ALTERATIONS IN SOME COAGULATION BIOMARKERS OF PULMONARY TUBERCULOSIS SUBJECTS IN THE SETTINGS OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION: AS SEEN IN MAIDUGURI NORTH EASTERN NIGERIA

[1]Obi SO, [1]Bukar A,[5]Amilo GI, [3]Digban KA, [1]Medugu JT, [1]Waziri G, [3]Osakue EO, [1]Peyo BA, [1]Abja UM, [2]Olaniyam MF, [4]Jeremiah ZA.

[1]Department of Medical Laboratory Science, University of Maiduguri,
[2]Department of Medical Laboratory Science, Achievers University, Owo,
[3]Department of Chemical Pathology, Igbinedion University Teaching Hospital, Okada,
[4]Department of Medical Laboratory Science, Niger Delta University, [5]Department of Immunology, Nnamdi Azikwe University.

Correspondence and reprint request to: **Mr Alhaji Bukar**
Department of Medical Laboratory Science, University of Maiduguri, Maiduguri, Borno State.
eMail:- alhajibukar@gmail.com **Phone number:-** +234-802-970-7067

ABSTRACT

Background: Synergistic association between Human Immunodeficiency virus (HIV) and pulmonary tuberculosis (PTB) infection has resulted in variable haematological manifestations including coagulopathies; these accelerated the morbidity and mortality burden of HIV/PTB co-infection.
Objectives: Based on this preposition, we prospectively evaluated some coagulation biomarkers in a case-controlled study of 102 HIV sero-positive subjects consistent with WHO clinical stages I and II, 56 HIV/PTB co-infected subjects; both groups were therapy naive. Also 104 HIV sero-negative healthy blood donors were recruited as control subjects.
Method: All participants were tested for platelet count (PLT), Plasma fibrinogen concentration (PFC), Protein C (PC), prothrombin time (PT) and Activated partial thromboplastin time (APTT).
Results: In HIV/PTB co- morbidity PT, APTT were prolonged ($P<0.001$); PLT and PFC were also elevated ($P< 0.001$), while PC % activity was down-regulated ($P<0.01$) all in comparison to the HIV group and the controls.
Conclusion: We asserted that alterations occur in some coagulation indices of PTB/HIV co-infected individuals found in our environment. Clinical findings are however, needed to shed more light on these findings to aid patient's management.

Keywords: PTB, HIV, Coagulation, Biomarkers

INTRODUCTION

Human Immunodeficiency Virus (HIV) infection exacerbated global incidence of Mycobacterium tuberculosis (MTB), the causative agent of Pulmonary Tuberculosis (PTB).[1,2,3] The risk of progression from latent to active tuberculosis was reported to be greater among people living with HIV compared to the general population.[4] On the other hand, tuberculosis bacterium infection is known to accelerate the progress of HIV infection thus suggesting a scenario of synergistic morbidity in HIV and TB co-infection.[5] Evidence exists in the literature that resource limited setting as in Sub-Saharan Africa a disproportionate burden of global HIV/PTB co-infection borne.[6,7,8] The reasons adduced for this in part was the concurrent HIV and TB morbidities in the region.[8,9] And the fact that despite expanded access to antiretroviral therapy ART many persons in the region requiring treatment initiate ART too late and often have

already developed clinically significant tuberculosis on presentation.[7,10] In the past decade it was reported that at least one million adults in Nigeria were co-infected with HIV/PTB.[11,12] On presentation therefore, many HIV/PTB co-infected individuals have need for concurrent ART and anti TB therapy. Treatment however, improves survival, but sometimes management challenges emerge such as shared drug toxicity, TB Immune Reconstitution Inflammatory Syndrome (IRIS)[13,14,15] and coagulopathies.[16,19]

Literature abundantly indicated various patho-physiological mechanisms promoting haemostatic anomalies in individuals infected with HIV and pulmonary tuberculosis (PTB); these include the emergence of various inflammatory cytokines from the granulomatous lesions in PTB[20] and from HIV viraemic challenges.[21,22] In addition, the presence of antiphospholipid antibodies such as lupus anticoagulant recently associated with inexplicable hypercoagulability,[21,23] and immune activation complexes were implicated.[3,24] With this background it was considered worthwhile to evaluate some coagulation biomarkers in HIV and HIV/PTB individuals in our environment.

MATERIALS AND METHOD

This was a case-control study conducted between August 2013 and October 2014 among HIV-sero-positive subjects with pulmonary tuberculosis (PTB) co-morbidity and HIV-sero-positive subjects. The study was conducted at the University of Maiduguri Teaching Hospital (UMTH) Borno State, Northeast of Nigeria. UMTH is designated a centre of excellence in immunology and infectious diseases.

Subjects comprised of 102 HIV-sero-positive participants with or without minor muco-cutaneous infection (WHO Stage I and II) who were HAART naïve and 56 HIV sero-positive subjects, co-infected with Mycobacterium tuberculosis. The control subjects, 104 in number were healthy blood donors' sero- negative for HIV antibodies and negative for M. Tuberculosis infection. Demographic data were obtained through semi-structured questionnaires and from the laboratory records. Data was collected prospectively after Informed consent and pre-test counselling were instituted. Ethical approval was obtained from university of Maiduguri teaching hospital research and ethics committee. The HIV/PTB subjects did not start ART or anti-tuberculosis therapy before laboratory investigations commenced; study was mainly laboratory based. Exclusion criteria included, use of tobacco, anticoagulant drugs, hormones or contraceptives; immobility, pregnancy or the presence of active thrombosis or haemorrhage.

Ten millilitres (10ml) of blood were collected from each subject; 4.5ml of blood was dispensed into plastic blood bottles containing 0.5ml (0.11 molar solutions) of sodium citrate to give a final blood citrate ratio of 9:1. Platelet poor plasma was obtained by centrifuging immediately at 3000g for 5minutes. Plasma was evaluated for PT, APTT, PFC and protein C (PC) by automation using coagulometer Sysmex 560 S/N 1016 (Sysmex Corporation Kobe Japan). Reagent kits acquired from Siemens Healthcare Diagnostic products GMbH 36041-Marburg Germany were used. 2.0ml of blood was dispensed into EDTA containers for platelet and WBC counts by automated haematology blood analyser Sysmex KX-21 S/NO A8893 (Sysmex corporation Kobe Japan). CD4+T cell count was performed with Partec Cyflow SL3 using kits acquired from Partec GMbH AM Flugp H_2 Germany. 3.5ml of blood was allowed to clot in plain blood bottles and serum obtained was used for HIV screening test using immuno-chromatographic kit (Chembio HIV 1&2, stat pack Medford New York, USA). Positive samples were further confirmed by Western blotting technique (QualiCode™ HIV 1&2 immunetics Inc. Botson, USA). Two serial sputum sample smears digested and concentrated by the use of 3% sodium hydroxide solution; were stained by Ziehl-Neelsen (ZN) method and acid fast bacilli identified microscopically.

Statistical analysis was carried out using SPSS package windows version 20. Data were presented as means ±SD. Comparison between variables was achieved with Kruskal wallis test (one-way ANOVA) and Newman-keul Post hoc analysis where applicable. A p value less than 0.05 was considered statistically significant.

RESULTS

Demographic information revealed that out of 102 HIV sero-positive subjects enlisted 58 (56.9%) were males and 44 (43.1%) female. Independently 56 HIV/PTB co-infected subjects 37 (57.1%) were males and 19 (33.9%) females. The age group 17-30 years had the largest number of participants accounting for greater than 50% in each group. Marital status revealed a tilt towards single status for males and female alike. The control subjects were sex and age matched with the HIV sero-positive subjects. The age ranges of all subjects were between 17 to 58 years with a median age of 35 years (table I). Table II displayed the total white cell and the $CD4^+$T count of all subjects. As expected the mean $CD4^+$T cell count of the controls were high at 610 ± 5.2 cell/µl compared to the HIV group (321±1.05) and HIV/PTB cohorts (210 ± 2.07). The total white cell count (WBC) was significantly higher in the HIV/PTB subjects compared to the controls and HIV only (P<0.001). The prothrombin time (PT) mean values of the controls 11.5 ± 0.05 seconds was significantly lower compared to the mean values for the HIV subjects 14.3 ± 0.32 (INR; 1.54) and the HIV/PTB subjects 16.2 ± 0.51 (INR; 2.0) (P<0.001). Correspondingly the activated partial thromboplastin time (APTT) mean value of the controls compared to the HIV and HIV/PTB subjects indicated similar comparisons (P<0.001). However, platelet counts (PLT) mean value was lowest in the HIV infection 107.0 ± 31.56/µl and highest in the HIV/PTB subjects 505±30.2, while the controls mean value stood at 223 ± 10.40. Multiple comparisons revealed statistically significant difference (P< 0.001). There was no significant difference between mean values of plasma fibrinogen concentration PFC between the controls 2.8 ± 0.31g/l and HIV subjects 2.3 ± 0.31 however, PFC mean value was significantly higher in the HIV/PTB subjects 4.8 ± 0.71 (P<0.001). Protein C (PC) mean value was significantly higher in the controls 95.32 ± 11.2% compared to the HIV infection 70.10 ± 3.5 and HIV/PTB co-infection 67.30 ± 5.5 (P<0.01) Table III.

TABLE 1: DEMOGRAPHIC INFORMATION OF STUDIED SUBJECTS

	CONTROL		HIV MONO-INFECTION		HIV/PTB CO-INFECTION	
SEX	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
	60(57.7)	44(42.3)	58(56.6)	44(43.1)	37(66.1)	19(33.1)
Total	**104**		**102**		**56**	
AGE RANGE:						
17-30	29(28.0)	23(22.1)	26(25.5)	27(26.5)	21(37.6)	10(17.7)
31-44	15(14.4)	16(15.4)	18(17.6)	13(12.5)	13(23.2)	6(10.5)
45-58	16(15.4)	4(3.8)	14(13.7)	4(3.9)	3(5.4)	3(5.4)
Total	**104**		**102**		**56**	
MARITAL STATUS						
Single	42(40.4)	23(22.1)	40(39.2)	23(22.5)	25(44.6)	11(19.6)
Married	19(18.3)	20(19.2)	18(17.6)	21(20.6)	12(21.4)	8(14.3)
Total	**104**		**102**		**56**	

TABLE 2: MEAN ± WBC AND CD4+T CELLS MEAN COUNT FOR ALL SUBJECTS

	SUBJECTS	WBC count (10^9/L) (4.0 - 11 X 10^9/L)	$CD4^+$T count (cells/µL) (400-1000cells/µL)
1.	Control	4.2 ± 0.30	610 ± 5.3
2.	HIV	3.5 ± 0.15	321 ± 1.50
3.	HIV/PTB	11.4 ± 0.31	210 ± 2.07
	F Statistics	23.56	168.63
	p Value	0.000^{xxx}	0.000^{xxx}
	1 vs 2 p Value	0.90	0.000^{xxx}
	1 vs 3 p Value	0.00^{xx}	0.000^{xxx}
	2 vs 3 p Value	0.00^{xx}	0.02^{x}

Key:
WBC - White Blood Cell; 1 vs 2 - Control vs HIV; 1 vs 3 - Control vs HIV/PTB; 2 vs 3 - HIV vs HIV/PTB. P Value – $^{xxx}p<0.001$, $^{xx}p<0.01$, $^{x}p<0.05$

TABLE 3: MEAN ± SD VALUE OF PT, APTT, PLT, PFC AND PC AMONG STUDIED SUBJECTS

	Subjects	PT (Seconds) 11-13.0 seconds	APTT (seconds) 32-45.0 seconds	PLT (150-450 X 10^9/l)	PFC (1.6 -3.2 g/L)	PC 70-140 (% activity)
1.	Control	11.5 ± 0.05	32.3 ± 2.0	223 ± 10.40	2.8 ± 0.31	95.32 ± 11.2
2.	HIV	14.3 ± 0.32 INR;1.54	42.0 ± 3.4	107 ± 31.56	2.3 ± 0.31	70.10 ± 3.5
3.	HIV/PTB	16.2 ± 0.51 INR; 2.0	67.3 ± 2.4	505 ± 30.2	4.8 ± 071	67.30 ± 5.5
	F Statistics	23.31	33.41	64.5	37.05	68.10
	p Value	000^{xxx}	000^{xxx}	000^{xxx}	000^{xxx}	0.00^{xx}
	1 vs 2 p Value	00^{xx}	00^{xx}	00^{xx}	0.63	0.00^{xx}
	1 vs 3 p Value	000^{xxx}	000^{xxx}	00^{xx}	000^{xxx}	0.000^{xxx}
	2 vs 3 p Value	00^{xx}	000^{xx1}	000^{xxx}	000^{xxx}	0.01^{x}

Key:
PT – Prothrombin Time; APTT – Activated Partial Thromboplastin Time; PLT – Platelet Count; PFC – Plasma Fibrinogen Concentration; PC – Protein; 1 vs 2 – Control vs HIV
1 vs 3 – Control vs HIV/PTB; 2 vs 3 – HIV vs HIV/PTB; P Value – $^{xxx}p<0.001$, $^{XX}p<0.01$, $^{x}p<0.05$

DISCUSSION

All our subjects were adults, with greater than 50% males in each study group. The age group 17-30 years accommodated a larger proportion of the subjects with mean age at 35 years. Marital status of the HIV infected subjects tilted towards the "singles".

HIV/PTB subjects had lower mean $CD4^+$T cell count compared to the HIV group and the control. Previous studies reported that HIV and TB infections act in synergy to accelerate decline of immunological profile of co- infected patients.[5,10,14] A very recent study indicated that in HIV/PTB co-infection, HIV disrupts the balance in the $CD4^+$Th subset diversity, which may play a role in impairing immunity in PTB infection by altering the equilibrium of M. tuberculosis specific $CD4^+$Th subsets [25]. Total white blood cell count mean value was higher in the HIV/PTB subjects compared to the other study groups; this in our opinion may relate to the leucocytosis associated with inflammation or acute bacterial infection.[26]

Our study also revealed a prolonged prothrombin time (PT) in the HIV subjects which was overtly pronounced in the HIV/PTB co-morbidity as indicated by its INR value. Similar report was documented by Kartaluglu et al.[20] These authors proposed that variable types of cytokines including tumour necrosis factor alpha (TNfα), interleukin 6 (IL6) emerging from the TB granulomatous lesions were thought to influence the prolongation of pro-coagulant biomarkers. Literature also documented a relationship between similar inflammatory cytokines and pro-coagulant pathways in HIV infection.[21,27]Activated partial thromboplastin time (APTT) was also prolonged in the HIV/PTB co-infection but was less affected in the HIV group. APTT is a phospholipid dependent coagulation marker, known to be prolonged by anti-phospholipid anti bodies such as lupus anti-coagulant.[19,21] Previous researchers had documented increased presence of lupus anti-coagulant in HIV infection, describing the phenomenon as a phospholipid response to HIV viraemic challenges.[21,23,28] A recent study asserted that lupus anti-coagulant was overtly increased in HIV patients co-infected with Mycobacterium tuberculosis.[19]

Platelet count was decreased in the HIV infection in comparison to the controls in our study, but was relatively increased in the HIV/PTB co-infection. Studies previously reported the presence of thrombocytosis in pulmonary tuberculosis patients.[10,18,20] In consonance with these finding Robson *et al*.,[16] earlier asserted that severe pulmonary tuberculosis was often complicated with reactive thrombocytosis, elevated fibrinogen with impaired fibrinolysis which appears to favour thrombotic events in PTB. These prepositions were not at variance with our observation because plasma fibrinogen concentration (PFC) was also elevated in PTB/HIV subjects in comparison to the controls and HIV group. Fibrinogen is an acute phase reactant protein and its elevation in pulmonary tuberculosis may be a response to acute phase phenomenon associated with PTB inflammatory processes.[16] The report of Famodu *et al*.[7] who also observed elevated PFC in PTB patients did not contradict this assertion.

Increased generation of microparticles common in HIV was attributed to immune activation and enhanced $CD4^+$ T cell apoptosis.[21,24] Protein S (a co-factor of protein C) can be bound by micro-particles rendering it inactive, this impart may explained down regulation or dysfunction of protein C (PC) in HIV morbidity.[21,22] In addition, of all the physiological anti-coagulant glycoprotein, protein C (PC) activation pathway was reported to be most affected by inflammatory cytokines-abundant in HIV and TB; PC dysfunction and reduced activity was reported in some systemic inflammatory conditions.[22,27,29,30] Percentage activity of protein C in our study was down-regulated in HIV subjects compared to the controls but was more efficient in the HIV only compared to the HIV/PTB co-morbidity.

Macrophage apoptotic process in PTB was also reported, authors asserted that the process could generate micro particles in the course of granulomatous lesion formation.[3,30,31] Speculatively therefore it can be stated that the down-regulation of protein C in this study may not be unrelated to a synergistic consequences of HIV and PTB co-morbidity.

In conclusion we opine that alterations in some coagulation biomarkers of PTB patients can occur and the risk may increase in the setting of HIV/PTB co-morbidity. Clinical findings however, may be necessary to shed more light on emerging coagulopathies.

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