IOSR Journal of Pharmacy and Biological Sciences (IOSRJPBS)
ISSN : 2278-3008 Volume 1, Issue 5 (July-August 2012), PP 24-29
www.iosrjournals.org

Effects of Malaria, HIV Infection and Antiretroviral Therapy on Some Coagulation Profiles

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Abstract*: This study was designed to determine the effects of HIV, Malaria and antiretroviral therapy on some Coagulation Profiles. The Participants were groups as follows; HIV on antiretroviral therapy (ART) with malaria (n=30), HIV on ART without malaria infection (n=29), HIV positive CDC classification stage I not on ART with malaria (n=30), HIV stage II not on ART with malaria (n=31), HIV stage I not on ART without malaria (n=31), HIV stage II not on ART without malaria (n=31), HIV negative without malaria and HIV positive with malaria control participants (n=61). HIV Screening, Malaria (Screening and density count), CD4 count, APTT, prothrombin time (PT), PCV, platelet, white blood cell counts were enumerated by standard laboratory techniques. The result showed that PCV were significantly different (p<0.05) among groups. On comparison within groups, PCV was significantly lower in HIV positive not on ART compared with control participants. Platelet count was significantly higher in HIV on ART compared with HIV positive not on ART (p<0.05). APTT was significantly higher in HIV positive stage II not on ART without malaria compared with HIV positive on ART without malaria. Also PT was significantly higher in HIV on ART with malaria compared with HIV positive stage II not on ART with malaria. This study suggests that the impact of ART improved PCV and Platelet levels and also the possible cause of mild prolongation in prothrombin time. Lupus anticoagulant is likely cause of observed prolonged APTT in HIV positive not on ART.*

I. Introduction

Malaria infection is associated with systemic endothelial activation, patchy endothelial damage (Turner et *al*, 1994). Low platelet counts, decreased levels of anticoagulant, generation of activated thrombin and procoagulant microparticles (Clemens *et al*, 1994). This strongly suggests a state of dysregulation of the coagulation system.

HIV infection is characterized by high rate of viral replication throughout the course of the infection with resulting viral and immune mediated destruction of CD4 cells (Ifeanyichukwu *et al*, 2011). Consequently, the infected individual becomes susceptible to opportunist infections, malignancies and neurological diseases (Ukibe *et al*, 2010; Uchaikin 1989). Morever, various abnormalities includes presence of antiphospholipid antibody, lupus anticoagulant, increased level of von Willebrand factor and D-dimer were found in HIV patients (Jason *et al*, 2011) and all these are coagulation markers.

In addition, bleeding has been reported among non-hemophilic patients, taking protease inhibitor treatment. Mucous membranes and gastrointestinal tract were the most common sites of bleeding, skin, soft tissue and intracranial bleeds were also reported (Racoosin and Kessler, 1999).

Considering this prior information, this study was designed to determine the effects of malaria, HIV infection and antiretroviral therapy (alone or as co-infection) on coagulation aspects of haematology.

II. Materials And Methods

Subjects:

A total of two hundred and forty three participants were recruited using stratified sampling method for the study.

One hundred and eighty two HIV infected patients were recruited from the Voluntary Counseling and Testing (VCT) Unit and HIV Clinic at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State Nigeria. They were aged 18-60 years. CDC classification for HIV infection based on CD4 count was used to

classify the HIV disease stages. Sixty one apparently healthy HIV seronegative participants were drawn from the VCT Unit of Nnamdi Azikiwe University Teaching Hospital Nnewi, who served as control. Blood sample; 4.5ml were collected from all the participants for the analysis of the parameters, for HIV screening by immunochromatography, CD4 counts by cyflowmetry, PCV, WBC total and differential count and malaria screening by standard routine method, 3ml was dispensed into EDTA container and mixed . Also 1.8ml of blood was added into 0.2ml of trisodium citrate tube (1 part of citrate to 9 parts of blood) for prothrombin and activated partial thromboplastin time assay by routine method. Informed written consent was obtained from those who participated in the study. Ethical approval of study design was obtained from Nnamdi Azikiwe University Teaching Hospital Nnewi Ethical Committee.

III. Methods National Algorithm For Serial Rapid Hiv Testingprinciple:

Algorithm is defined as combination and sequence of specific tests used in a given testing strategy. An HIV positive status should be based upon the outcome of two or more tests. Serial testing means sample tested by first test kit, the results of the first determine whether additional testing is required. If the first test kit showed a non-reactive result; the tested sample was reported as HIV negative .If the first test kit showed a reactive result, the sample was tested further by a second test kit; if the second test kit showed a reactive result, the tested sample was reported as HIV positive. When two test results disagree (the first is reactive and second is non-reactive). In this case a third test was performed; the result of the third test was the final test result. For this study, Determine, Stat Pak and Uni-Gold were the first, second and tie breaker used respectively.

Test Of Hiv Infection Using: Determine Hiv1/2 Rapid Test Kit By Immunochromatography

Procedure :

Fifty microlitres of plasma samples was dispensed into the specimen pad of the test strip. The reaction will allowed for 15 minutes (up to 30 minutes), the appearance of distinct red lines on test region and control region of the kit suggest positive HIV test while one distinct red line in the region of control suggest HIV negative test. Appearance of the distinct red line on the control region validates the result without that the kit is assumed to be non-functional.

Stat Pak Hiv 1/2 Rapid Test Kit By Immunochromatography

Procedure:

HIV 1/ 2 stat pak test device was removed from its pouch and was placed on a flat surface. Five microlitres of sample (serum, plasma or whole blood) was added into the sample pad. Also 3 drops of buffer was dropped into the sample well. Result was read 10 minutes after the addition of the buffer.

Uni-Gold Hiv1/2 Rapid Test Kit By Immunochromatography

Procedure:

The test device was removed from protective wrapper. Over the sample port 60µl of serum sample was added. Also two drops of wash buffer reagent. Result was read after 10 minutes.

CD4+ T CELL COUNT BY CYFLOWMETRY

Twenty microlitres EDTA whole blood was collected into partec test tube (Rohren tube). Then twenty microlitres of CD4+ T cell antibody was added into the tube. The content was mixed and incubated in the dark for 15 minutes at room temperature. Eight hundred microlitres of CD4 buffer was added into the mixture and mixed gently. The partec tube was plugged on cyflow counter and the CD4+ T cells were displayed as peaks and interpreted as figures.

IV. Malaria Screening

The test device was at room temperature by allowing for 15 minutes. Twenty microlitres of whole blood sample was added to sample well. Also 3 drops of clearing buffer was dispensed into buffer well and the reaction was allowed for 10 minutes with the whole blood, the appearance of distinct red lines at the test region and control region of the kit suggest that the specimen contains plasmodium falciparum or vivax antibody (depending where the red line appeared) while one distinct red line in the control region suggest absence of plasmodium falciparum or vivax antigen on specimen. Appearance of the distinct red line on the control region validates the result without that the kit is assumed to be non- functional.

V. Malaria Parasite Density Count.

The determination of the number of circulating parasites is exceedingly important for clinical purposes to monitor the evolution of the disease and the efficacy of the therapy. This is achieved by estimating parasite numbers per microlitre of blood by counting parasites against white cells. A part of the thick film where the white cells were evenly distributed and parasites well stained was focused and using oil immersion objective, 200 white blood cells (WBC) were counted at the same time estimating the numbers of parasites (asexual) in each field (McKenzie *et al*, 2005). The numbers of parasites per microlitre of blood was calculated as follows.

Total WBC x parasites counted against 200 WBC/ 200 = Parasites/µl of blood.

VI. Prothrombin Time Test

As described by the manufacturer of the kit linear chemical, Barcelona, Spain. The reconstituted prothrombin time reagent was pre- incubated at 37^0C for 10 minutes. Fifty microlitres of sample or control plasma was then pipette into test curvette and incubated at 37^0C for 2 minutes. One hundred microlitres of pre-incubated prothrombin time reagent was rapidly added and clotting time in seconds was recorded.

Activated Partial Thromboplastin Time Test

As described by the manufacturer of the kit linear chemical, Barcelona, Spain. Calcium chloride (0.02mol/l) was pre-incubated at 37^0C for 10 minutes, and 50µl of plasma or control plasma were then be pipetted into test curvette and incubate at 37^0C for 3 minutes. Fifty microlitres of APTT reagent was added into to the curvette containing the plasma and the mixture incubated at 37^0C for 3minutes. Fifty microlitres of the pre incubated calcium chloride was rapidly added and timer simultaneously started and clotting time in seconds was recorded.

Packed Cell Volume By Microhaematocrit Method

Blood samples was thoroughly mixed and allowed to flow into plain capillary tube by capillary action to about 2/3 length of the tube. The unfilled end of the tube was flamed sealed using a Bunsen burner. The sealed tubes was placed in the racial grooves of a microhaematocrit centrifuge and spin for 5 minutes at 12,000 revolutions per minute (rpm).The packed cell volume was read using Haematocrit Reader.

Platelet Count By Routine Method

A Well Mixed Anticoagulated Venous Blood, 0.02ml Was added to 0.38ml of I% ammonium oxalate. The counting chamber was charged and filled with the well mixed sample and left undisturbed for 20 minutes. To prevent drying of the fluid, the chamber was placed in a Petri-dish and covered with a lid. The platelets were counted using the x 40 objectives of binocular light microscope

Total White Cell Count By Routine Method

The mixed blood sample was diluted 1 in 20 with Turk's solution (i e 20µl of blood and 380µl of Turk's solution) using automated pipette into test tube. The content of the tube was gently mixed and the Improved Neubauer chamber, charged with the diluted blood.

The charged chamber was placed in moist condition for 2 minutes for the cells to settle. The total white cell count was performed using x 10 objectives of binocular light microscope.

Statistical Method

Statistical analysis was done using software; statistics package for social sciences (SPSS) version 16. ANOVA was used to compare parameters among groups. Student t-test was used to compare parameters within groups. Pearson correlation coefficient was also applied. $P< 0.05$ was considered as level of significance.

VII. Results

The mean ±S.D values of packed cell volume (%) in HIV negative participants without malaria infection 38±4.7, in HIV positive on ART without malaria infection participants 38 ± 3.9, in HIV positive (CDC) classification stage I not on ART without malaria infection participants 36±4.6 and HIV positive (CDC) classification stage II not on ART without malaria participants 36 ±4.4 were significantly different ($F=4.495$; $P<0.05$), (Table 1).

Within groups comparison showed mean ±S.D values of packed cell volume was significantly higher in HIV negative without malaria 38±4.7 compared with mean ±S.D values of packed cell volume in HIV positive stage I not on ART without malaria 36 ±4.6 ($P<0.05$), (Table 1). Furthermore, the mean ±S.D values of PCV was significantly higher in HIV negative without malaria 38±4.7 compared with HIV positive stage II not ART without malaria 36±4.4 ($P<0.05$). In addition, mean ±S.D values of PCV in HIV positive on ART without malaria 38±3.8 compared with HIV positive stage I not on ART without malaria 36±4.6 showed significant higher value. ($P<0.05$), (Table I). The mean ±S.D values of PCV in HIV positive on ART without malaria 38 ±3.8 compared with HIV positive stage II not ART without malaria 36±4.4 showed significant higher mean ($P<0.05$), (Table 1). However the mean ±S.D values of PCV in HIV negative without malaria 38±4.7 and in HIV positive on ART without malaria 38 ±4.7 compared showed similar statistical values. Furthermore mean ± S.D values of PCV in HIV Positive stage I not on ART without malaria 36±4.6 compared with values of HIV positive stage II not on ART without malaria 36±4.4 respectively were not significant ($P>0.05$),(Table 1).

The mean ±S.D values of platelet ($x10^9$/l) in HIV positive on ART without malaria participant 253±77 was significantly higher when compared with values in HIV positive stage I not on ART without malaria 209±65.

(P<0.05), (Table I). However, the mean ±S.D values of platelet compared amongst all other groups were not significant (P>0.05), (Table I).

The mean ±S.D values of activated partial thromboplastin time in HIV positive stage II not ART without malaria 37.1±1.3 was significantly higher (P<0.05) than mean ± S.D values of APTT in HIV positive on ART without malaria 35.2±4.5. (Table I). However mean ± S.D APTT compared amongst all other groups had similar statistical values (P>0.05). (Table I).

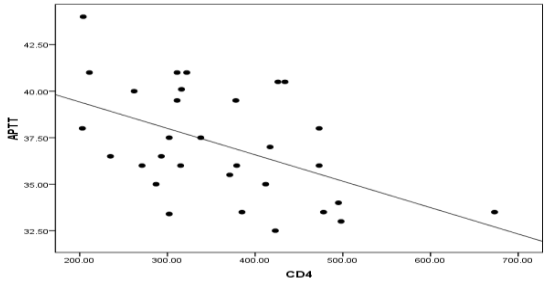
However, mean ± S.D values of promthrombin time (PT) (sec) in HIV negative without malaria , HIV positive on ART plus malaria negative participants, HIV positive (CDC) classification stage I not on ART plus malaria negative participants and HIV positive (CDC) classification stage II not on ART plus malaria negative participants compared were statistically not significant (in each case) amongst the groups. (Table I).

Mean ±S.D values of activated partial thromboplastin time and CD4 count correlated in HIV positive stage II not on ART without malaria shown a significant negative correlation (fig. 4.1).

TABLE 4. I Mean (±S.D) of some coagulation profiles and haematological parameters of HIV patients without malaria.

	APTT (sec)	**PT (sec)**	**PCV (%)**	**WBC ($x10^9$ /l)**	**PLATELET ($x10^9$ /l)**
(A)HIV negative without malaria. (N=31)	36.0 ±3	19.1 ±1.7	38±4.7	5.3±1.8	204±77
(B) HIV positive on ART without malaria. (N=29)	35.2±4.5	19.2 1.8	38±3.8	5.3±1.4	253 ±77
(C) HIV positive (CDC) stage I not on ART without malaria (n =29)	36.6 ±2.6	18.5±1.5	36 ± 4.6	5.6±2	209±65
(D) HIV positive (CDC) stage II not ART without malaria.(N=31)	37.1 ±3.0	18.5±1.7	36±4.4	4.8±1.5	217±81
F (P)	1.916(0.131)	1.287(0.282)	4.495(0.04) *	1.164(0.326)	2.534 (0.060)
AvsB p-value	0.262	0.989	0.873	1.000	0.065
AvsC p-value	0.810	0.210	0.04*	0.529	0.796
AvsD p-value	0.643	0.225	0.010*	0.252	0.528
BvsC p-value	0.147	0.141	0.02*	0.465	0.02*
BvsD p-value	0.044 *	0.160	0.04*	0.123	0.09
CvsD p-value	0.927	0.998	1.000	0.257	0.976

- Key F(P) = HIV negative without malaria , HIV positive on ART without malaria , HIV positive (CDC) stage I not on ART without malaria and HIV positive stage II not on ART without malaria compared. (Using ANOVA).
- AvsB= HIV negative without malaria compared with HIV positive on ART without malaria (using student t-test).
- AvsC =HIV negative without malaria compared with HIV positive (CDC) stage I not on ART without malaria (using student t-test)
- AvsD= HIVnegative without malaria compared with HIV positive (CDC) stage I not on ART without malaria (using student t-test).
- BvsC= HIV positive on ART without malaria compared with HIV positive (CDC) stage I not on ART without malaria (using student t-test)
- BvsD = HIV positive on ART without malaria compared with HIV positive (CDC) stage II not on ART without malaria (using) student t-test
- CvsD = HIV positive CDC stage I not on ART without malaria compared with HIV positive stage II not on ART without malaria (using student) test).



r= -0.500 p<0.05

Fig.4.1. Scatter diagram of significant negative correlation of Activated partial thromboplastin time and CD4 count in HIV positive (CDC) stage II not on ART without malaria.

VIII. Discussions

In this study packed cell volume was slightly reduced in both HIV positive stages I participants not undergoing antiretroviral therapy (ART) and infected by malaria parasite and HIV positive stage II participants not undergoing ART treatment and not infected by malaria parasite, compared with normal control subjects. This shows that HIV/AIDS has effects on progenitor cells of haemopoietic cells involved in red blood cells production as well as in the survival and half life of the red cells (Blockman, 1991). But the effect of ART reverted the effect of HIV/AIDS on red blood cell production to normal. This observation shows that the effect of antiretroviral therapy has disabled the deranging actions of the HIV/AIDS on red blood cell production and survival. This is in agreement with independent research conducted in Benue State, Nigeria (Amegor *et al,* 2009).However, this is in contrast, as several authors have reported that zidovudine (AZT) used as ART had caused anaemia by inhibition of haemoglobin synthesis and toxicity to bone marrow cells, particularly erythroids lines (Moyle, 2002; Omoregie *et al,*2008).

The platelet count in HIV positive on ART participants either infected or not infected by malaria parasite was significantly higher compared to HIV positive participants not undergoing ART treatment and not infected by malaria parasite. It has been reported that zidovudine improve platelet production in HIV patient with thrombocytopaenia (Ballem *et al,* 1992) This is in consonance with another independent work conducted by (Chukwurah *et al,* 2007), that reported that ART and nutrition increase platelet count than ESR and PCV in HIV positive on ART. Although it has been reported that patients with AIDS have decreased platelet production whereas patients with early onset of HIV infection are more likely to have increased peripheral destruction of platelet by antiplatelet antibodies (Karpatkin *et al,* 2002). Mechanism of action of the ART are not known, but this higher value may be due to the fact that these therapies includes two nucleoside reverse transcriptase inhibitors with a protease inhibitor and could normally slow down the action of the enzyme reverse transcriptase possessed by the HIV virus. Hence ART may provide the opportunity for haemopoiesis rejuvenation, giving way to normalizing the platelet production.

In this present study also show significantly higher APTT in HIV positive (CDC) stage II participants, who are not undergoing ART treatment and are not infected by malaria parasite compared to HIV positive participants, who are undergoing ART treatment and are not infected by malaria parasite. Though still within the reference range (30-40sec). The higher value may be due to various abnormalities found in HIV patients which include presence of antiphospholipid antibody, lupus anticoagulant (Jason et *al,* 2011). Lupus anticoagulant has been associated with the catastrophic antiphospholipid syndrome in Human Immunodeficiency Virus (HIV) – infected patient (Petrovas et *al,* 1999). There is marked difference between lupus anticoagulant in HIV patient not on ART and HIV patient on ART, there is higher prevalence of lupus anticoagulant in HIV patient not on ART. ART may prevent development of lupus anticoagulant in HIV-infected patient (Awodu *et al,* 2010). Lupus anticoagulant (also known as lupus antibody or lupus inhibitor) is an immunoglobin that binds to phospholipid and protein associated with the cell membrane. It targets phospholipids and is the most common cause of isolated prolongation of activated partial thromboplastin time, which may predispose patients to thrombosis rather than haemorrhage (Galli *et al,* 2003). Although, the prothrombin time requires phospholipid but the concentration of phospholipid in the prothrombin time reagent is high and so it frequently neutralizes the lupus anticoagulant and therefore prothrombin time is not prolonged during testing (Triplett and Brandt, 1989). This may be possible reason of mild increase in APTT and normal value of PT in HIV patient stage II participants, who not undergoing ART treatment. The correlation between CD4 count and coagulation profiles analyzed showed that only APTT was significantly correlated negatively with CD4 count of HIV patient CDC stage II not undergoing ART treatment and not infected by malaria parasite. It seems likely that APTT could be used as a means of HIV disease progression in resource poor setting where CD4 count is impossible, just as anaemia is used as a marker of disease progression (Moyle, 2004).

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