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HIV Mono and Hepatitis B Virus (HBV) Coinfection: The Impact on Some Coagulation Biomarkers

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Abstract: ***Co-infection of hepatotropic virus such as Hepatitis B Virus (HBV) has been associated with a reduced survival rate and an increased risk of progression to severe liver disease. In Nigeria, information on this is sparse. This study was design to determine the impact of HIV/HBV co-infection on the coagulation parameters, platelet count (PLT), prothrombin time (PT), activated partial thromboplastin time (APTT), protein C (PC), protein S (PS) and lupus anticoagulant in a prospective cohort study of patients confirmed as HIV seropositive by Western blot analysis in University of Maiduguri Teaching Hospital. Demographic data was obtained from 109 HIV patients, HBsAg was present in 14 (12.8%), males 10 (9.2%) and females 4 (3.6%). HIV/HBV co-infected patients had PLT, PC and PS lower than values in HIV mono infection (P ‹ 0.01). The mean values in the control subjects were higher compared to the HIV groups (P‹0.001). PT, APTT and LA mean values were higher in the HIV/HBV patients compared to the HIV mono infection (P ‹ 0.01) while the control subject mean values were lower compared to the HIV group (P ‹ 0.001). Lupus anticoagulant was significantly elevated in the HIV mono and HIV/HBV co-infected subjects. The deranged coagulation biomarkers in the HIV/HBV patients inform the need for routine screening of these haemostatic markers in our environment.***

Keywords: HIV, HBV, Mono-infection, Co-infection Coagulation

1. Introduction

Reports on HIV infection in Nigeria indicate that seroprevelence rate has stabilized within the range of 4.4 and 4.1 from 2005 t0 2010 [1]. Also previous studies have stated the prevalence of 5.5 – 12.3% for HIV and hepatitis B virus (HBV) co-infection [2]-[5]. HIV immunopathogenesis in infected individuals is known to be associated with enormous haematological consequences. These in the recent time also include coagulation abnormalities [6]. The extent of this haemostatic dysfunction has been shown to correlate with the severity of HIV immunosuppression as evidenced by decreased CD4 + lymphocyte counts [7].

Coagulation abnormalities may be multiple and include quantitative and qualitative platelet abnormalities, defects of prothrombotic activation complex leading to prolonged Prothrombin Time (PT) and activated partial thromboplastin (APTT), decrease in the plasma levels of some natural anticoagulant proteins such as protein C (PC) and protein S (PS) [8]-[10]. Immune bases have been suggested as the reason for thrombocytopenia because of the presence of platelet associated IgG/complement complex [11]. Reduced counts in addition may also follow marrow impairment and drug toxicity [10], [12].

PT and APTT may be prolonged in HIV disease due to the presence of an inhibitor such as Lupus anticoagulant (LA), an antiphospholipid associated with HIV infection in greater than 70% of cases [13], [14]. LA is an IgG/IgM antibodies of phospholipid specificity and can bind phospholipids active in coagulation [15].

Co-infection of HIV and hepatotropic viruses such as HBV could occur since both share common principal route of infection. It was been observed that as deaths caused by HIV disease are declining due to improved management scheme including the use of Highly Active Antiretroviral Therapy (HAART), diseases associated with co-infection by HBV are increasing the morbidity and mortality burden of HIV [6]. Coagulation abnormalities are often measured by the prolongation of global screening test such as PT and APTT [16]. Prolonged PT and APTT are related to the severity of liver failure and serves as prognostic indices in CLD [17]. PT determines vitamin K dependent extrinsic factors VII, X, II and fibrinogen. While APTT measures the activities of the intrinsic pathway of coagulation cascade and most sensitive to factors VIII, IX, XI, XII and the contact system [18]. APTT is more often prolonged in advanced CLD [19], [20].

The incidence of hypercoagulable state particularly thrombosis in HIV patients was reported to have increased 2 - 10 folds compared to healthy control population of the same age [21]. The risk appears to increase in advance HIV disease [10]. HIV related thrombosis has been associated with reduced plasma level of glycoproteins, Protein C (PC) and protein S (PS). These are vitamin K dependent glycoprotein synthesized in the liver. They are involved in the haemostatic balancing mechanism which ensures non occlusion of the vasculation by blood clot [21].

As coagulation abnormalities increasingly emerge as clinical issue in HIV patients, we considered worthwhile to investigate some haemostatic biomarkers in HIV and HIV/HBV patients in our environment.

2. Subjects and Methods

This is an hospital based prospective study conducted between October 2012 and May 2014 at University of Maiduguri Teaching Hospital (UMTH) a Centre of Excellence in Infectious Disease and Immunology. The Hospital is located in Northeastern Nigeria.

Patients confirmed to be seropositive for human immunodeficiency virus (HIV) infection were recruited for the study. They were all WHO stage II patients with CD4 count greater than 200cells/ul. These HIV positive subjects were also screened for hepatitis B virus and (HBV) infection. Out of the 109 HIV subject recruited, 14 (12.8%) were positive for HBV, 10 (71.4%) were male and 4 (28.6%) females. The HIV mono-infected were 95, male 65 (68.4%) and female 30 (31.6%). The age of the patients ranged from 16 to 59 with a mean age of 36±20 years for both sexes.100 age and sex matched apparently heathy blood donors male 78 (78%), female 22 (22%) seronegative for HIV and HBV infections were also recruited as control subjects.

The confirmed HIV positive patients were on triple combination Highly Active antiretroviral Therapy (HAART), while different drug combination was administered to patients co-infected with HBV. Demographic information, sexual behavior, intravenous drug use and blood transfusion data were obtained through brief structured questionnaire and laboratory records. Informed written consent and pretest counseling were instituted before the study commenced.

Ten milliliters of blood was collected from each subject, 4.5ml of blood was dispensed into plastic blood bottle containing 0.5ml (0.11 molar solution of sodium citrate to give a final blood/citrate ratio of 9:1. Platelet poor plasma obtained by centrifuging immediately at 3000g for 5minute was used for Prothrombin Time (PT), activated Partial Thromboplastin Time (APTT), Protein C (PC), Protein S (PS) and Lupus Anticoagulant (LA) assay by automated coagulometer Sysmex 560, S/No. 1016 manufactured by (Sysmex Corporation, Kobe, Japan). Reagent kits acquired from Siemens Healthcare Diagnostic Products GMbH 36041-Marburg, Germany were used. 2.0ml of the blood collected was dispensed into EDTA containers for platelet (PLT) count using automated Haematogy blood analyser Sysmex KX-21 S/No. A8893 manufactured by Sysmex Corporation, Kobe, Japan. 3.5ml of the blood was allowed to clot in plain blood bottle and serum obtained used for HIV screening test using immunochromatographic Kit (Chembio HIV 1 and 2 stat pack Medford New York, USA). Positive samples were further confirmed by Western Blotting (Qualicode^{TM} HIV 1 and 2 Immunetics Inc. Boston, USA and HBV (HBsAg) by enzyme-linked immunosorbent Assay (ELISA) using the kit BIORAD Monolisa HBsAg ULTRA EIA 2430 Marnes-LA-Coqiette, France.

3. Statistical Analysis

Results obtained were analyzed with Statistical Package for Social Science (SPSS) Version 20 Software Data was presented as descriptive mean (x) and standard deviation (SD). Statistical comparison were performed by ANOVA (one-way). The level of significant was taken at 95% confidence interval and P values less than ‹ 0.05 was considered significant.

4. Results

Table 1: Demographic Characteristics of Subjects (HIV Positive – n = 109, Control – n = 100).

Variables	*HIV mono-infection*	*HIV co-infection*	*Control*
Gender			
Male	65 (59.7%)	10 (9.2%)	68 (68%)
Female	30 (27.5%)	4 (3.6%)	32 (32%)
Total	95 (87.2%)	14 (12.8%)	95 (100%)
Age group			
16 – 26	6 (6.3%)	1 (7.2%)	10 (10%)
27 – 37	49 (51.6%)	8 (57.1%)	50 (50%)
38 – 48	38 (40.0%)	5 (35.7%)	32 (32%)
49 – 59	2 (2.1%)	0 (0.0%)	8 (8%)
Total	95 (100%)	14 (100%)	100 (100%)
Marital Status			
Single/Never married	52 (54.7%)	7 (50.0%)	44 (44%)
Married	12 (12.6%)	1 (7.1%)	43 (43%)
Widow/Widower	31 (32.7%)	6 (42.9%)	13 (13%)
Total	95 (100%)	14 (100%)	100 (100%)

Table I presents the demographic characteristics of all subjects. A total of 14 (12.8%) of the 109 patients were positive to HBsAg (a biomarker for HBV) out of which 10 (9.2%) were male and 4 (3.6%) female. Among the HIV mono infection patients, males were 65 (59.7%) and females 30 (27.5%). The age group of both sexes range from 16 to 59 with a mean age of 34.0 ± 18 years. The age group 27 – 39 years had the highest number of HIV mono infection 49 (51.6%) and HIV/HBV 8 (57.1%). The control subjects appeared age and sex matched with the HIV groups. About half of th HIV mono-infection 52 (54.7%) and HIV/HBV co-infected 7 (50%) were single or never married. The control subjects showed a different pattern with approximate representation in the single 44 (44%) and married 43 (43%)

Table 2: Coagulation Parameters in HIV monoinfection, HIV/HBV co-infection and their Controls (HIV Positive – n = 109, Control – n = 100)

Parameters	*HIV mono-infection*	*HIV co-infection*	*Control*
PLT (x 10^9/L)	167.20 ±33.6	108.15 ±31.68	236.50 ±63.0
PT (Second)	14.00 ±0.07	16.60 ±0.87	12.30 ±1.40
APTT (Second)	38.74 ±12.20	50.34 ±2.30	32.40 ±2.70
PC (% Activity)	80.34 ±3.43	68.30 ±2.40	98.34 ±4.40
PS (% Activity)	80.06 ±11.3	60.32 ±0.50	96.42 ±3.20
LA (Ratio)	1.21 ±0.84	1.30 ±0.73	84.0 ±2.10

Key :- PLT = Platelet, PT = Prothrombin Time, APTT = Activated Partial Thromboplastin Time, PC = Protein C, PS = Protein S, LA = Lupus Anticoagulant.

Table 2 displays coagulation biomarker of HIV mono-infection and HIV/HBV co-infected subjects and their controls. The mean PT and APTT values were significantly higher in the HIV mono infection and HIV/HBV co-infections compared to the controls (P ‹ 0.001), similarly HIV/HBV mean values compared to HIV mono infection was significantly higher (P ‹ 0.01). The PLT, PC and PS mean values were significantly higher in the Controls compared to the HIV mono-infected and HIV?HBV co-infected subjects (P ‹ 0.01). These mean values are similarly significantly higher in the HIV mono infection compared to the HIV/HBV co- infection (P ‹ 0.01). PC and PS mean values are deranged in the HIV/HBV group (Normal reference ≥ 70% activity Level). LA mean ratio was lower in the Controls when compared to the HIV and HIV/HBV group (P > 0.001) There was also statistically significant difference between the LA mean ratio of the HIV mono infection and HIV/HBV co-infection (P ‹ 0.05). LA mean ratio of the HIV groups were deranged (Normal reference ratio = ‹ 1.20).

5. Discussion

This study revealed that more males may be suffering HIV co-infection giving the male:female ratio of 1.8:1 in our environment. This is however different from the report of Balla et al 2012 [4] with a male:female ratio of 1:1.4. The age group 27 – 37 appears to suffer more from the co infection, this is however similar to the report of previous author [4]. More than half of the study population of HIV mono infection (51.6%) and HIV co infection (57.1%) were single or have never married. This marital status are more likely to be associated with multiple sex partners which may favour sexually transmitted infections such as HIV and hepatotropic viral infection such as HBV. In greater than 80% of the cases, the cause of liver impairment such as chronic liver disease included infection by hepatitis B virus (HBV) [22].

Thrombocytopenia has been associated with HIV infection and immune basis was strongly suggested by the presence of platelet and complement complex [11]. Our study similarly revealed decrease in platelet count in the HIV mono infected patients while patients with HBV co infection had a greater level of reduction in mean platelet count. In liver impairment, sequestration related thrombocytopenia is not uncommon [23], [24].

In this study, prothrombin time (PT) was mildly prolonged in the HIV mono- infection but overtly prolonged in subjects co infected with HBV. Deranged PT and APTT in HIV infection was related to the inhibitory effect of lupus anticoagulant an antiphospholipid specific antibody which has been reported to bind phospholipids active in coagulation, APTT is more affected than PT [24]. Stimmiler et al [13] and Ankri et al [14] previously asserted that LA was detected in greater than 70% of HIV/AID cases. In our study, LA ratio showed significantly elevated ratio in the HIV mono infection and HIV/HBV co infection compared to the control. The presence of LA in HIV infection was discribed as antiphospholipid response to viramic challenges [20]

Natural anticoagulants protein C and protin S were significantly reduced in the HIV subjects compared to the control. However in the HIV/HBV co infection, PC and PS revealed deranged percentage plasma level activities (‹ 70% activity is abnormal). Down regulated PC and PS have been reported in HIV infection [25]. Various pathophysiological mechanisms have been suggested to explain this down regulation. Klein et al [20] reported that the presence of antiphospholipid antibodies such as LA which has the ability to inhibite the action of activated protein C (APC) could interfere with PC pathway.

It was also observed that inflammatory cytokines such as tissue necrosis factor alpha (TNα) released in chronic HIV disease may lead to down regulation of natural anticoagulants such as PC and PS [25]. Of all the natural anticoagulant pathway, PC appear to be most affected by inflammatory responses [26].

The presence of increased levels of compliment binding protein 4 in HIV disease which could bind PS inactivating it has also been reported [10]. Immune activation and increased apoptosis of circulating T cells can generate microparticles that can bind PS rendering it inactive as a co-factor of PC [27]. Protein C (PC) and protein S (PS) are both vitamin K dependent glycoptrotein synthesized mainly by the liver [23]. This may explain the deranged percentage plasma activity levels of PC and PS obtained in our study among HBV co-infected HIV subjects. Liver disease as a result of HIV/HBV co-infection is on the rise in our environment, therefore, more research in this area of studies are warranted in order to increase our understanding of the phenomena.

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