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CD4$^+$ T-cells count in HIV-malaria co-infection in adult population in Nnewi, South Eastern Nigeria

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ABSTRACT

The study was designed to evaluate CD4$^+$ T-cells count in subjects with HIV-malaria co-infection in Nnewi, South Eastern Nigeria and to assess the effects any changes in CD4$^+$ counts has on the prevalence and or severity of both illness. Two hundred and eighty-five participants aged between 16 and 72 years were recruited for the study and grouped as symptomatic HIV subjects, asymptomatic HIV subjects, HIV/AIDS subjects on ART (Antiretroviral Therapy) and HIV-seronegative subjects. HIV and malaria parasite screening, CD4$^+$ T-cell count and parasite density were determined using standard laboratory methods. The result showed that the prevalence of malaria infection was 75% in symptomatic HIV, 46.7% in asymptomatic HIV and 59.6% in HIV/AIDS subjects on ART respectively as opposed to 26.9% observed in the control ($P<0.001$). The CD4$^+$ T-cell count was significantly lower in both symptomatic and asymptomatic HIV-malaria infected subjects when compared with the malaria-infected control subjects (238 ± 176, 312 ± 144, $P<0.01$) respectively. CD4$^+$T-cells count was also significantly lower in malaria-infected HIV/AIDS on ART when compared to the malaria-infected control subjects (315 ± 195, $P<0.01$). The study concludes that malaria prevalence is increased in subjects with HIV/malaria co-infection and is accompanied by a significant reduction in CD4$^+$T-cell counts, which might worsen the severity and prognosis in these subjects. Other public health implications are discussed.

Keyword: CD4+ T Cells, Prevalence, HIV, AIDS, Malaria.

INTRODUCTION

Malaria and HIV are leading causes of morbidity and mortality particularly in sub-Saharan Africa. Both diseases are highly endemic and have a wide geographical over lap. The National Centre for Infectious

Disease Control (CDC) states that a small effect of malaria on HIV or vice-versa could have substantial population level implication (CDC, 2003).

Both HIV and malaria generate immune response from the host. Whereas the immune response to malaria involves prevention and/or suppression of infection and clinical disease (Good et al., 1989). HIV-I causes progressive immuno-suppression thereby leading to impairment in malaria immune response, although laboratory based studies suggest that some components of the human immune response to malaria remain unaffected in HIV infection (Migot et al., 1996; Moore et al., 2000; FHI, 2005).

The primary target of HIV is the $CD4^+$ T-cells, which are destroyed, and the number depleted. Unfortunately, malaria has also been associated with reduced $CD4^+$ T-cell counts (Ho et al., 1986; Hviid et al., 1991; Chirenda, 1999). The implication of this is that HIV-malaria co-infection presents a heavy burden on the immune system and eventually potentiates each other.

$CD4^+$T-cell counts have been crucial to the diagnosis and management of HIV disease. $CD4^+$T-cells are helper T-lymphocytes that carry $CD4^+$ receptors on their surfaces. Normal $CD4^+$ counts in South Eastern Nigerians are between 800-1200 /µl (Anyiwo et al., 2006). It has to be noted that $CD4^+$ T-lymphocytes are constituents of the total WBC (White Blood Cells), which eventually falls low as these cells (CD4) are being depleted. However, Malaria infection has been associated with raised WBC and parasite density in non-immune subjects with HIV co-infection (Onyenekwe et al., 2008). The present study however seeks to evaluate the situation in an endemic area with stable malaria transmission and to assess the effects any changes in $CD4^+$ counts has on the prevalence and severity of HIV and malaria co-infection.

MATERIALS AND METHODS

Subjects

The study was conducted at the VCT (Voluntary Counseling and Testing) unit and HIV clinic in Nnamdi Azikiwe University Teaching Hospital Nnewi, South Eastern Nigeria (NAUTH).

A total of 285 participants aged between 16 and 72 years were recruited. Blood samples collected from all the participants were used for double HIV screening and confirmation, *P. falciparum* malaria parasite screening, thick and thin blood smear, parasite counts, $CD4^+$T-cells count and white blood cell count. The participants were grouped based on the result of the HIV and malaria parasite screening and using the World Health Organization (WHO) criteria for staging HIV as:

i. Symptomatic HIV subjects consisting of 100 participants (male = 37, female = 63). 75 of these had malaria parasite co-infection. These participants were regarded as HIV stage II.
ii. Asymptomatic HIV subjects consisting of 60 participants (male = 25, female = 35). 28 of these participants had *P. falciparum* malaria parasitaemia. They were regarded as HIV stage I.
iii. HIV/AIDS subjects consisting of 47 participants (male = 15, female = 32). 28 of these had *P. falciparum* malaria parasite infection. These participants have been placed on antiretroviral therapy (ART).
iv. HIV-seronegative subjects consisting of 78 participants (male = 32, female = 46). 21 of these participants had malaria parasite infection. These participants were known as control subjects. Informed consent was obtained from the entire participants and the Board of ethical committee Nnamdi Azikiwe University Teaching Hospital approved the study design. Only those subjects adjudged as HIV stage II and I were used for the study.

Methods

Detection of antibodies to HIV-1 and HIV-2 in human plasma

Two different methods were used namely Abbott determine HIV 1 & 2 kit which is an in vitro visually read immunoassay (Abbot Japan C. Ltd. Tokyo, Japan) and HIV 1 & 2 STAT-PAK Assay kit, which is an immunochromatographic test for the qualitative detection of antibodies to HIV-

1 and HIV-2 in human plasma (CHEMBIO Diagnostic system, INC, New York, USA). For the Abbott determine HIV-1 & 2 the procedure as described by the manufacturer was used for the analysis. Briefly, 50 µl of participants' plasma samples separated from corresponding whole blood samples in EDTA (Ethylenedimethyltetraacetic acid) were applied to 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 & 2 it is used as a point of care test. The procedure as described by the manufacturer was used for the analysis. In brief, 5 µl of participant's plasma were dispensed into the sample wells; buffer supplied by the manufacturer was added drop-wise into the appropriately labeled sample wells. The results of the tests were read at 10 minutes after the addition of the running buffer. This method has inherent quality control that validates the results. HIV positive results' using these two methods was used to classify participants as presenting with HIV infection.

Diagnosis of P. falciparum malaria

Whole blood was used for the diagnosis of *P. falciparum* malaria using Malaria *Plasmodium falciparum* Rapid Test Device (Para check, Orchid Biomedical systems, Vena Goa, India) and Giemsa stained thick blood smears for microscopic detection of *P. falciparum* parasites. The parasites density was determined as previously reported (Onyenekwe et al., 2002) and density expressed per liter of blood. However, the principle of the *P. falciparum* antigen detection is based on a rapid chromatographic immunoassay, for the qualitative detection of circulating *P. falciparum* antigen in the whole blood. This method utilizes Gold conjugate to selectively detect *Plasmodium* antigen. The procedure was as described by the manufacturer. Briefly, 10 µl of the whole blood specimen from the participant were transferred into appropriately labeled specimen cassettes containing sample well. Subsequently, 3 drops of buffer supplied by the manufacturer (approximately 120 µl) was added into the sample wells. After 15 minutes the results were read.

The test device has inherent quality control that validates the result. The presence of two pink lines at the region of the control and test sample signifies presence of *P. falciparum* malaria infection while the presence of only 1 pink line in the control region signifies absence of *P. falciparum* malaria.

Determination of $CD4^+$ T-cells count by Cyflow SL Green

About 50 µl of whole blood in EDTA anti-coagulant was dispensed into a partec test tube and 10 ml of CD4 PE antibody was added. The reaction mixture was homogenized and incubated in the dark for 10-15 min. After the incubation, 800 µl of the already prepared diluted buffer was added to each reaction tube and mixed gently. 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 WBC counts using Turks solution

Into appropriately labeled test tubes containing 950 µl of Turks solution (2% glacial acetic acid and 50 µl of gentian violet), 50 µl of EDTA anti-coagulated blood sample of each participant was added respectively. The solution was allowed to stand for 5 min and through capillary action was loaded unto the new improved newbaeur chamber. The population of WBC in the four corner cells was read under the microscope using x 10 objectives lens. The amount of WBC was then

calculated for each participant adjusting for the dilution.

Statistical analysis

The variables were expressed as mean (±SD). The independent student t-test was used to assess significant mean differences. Chi – square (x^2) test was employed to establish any relationship between Plasmodium infection and HIV subjects. Significant level were considered at <0.05 – and <0.01.

RESULTS

A total of 285 (male=109, female=176) participants were recruited for the study. Out of 100 symptomatic HIV subjects, 75(75%) had *P. falciparum* malaria co-infection with malaria parasite density of 604 ± 440 while 25 (25%) had no malaria parasite infection. 28 (46.7%) of the 60 asymptomatic HIV subjects had malaria co-infection with malaria parasite density of 803 ± 526 while 32 (53.3%) had not. Out of the 47 HIV/AIDS subjects on ART, 28 (59.6%) had malaria co-infection with malaria parasites density of 690 ± 480 while the remaining 19 (40.4%) did not have malaria parasite infection. Furthermore, 21 (26.9%) of 75 control HIV-seronegative subjects had malaria parasite infection with malaria parasite density of 890 ± 380 while the remaining 57 (73.1%) did not have malaria parasite infection. The prevalence of malaria infection was statistically significant in symptomatic HIV (75%) than in asymptomatic HIV, HIV/AIDS and control subjects (P<0.001) while the least prevalence was observed in the control (26.9%) (Table 1)

There was no significant difference in malaria parasite densities amongst all the groups studied, (P>0.05) (Table 2, 3, 4). Furthermore, there was no significant difference observed when malaria-infected symptomatic HIV, Asymptomatic HIV and HIV/AIDS subjects on ART were compared with their malaria-uninfected counterparts (P>0.05) in each case (Table 2). Conversely, there was significant reduction in the mean value of $CD4^+$ T-cell count in malaria-infected symptomatic, asymptomatic HIV and HIV/AIDS subject on ART compared with malaria-infected control subjects (P<0.01, <0.01, <0.05) respectively. Similarly, when the malaria-uninfected Symptomatic and HIV AIDS on ART were compared with their corresponding control subjects, the mean value of $CD4^+$ cell count were significantly different (P<0.01, <0.05) in each case except in malaria-uninfected asymptomatic HIV which were not significantly different from the malaria-uninfected control subjects (P>0.05) (Table 3). There was no significant difference observed between groups (P>0.05) (Table 4).

Table1: Malaria prevalence amongst symptomatic, asymptomatic, HIV/AIDS and control subjects

Group	Malaria Infected (%)	Malaria Uninfected (%)	Total (%)
Symptomatic HIV	75 (75)	25 (25)	100 (100)
Asymptomatic HIV	28 (46.7)	32 (53.3)	60 (100)
HIV/AIDS	28 (59.6)	19 (40.4)	47 (100)
Control	21 (26.9)	57 (73.1)	78 (100)

Calculated x^2 =71.6, Degree of freedom (df) =3 (P<0.001) statistically significant.

Table 2: CD4+T cells count, malaria parasite density in symptomatic, asymptomatic, HIV/AIDS on ART and control groups.

Parameters	CD4+ T cells count (/mm^3)	MP density (/µl)
Symptomatic group		
Symptomatic HIV Malaria Infected (n=33)	238 ± 176	604 ± 440
Symptomatic HIV Malaria Uninfected (n = 35)	247 ± 178	-
P – value	> 0.05 (ns)	nc
Asymptomatic group		
Asymptomatic HIV Malaria Infected (n=30)	312 ± 144	803 ± 526
Asymptomatic HIV Malaria Uninfected (n = 20)	403 ± 226	-
P – value	> 0.05 (ns)	nc
HIV/AIDS group on ART		
HIV/ AIDS Malaria Infected (n=28)	315 ± 195	690 ± 480
HIV /AIDS Malaria Uninfected (n = 19)	343 ± 216	-
P – value	> 0.05 (ns)	nc
Control group		
Control Malaria Infected (n=20)	504 ± 181	890 ± 380
Control Malaria Uninfected (n = 20)	518± 237	-
P – value	> 0.05 (ns)	nc

ns = not significant; nc = not compared; MP = malaria parasite. Tabulated values are means ± SD

Table 3: Comparison of CD4+T cells counts and malaria parasite density between the symptomatic, asymptomatic, HIV/AIDS on ART and control groups.

Parameters	CD4+ T cells count (/mm^3)	MP density (/µl)
Control malaria infected	504 ± 181	890 ± 380
Symptomatic malaria infected	238 ± 176	604 ± 440
P- value	<0.01	>0.05 (ns)
Control malaria uninfected	518 ± 237	-
Symptomatic malaria uninfected	247 ± 178	-
P- value	<0.01	nc
Control malaria infected	504 ± 181	890 ± 380
Asymptomatic malaria infected	312 ± 144	803 ± 526
P- value	<0.01	>0.05 (ns)
Control malaria uninfected	518 ± 237	-
Asymptomatic malaria uninfected	403 ± 226	-
P- value	>0.05 (ns)	nc
Control malaria infected	504 ± 181	890 ± 380
HIV/AIDS malaria infected	315 ± 195	690 ± 480
P- value	<0.05	>0.05 (ns)
Control malaria uninfected	518 ± 237	-
HIV/AIDS malaria uninfected	343 ± 216	-
P- value	<0.05	nc

ns = not significant; nc = not compared; MP = malaria parasite. Tabulated values are means ± SD

Table 4: Comparison of CD4+T cells counts and malaria parasite density between the symptomatic, asymptomatic, HIV/AIDS on ART groups.

Parameters	CD4+ T cells count (/mm^3)	MP density (/µl)
Symptomatic HIV malaria infected	238 ± 781	890 ± 380
Asymptomatic HIV malaria infected	312 ± 144	604 ± 440
P- value	>0.05 (ns)	>0.05 (ns)
Symptomatic HIV malaria uninfected	247 ± 178	-
Asymptomatic malaria uninfected	403 ± 226	-
P- value	>0.05 (ns)	nc
Symptomatic HIV malaria infected	238 ± 781	890 ± 380
HIV/AIDS malaria infected on ART	315 ± 195	690 ± 480
P- value	>0.05 (ns)	>0.05 (ns)
Symptomatic malaria uninfected	247 ± 178	-
HIV/AIDS malaria uninfected on ART	343 ± 216	-
P- value	>0.05 (ns)	nc
Asymptomatic HIV malaria infected	312 ± 144	604 ± 440
HIV/AIDS malaria infected	315 ± 195	690 ± 480
P- value	>0.05 (ns)	>0.05 (ns)
Asymptomatic HIV malaria uninfected	403 ± 226	-
HIV/AIDS malaria uninfected on ART	343 ± 216	-
P- value	>0.05 (ns)	nc

ns = not significant; nc = not compared; Tabulated values are means ± SD

DISCUSSION

The high prevalence of malaria observed in HIV subjects when compared with the control group in the present study is consistent with previous reports (French et al; 2001; Patnaik et al., 2005; Laufer et al., 2006) the significant reduction in CD4+Tcell Count of all the HIV groups studied is also consistent with previous findings (Siridama et al., 1982; Johnstone et al., 1994; Elna et al., 1998; Kapiga et al., 2000). Both malaria and HIV infections are two important diseases that exert a lot of pressure on the immune system. The primary target of HIV virus is the CD4+T cells, which are consequently destroyed, and the number depleted (Balter, 1997). The resultant reduction in cellular immunity in affected individuals leads to increases vulnerability to malaria infection hence the increased malaria prevalence observed in the present study. The insignificant difference in malaria parasite density however, suggests that the subjects were simply asymptomatic malaria parasite carriers devoid of the clinical illness, considering the fact that the study area is endemic for malaria.

However, studies have shown that frequent attacks of malaria in HIV positive subjects have been associated with increased HIV-1 replication and viral load (Kublin et al., 2005; Mermin et al., 2006) which leads to a further depletion in CD4+Tcells count especially in an endemic area where both illnesses exist as co-infection. Xiao et al. (1998) believe that the increased HIV-1 RNA replication may be due to the presence of malaria antigens and pigments, which was usually associated with increased expressions of the cytokine, tumour necrosis factor (TNF). Previous studies have shown that 95% of HIV-1 in the plasma is produced by infected activated CD4+ lymphocytes (Perlson et al., 1997). Since *P. falciparum* malaria causes increased CD4+T cell activation, (Worku et

al., 1997), it means that the number of susceptible target cells for HIV-1 infection increases thereby resulting to increased HIV-1 RNA levels. However, the negative effect of malaria on CD4+T cells count is said to be reversible in HIV-negative individuals (Greenwood et al., 1997). This means that that the malaria induced reduction in CD4+ count in affected individuals is transient and returns to normal as soon as such subjects are treated and the malaria parasites eradicated. This is probably why there is insignificant effect of malaria on CD4+ count in the present study, since most of the participants in the study were asymptomatic carriers of malaria parasite and hence had low parasite density. The use of ART however has been associated with improvement on CD4+ cells due to the fact that opportunistic infections are reduced and more CD4+ T cells are mobilized from the tissues to the peripheral circulation and cell death (apoptosis) is reduced.

Furthermore, the resurgence of tuberculosis in sub-Saharan Africa recently among subjects suffering from HIV infection has been linked to further depletion of $CD4^+$ T-cells by the tubercle organisms (Beck et al., 1985; Freeney et al., 1995). In poor communities like the study area, malnutrition is rampant among HIV infected subjects and could also explain the low $CD4^+$ T-cells count reported (Hegde et al., 1999). The psychological trauma associated with diagnosis of HIV infection in some individuals has also been associated with further reduction of $CD4^+$ T-cells (Castle et al., 1995). The contributory effects of this in the study environment may be significant given the fact that most subjects who have been diagnosed HIV positive find the situation "unbelievable" and are highly tensed up.

There has been a controversy in the use of CD4+ count of 200/ul as the lower limit to initiate ART in subjects with HIV/AIDS (FPI, 2005). It is believed that this policy will exclude a lot of people who really need the drug to stabilize their condition.

In conclusion, the increased prevalence of malaria observed in HIV infected subjects in the present study may be due to the reduction in CD4+T cells count, a vital component of cellular immunity which is responsible for clearing the blood of malaria parasites. However, the insignificant difference in parasite density suggests that severe clinical malaria was not recorded since the study area is endemic for malaria and subjects in early stages of HIV disease were used. However, the need to control malaria in the study area is a topical public health issue, which deserves more attention than is being given presently. While reduction of poverty and improved nutrition are important factors, further research is suggested in the area of effective control measures for the two diseases to reduce the impact of co-infection.

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