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Evaluation of Pcv, Cd4$^+$ T cell Counts, ESR and WBC Counts in Malaria Infected Symptomatic HIV (Stage 11) Male HIV/ Aids Subjects on Antiretroviral Therapy (Art) In Nnewi, South Eastern Nigeria

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Abstract—This study was designed to assess the values of PVC, CD4$^+$ T cell counts, ESR and WBC counts in malaria infected HIV male subjects on ART. A total of 273 male participants aged between 18 and 60 (42±13) years were randomly recruited for the study. The Based on WHO HIV staging and classification, 69 participants were classified as symptomatic HIV (stage 11) male participants on ART (Lamivudine, Stavudine and Nevirapin); 69 participants were classified as symptomatic HIV (stage 11) male participants not on ART; 68 participants as asymptomatic HIV male groups and 68 participants as HIV seronegative controls. Blood samples were collected from the participants for the determination of HIV status by immunoassay and immunochromatography; CD4$^+$T cell counts by Cyflow method, WBC counts, packed cell volume (PCV), and Erythrocyte sedimentation rate (ESR) by standard laboratory methods. The CD4$^+$T cell counts, PCV and ESR values were significantly different when observed amongst the malaria infected groups ($p<0.05$) respectively. Nevertheless, the value of WBC count was not significantly different when observed amongst the groups ($p>0.05$). The implication of this finding is that both HIV and malaria co- infection has an adverse effect on the values of CD4$^+$T cell counts, PCV and ESR in the host.

I. INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is an endemic disease that causes death among young adults worldwide (Amegor *et al*., 2009). It is caused by Human immunodeficiency virus (HIV) (Onyancha, 2009).

Malaria is a life threatening disease that causes major health problem in the tropics. It is caused by Plasmodium species. Malaria was observed to be endemic in Nigeria and it is the leading cause of morbidity and mortality in the Country (Onwujekwe *et al*., 2000; FMH, 2001). Cohen *et al*, (2005) observed an increased prevalence of severe malaria in HIV-infected adults' subjects in South Africa. While Onyenekwe *et al.,* (2007) also observed an increased prevalence of severe malaria in HIV-infected malaria subjects in endemic area of Southern Nigeria.

Presentation of severe malaria has been observed in HIV subjects with CD4$^+$T cells count less than 2000 x 10^6 cells/ l (Cohen *et al*, 2005). Onyenekwe *et al*., (2007) reported a tripled prevalence of malaria infection in symptomatic HIV-infected subjects. Some researchers have observed that HIV/AIDS causes anaemia (Sullivan, 2002; Volberding, 2002). The explanation given by some researcher was that the anaemia observed in HIV/AIDS subjects may be as a result of the direct attack of the reticuloendothelial cells by the virus (Jacobson *et al*., 1990). Anaemia has been shown to be statistically significant predictor of progression to the acquired immunodeficiency syndrome and it is related with increased risk of death in subjects with HIV infection (Belperio and Rhew, 2004). Hence, this study was designed to determine the values of PCV, CD4$^+$T cell counts, WBC and ESR levels in HIV and malaria co-infection.

II. MATERIAL & METHODS

Subjects

A total of 549 adult male participants aged between 18 and 65 (39 ±12) years were randomly recruited at the Voluntary Counseling and Testing (VCT) Centre in Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria. Based on World Health Organisation (WHO) criteria for staging HIV, the participants were grouped as follows: 139 symptomatic HIV (stage 11) participants on ART, 136 symptomatic HIV (stage 11) participants not on ART, 136 asymptomatic HIV participants and 136 HIV seronegative male participants.

Hematological analysis

Five (5) ml of EDTA blood sample were collected from each subject and were analysed for malaria parasite (MP), haematocrit (PCV), white blood cell count (WBC), CD4+T-cell counts, HIV screening with confirmation and erythrocyte sedimentation rate (ESR).

III. METHODS

Determination of Antibodies to HIV-1 and HIV-2 in Human plasma.

Two different methods were used, namely, Abbott determine ^{TM} HIV -1 and HIV-2 kit, which is an in-vitro visually read immunoassay (Abbott Japan Co.Ltd.Tokyo, Japan) and HIV-1 and 2 STAT-PAK Assay kit, which is an Immunochromatographic test for the quantitative detection of antibodies to HIV-1 and HIV-2 in Human plasma (CHEMBIO Diagnostic system, Inc, New York, USA). For the Abbott determine ^{TM} HIV -1 and HIV-2 kit, the procedure described by the manufacturer was used for the analysis. Briefly, 50 µl of participant serum samples separated from the corresponding whole blood samples in EDTA were applied to the appropriately labeled sample pad. After 15 minutes but not more than 60 minutes of sample application, the result was read. This method has inherent quality control that validates the results. For the Immunochromatographic method for HIV -1 and HIV-2, the procedure described by the manufacturer was used for the analysis. In brief, 5 ml of participant's plasma was dispensed into the sample well in the appropriately labeled sample pad. Three drops of the buffer supplied by the manufacturer was added into the appropriately labeled sample pad. The results of the test were read at 10 minutes after the addition of the running buffer. This method has inherent quality control and validates the results.

Determination of malaria parasitaemia by Thick and thin film as will be described by WHO (1995).

Thick and thin films will be prepared for each participant's blood sample. The thin films were fixed with methanol and both thick and thin films were stained with Giemsa (1 in10 dilution) for 10 minutes, after which they were examined microscopically with oil immersion (x 100) objective. The malaria parasite counting was done using the thick blood films while the thin blood films were used for species identification. Malaria parasites were counted according to the method of World Health Organisation (1995). 200 leukocytes were counted and if 10 or more parasites were identified, then the number of parasites per 200 leucocytes was recorded; but if after counting 200 leukocytes and 9 or less parasites identified then, 500 leukocytes was recorded. In each case the parasite count in relation to the leukocyte count was converted to parasite per microlitre of blood using this mathematical formular:

$$\text{Malaria parasite density /µl} = \frac{\text{number of parasites x 8000}}{\text{Number of leukocytes}}$$

Where 8000, is the average number of leukocyte per microlitre of blood, which is taken as the standard (WHO, 1995).

Determination of the packed cell volume as described by cheesbrough, 2000

EDTA blood sample from each participant was aspirated into micro-haematocrit capillary tubes. One end of the capillary tube was subsequently sealed with plastercin and placed in the micro-haematocrit centrifuge. A steady packing of the red blood cells was achieved with a centrifugation speed of 12000 g for 5 min. The PCV was measured using a Haematocrit reader and reported as a ratio of the whole blood volume in litre/litre.

Determination of WBC counts using turk's solution as described by cheesbrough, 2000

Into appropriately labeled test tubes containing 0.38ml of Turk's Solution (1% ammonium oxalate, 2ml of Glacial acetic acid, 100ml of distilled water and 2drops of aqueous voilet) was added 20µl of EDTA Blood of participant respectively. The solution was allowed to stand for 5 min and through capillary action the mixture was introduced into charged new improved neubauer chamber. The population of WBC in the four corner cells was read under the microscope using x10 objective lens. The amount of WBC was then calculated for each participant adjusting for the dilution.

Determination of CD4+T-cells count by Cyflow SLGreen

50 µl of whole blood in EDTA anticoagulant was dispensed into a partec test tube and 10 ml of CD4 PE antibody was added. The reaction mixture was mixed and incubated in the dark for 15 min. After the incubation, 800 µl of the already prepared diluted buffer was added to each reaction tube and mixed. The partec tubes containing these reactions were plugged in position in the Cyflow SL Green (Partec, Germany), which has already been connected to flow max software, CD4 count template data file and CD4 count instrument. The test was run on the Cyflow for 90 sec. The results were displayed as histogram and printed. The CD4+ T-cell count was read off the histogram correcting for the dilution factor.

Determination of erythrocyte sedimentation rate (ESR) by westergren as described by cheesbrough, 2000

0.4ml of sodium citrated anticoagulant was pipetted into a clean test-tube. 1.6ml of the Blood in the EDTA bottle was added into the test-tube and its content mixed. A westergren pipette was inserted into the test-tube and Blood was drawn to the zero mark with the aid of a rubber sucker. The filled ESR tube was allowed to stand vertically on the ESR stand for 1 hour. The area where the plasma stopped after 1 hour was read off as ESR and the result recorded accordingly.

Statistical analysis

The result of the analysis was statistically analysed. Students't-test and one way analysis of variance (ANOVA) were used to compare means. The analysis was performed with the use of Statistical *Package for Social Sciences* (SPSS) statistical software package, version 13.0. P <0.05 was considered statistically significant.

RESULT:

The mean (±SD) PCV (l/l) values in malaria infected male participants were: 0.38 ± 0.04 in HIV participants on ART; 0.34 ± 0.09 in HIV participants not on ART; 0.38 ± 0.05 in asymptomatic HIV participants and 0.42 ± 0.04 in HIV seronegative participants. There was significant difference observed amongst the groups ($p<0.05$). Also, there was significant difference observed between groups ($p<0.05$) respectively but similar value was observed between symptomatic HIV participants on ART and asymptomatic HIV participants ($p>0.05$).

The mean (±SD) CD4$^+$T cell counts (/ml) in malaria infected male participants were: 258.74 ± 174.81 in HIV participants on ART; 189.86 ± 59.46 in HIV participants not on ART; and 382.15 ± 152.23 in asymptomatic HIV participants. There was significant difference observed amongst the groups ($p<0.05$). Also, there was significant difference between the groups ($p<0.05$) respectively.

The (±SD) ESR values (mm/h) in malaria infected male participants were: 27.84 ± 22.85 in HIV participants on ART; 38.36 ± 4.61 in HIV participants not on ART; 25.66 ±20.40 in asymptomatic HIV participants and 8.85 ± 8.74 in HIV seronegative participants. There was significant difference observed amongst the groups ($p<0.05$). Nevertheless, the ESR value was significantly higher in HIV participants on ART compared with that in HIV seronegative participants ($p<0.05$). The ESR value was also significantly higher in HIV participants not on ART and asymptomatic HIV participants compared with that in HIV seronegative participants ($p<0.05$, in each case).

The (±SD) WBC counts (x 10^9 / ml) in malaria infected male participants were: 4.19± 0.99 values in HIV participants on ART; 4.65 ± 1.62 in HIV participants not on ART; 4. 30 ± 0.90 in asymptomatic HIV participants and 4.94 ± 1.00 in HIV seronegative participants. There was similar value observed amongst the groups ($p>0.05$). (See table1).

Table 1: Mean (±SD) Hematological parameters in symptomatic HIV (stage 11) malaria infected male participants with and without ART, asymptomatic HIV male participants and HIV seronegative control group.

Variables	PCV (l/l)	CD4 counts (cell/ml)	ESR (mm/h)	WBC counts (x 109/ml)
(A) Symptomatic HIV male participants on ART (n=139)	0.38± 0.04	258.74± 174.81	27.84 ± 22.85	4.31 ± 0.99
(B) Symptomatic HIV male participants not on ART (n=138)	0.34± 0.09	189.86± 59.46	38.36± 4.61	4.55 ± 0.83
(C) Asymptomatic HIV participants (n=136)	0.38 ± 0.05	382.15± 152.23	25.66 ± 20.44	4.39 ± 0.83

(D) Seronegative Control participants (n=136)	HIV	0.42± 0.04	-------	8.85 ± 8.74	4.53 ± 1.07
F (p) value		17.97 (<0.05)	34.00 (<0.05)	10.40 (<0.05)	1.00 (>0.05)
A V B		(<0.05)	(<0.05)	(>0.05)	-------
A V C		(>0.05)	(<0.05)	(>0.05)	-------
A V D		(<0.05)	--------	(<0.05)	--------
B V C		(<0.05)	(<0.05)	(>0.05)	--------
B V D		(<0.05)	---------	(<0.05)	--------
C V D		(<0.05)	---------	(<0.05)	--------

Key: F (p) value = symptomatic HIV male on ART, symptomatic HIV male not on ART and HIV seronegative control compared (using ANOVA).
A V B = symptomatic HIV male on ART compared with symptomatic HIV male not on ART (using student's t-test).
A V C = symptomatic HIV male on ART compared with asymptomatic HIV male participants (using student's t-test).
A V D = symptomatic HIV male on ART compared with seronegative control (using student's t-test).
B V C = symptomatic HIV not on ART compared with asymptomatic HIV male participants (using student's t-test).
B V D = symptomatic HIV not on ART compared with HIV seronegative control subjects (using student's t-test).
C V D = Asymptomatic HIV male participants compared with HIV seronegative control subjects (using student's t-test).

IV. DISCUSSION

In this study, the PCV value was significantly reduced in malaria infected HIV/AIDS participants on ART when compared with that in HIV participants not on ART. This reduced value may be due to the fact that immune systems of those not on antiretroviral therapies are more depressed or immunocompromised than the immune system of those not on ART, hence the lower value of PCV observed in them. This finding support the fact that ART improves Hematological parameters, hence the improved level of PCV on those on ART than on those not on ART (Amegor, 2009 *et al.*, 2009; Byomakesh *et al*., 2009).

The study also observed an improved $CD4^{+}$T cell counts on malaria infected HIV participants on ART than in other HIV seropositives. The reduced $CD4^{+}$T cell counts observed in HIV seropositives may be attributed to cell death caused by the HIV infection (Mark et al., 2005). Ifeanyichukwu *et al*., (2011) observed that the degree of T cell activation and rate of $CD4^{+}$T cell depletion in HIV infection appears to associate with the disease progression.

In this study, the ESR level was observed to be higher in malaria infected HIV positive groups. Higher level was observed in participants not on ART. This may be due to immune depletion by the viral infection which may allow the invasion of opportunistic infection (Ukibe *et al*., 2010) and even the presence of malaria infection itself. ESR has been reported to be increased in febrile conditions as a result of presence of infection (Cheesbrough, 2000).

V. CONCLUSION

The findings in this study, led to the conclusion that HIV and malaria co-infection are capable of lowering the values of $CD4^{+}$T cells, PCV and increasing the value of ESR host.

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