ($p< 0.001$). Also the difference between the mean values of HIV-mono-infection and HIV/PTB subjects show significant difference ($p<0.001$). Table 4 represents Pearson correlation analysis to determine the relationship between lupus anticoagulant (LA), CD4 count, HIV/PTB and HIV mono-infection. There was a significant but negative correlation between LA and CD4 count ($r = -0.158$, $p<0.05$). There was also strongly significant but positive relationship between LA and HIV/PTB co-infection ($r=0.883$, $p<0.001$). Similarly, there was a positive and significant association between LA and HIV mono-infection ($r = 0.190$, $p< 0.3$).

Table 2: Mean ± SD of CD4 ±T lymphocyte count for all Subject

S/N	Subjects	CD4 + T count	PLT count
1	Control	504.12 ± 213.89	274.0±85.24
2	HIV/No PTB	333.11± 91.9	176.3±64.06
3	HIV/PTB	212.00± 49.29	105.6±31.57
	F Statistics	282.65	98.6
	P Value	0.000***	0.000^{xxx}
	1 Vs 2 P Value	0.00**	0.00^{xx}
	1 Vs 3 P value	0.000**	0.00^{xxx}
	2 Vs 3 P value	0.00**	0.00^{xx}

Key: Data are expressed as mean ± SD. Significant p-values *** $p <$ 0.001, ** $p<0.01$.
1 vs 2 Control vs HIV-no PTB
1 vs 3 Control vs HIV/PTB
2 vs 3 HIV-no- PTB vs HIV/PTB

Table 3: Mean ±SD value and range of lupus anticoagulant in the controls, HIV mono-infection and HIV/PTB subjects

S/N	Subjects	LA Ratio/Range	APTT
1	Control	0.85 ± 0.05 (0.83 -1.07)	31.9±5.74
2	HIVmono-infection	1.21 ± 0.01 (0.96 – 1.35)	37.3±3.37
3	HIV/PTB	1.86 ± 0.34 (1.25 – 2.0)	54.2±0.43
	F Statistics	95.42	23.51
	p value	0.000***	0.000^{xxx}
	1 vs 2 P Value	0.001**	0.00^{xx}
	1 vs 3 P value	0.000***	0.000^{xxx}
	2 vs 3 P value	0.000***	0.000^{xxx}

Key: Data is expressed as mean value and range
p value: *** $p< 0.001$ ** $p<0.01$
LA = Lupus anticoagulant.
APTT= Activated partial thromboplastin time
1 vs 2 = Control vsHIVmono-infection
1 vs 3 = Control vs HIV/PTB
2 vs 3 = HIVmono-infection vs HIV/PTB

Table 4: Correlation analysis between lupus anticoagulant, CD4 count, HIV/PTB co-infection and HIV mono-infection

Variables	Co-efficient	p-value
LA vs CD4	–0.158	0.01*
LA vs HIV/PTB	0.883	0.000***
LA vs HIV mono-infection	0.190	0.001**

DISCUSSION

All the subjects in this study were adults with the greater proportion in the age group 17–30 years. More than 50% of the subjects in each group studied (control, HIV mono-infection and HIV/PTB co-infection respectively) were males, this, however, is not in keeping with the demographics of attendance to government hospitals as documented by previous workers [18]. This may be due to the recruitment pattern in this research which excluded more females for reasons of pregnancy and contraceptive use.

About half (52%) of the HIV group were unmarried and also belong to the age group 17–30 in this study. Multiple sex partnering is usually common among the unmarried age group and sexually active persons are also common in the observed age group. This study revealed significantly increased LA mean value in the HIV mono-infected subjects. This observation is supported by the reports of Cohen et al. [3] and Hassoun et al. [18]. They later found an incidence rate greater than 20% in HIV patients particularly infected with *Pneumocystis carinii*, an opportunities pulmonary infection common in HIV infected patients. Our study also observed that 22.4% of the HIV positive subjects were also co-infected with pulmonary tuberculosis PTB. This is in agreement with the previous reports [11–13]. In our study also, HIV/PTB subjects had overtly increased LA levels with mean value of 1.86±0.34 and range of (1.29–2.0). These represent strong, positive status for LA [16, 17]. Previous studies have associated LA with opportunistic infection by bacterial and viral agent. This was seen to represent antiphospholipid response to varemic challenges [5, 7, 18]. Front-line coagulation marker, APTT was significantly prolonged, while platelet count was significantly reduced in the test subjects in comparison to the controls in this study. This is not unexpected because these are phospholipid dependent and the presence of LA, an antibody with phospholipid specificity is known to interfere with such coagulation tests in vitro [1, 9].

Correlation analysis supported this finding, presenting a strong and positive association between the presence of lupus anticoagulant and HIV/PTB co-infection. Lupus anticoagulant also correlated significantly but negatively with CD4+ T cell counts. The later may suggest that LA incidence rate increases as immune status of the

subjects diminished, this is in consonance with previous studies [10, 14, 19]. The presence of LA is known to relate to increase tendency for thrombophilia [5, 10, 14]; while paradoxically prolongs phospholipid dependent coagulation markers in vitro [20, 21]. Lupus anticoagulant is now considered to be a significant risk factor in patients with otherwise unexplained thrombotic events including women who have recurrent fetal loss [5]. The mechanism of the thrombophilia associated with LA in vivo is yet not very clear but may be related to the antibody's ability to cause endothelia cellular activation with an up regulation of tissue factor expression [1]; or by virtue of LA disrupting the interaction of β2 glycoprotein 1 with anticoagulant factors such as activated protein C (APC) [3]. Pulmonary embolism (PE), very fatal sequelae of thrombosis can be confused clinically with opportunistic pulmonary pneumonia due to opportunistic infections such as PTB or *pneumocystis carinii* [14, 21]. Fultze et al. had asserted that a high degree of suspicion may be required from those managing such patient to be able to differentially investigate, detect and initiate early treatment so as to ameliorate complications that could be associated with this dilemma in HIV infection [21].

CONCLUSION

The strong association among lupus anticoagulant (LA), HIV infection and HIV/PTB co-infection as revealed by this study in our opinion suggests that routine investigation of LA and HIV associated pulmonary complications in our environment are required.

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Obi Simon Osita – Conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Critical revision of the article, Final approval of the version to be published
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Guarantor
The corresponding author is the guarantor of submission.

Conflict of Interest
Authors declare no conflict of interest.

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