



Packed cell volume and serum iron in subjects with HIV-malaria co-infection in Nnewi, South-Eastern Nigeria

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ABSTRACT

The present study was designed to assess the PCV and serum iron in HIV-malaria co-infected subjects in Nnewi, South Eastern Nigeria. 207 participants aged between 16-72 (44 ± 28) years were recruited and classified as follows based on standard screening and WHO criteria: (i) Asymptomatic HIV stage I subjects with or without malaria. (ii) Symptomatic HIV stage II subjects with or without malaria and not on (ART). (iii) HIV/AIDS subjects with or without malaria and on ART. (vi) HIV seronegative control subjects with or without malaria. Blood sample from these participants were analyzed for HIV seroreactivity, *Plasmodium falciparum* antigen, parasite density, serum iron concentrations and PCV using Standard Laboratory methods. The result showed that serum iron and PCV were significantly reduced amongst all the groups studied when compared with the control ($p<0.01$, <0.05 , <0.01) respectively. No significant difference was observed in malaria parasite densities amongst all the groups studied. The present study shows that serum iron could possibly be one of the most significant predictors of anaemia in subjects presenting with HIV-malaria co-infection, other factors notwithstanding. Effective antimalarial drugs in conjunction with antiretroviral therapy could go a long-way in reducing the incidence of anaemia associated with HIV/Malaria co-infection

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Keywords: HIV, Malaria, Serum iron, PCV.

INTRODUCTION

The emergence of HIV infection in the early eighties has complicated the problem of malaria in endemic areas. The interaction between malaria and HIV infection has been controversial (Whitworth et al., 2005) though it has been established that the two diseases potentiate each other with greater risks of complications (Froebel et al., 2004; Xiao et al.; 1998; Kublin et al., 2005; Tkachuk et al., 2001; Mermin et al., 2006; Te Kuile et al., 2004). The most notorious complication of malaria is anaemia (Mendez et al., 2004; Eliades et al., 2004; Okwa et al., 2004). Malaria causes anaemia by increased destruction of parasitized red blood cells by the reticuloendothelial system. The iron produced as a byproduct of erythrocytes destruction is essentially lost and this increases the chances of iron deficiency anaemia especially when the rate of red blood cell destruction exceeds the rate of bone marrow formation of new ones.

Studies conducted in malaria endemic areas have shown that HIV infection increases the rate of clinical malaria with greater risks of anaemia (low PCV) and derangement in serum iron concentration. (Whithwort et al., 2000; Lütfi et al., 2006; Patnaik et al., 2005). HIV infection can also induce anaemia by bone marrow suppression either as a result of the virus itself or due to the effects of the anti retroviral drugs used to treat the illness. A study from Italy suggests that intestinal malabsorption of iron is common among anemic HIV-infected subjects (Calstaldo et al., 1996). Loss of appetite due to advanced HIV infection or severe malaria can also lead to iron deficiency state and anaemia.

The public health importance of HIV-malaria co-infection cannot be over emphasized, especially in sub-Saharan Africa where it is estimated that 38 million people are infected with HIV-1 virus (UNAIDS 2004) whereas 300-500 million people are estimated to suffer from malaria each year (WHO 2006).

The essence of the present study is therefore to highlight the significance of the

co-morbidity of malaria and HIV virus in an endemic area with stable malaria transmission, where high morbidity is attributable to anaemia especially among children and pregnant mothers (Okwa et al., 2004; Eliades et al., 2004) other indices of anaemia notwithstanding.

MATERIALS AND METHODS

Subjects

A total of 207 (male = 100, female = 107) subjects aged between 16-72 (44 ± 28) years randomly recruited at the VCT and HIV clinic in NAUTH Nnewi participated in the study. Blood samples were collected from all the participants and whole blood were used for double screening for HIV seroreactivity, *P. falciparum* malaria antigen seroreactivity, PCV, serum Iron concentration and thick and thin blood smears. Based on the result of the malaria parasite screening and WHO staging for HIV, the participants were grouped into:

- (i) Asymptomatic HIV stage 1 subjects (n=67) thirty-five of the participants had *P. falciparum* malaria. They have not been placed on antiretroviral therapy (ART).
- (ii) Symptomatic HIV stage 11 subjects (n=53). 28 of these participants had malaria. They have not been placed on ART.
- (iii) HIV/AIDS subjects (n=47) on ART. 28 of these participants had *P. falciparum* malaria parasite infection. The antiretroviral combinations used included zidovudine (ZDV) or Azithrothymidine (AZT), Lamivudine (ZTC Epivir), Stavudine (d4T, Zerit) and Nevirapine (NVP, viramine) all in standard dosages.
- (iv) HIV seronegative subjects (n = 40). They served as control subjects. 20 of the participants had *P. falciparum* malaria.

The Ethical Committee of Nnamdi Azikiwe University Teaching Hospital Nnewi approved the study design and the participants gave informed consent.

Methods

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

Abbott DetermineTM HIV 1 & 2 method: It is a visually, read immunoassay (Abbott Japan C Ltd. Tokyo, Japan). The procedure as described by the manufacturer was used for the analysis. Briefly, 50µl of participants' plasma samples separated from corresponding whole blood samples in EDTA were applied to appropriately labeled sample pad. After 15 minutes of sample application, the result was read. This method has inherent quality control that validates the results. HIV seropositive results using this confirmatory test method were 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 makes use of Gold conjugate to selectively detect *Plasmodium* antigen. The procedure was as described by the manufacturer. Briefly, 10µl of the whole blood specimen from the participant were transferred into appropriately labeled specimen cassettes containing sample wells. 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.

Determination of the Packed Cell Volume

Anti-co-agulated (EDTA) blood sample from each participant was aspirated into micro-haematocrit capillary tubes. One end of the capillary tube was subsequently sealed and placed in the micro-haematocrit centrifuge. A constant packing of the red

blood cells was achieved with a centrifugation speed of 1200 x g for 3 min. the PCV was measured and reported as a ratio of the whole blood volume in litre/litre.

Serum iron determination by colorimetric method

The procedure was as described by the manufacturer of the kit (Chromatest, Barcelona, Spain). In brief, 200 µl of the sample from each participant was put in appropriately labeled test tube containing 1.0 ml of working solution [containing one part of chromogen solution (ferozine 40 mmol/L and sodium acetate 400 mmol/L and four parts of buffer/reductant solution (Guanidine chloride 1.0 mol/L; hydroxylamine 0.6 mol/L; acetate buffer 400 mmol/L pH 4.0; teepol mixture)]. Similar procedure was used for the reagent blank and standard test tubes, except that the sample blank was prepared with addition of 1.0 ml of the buffer/reductant solution. The reactions were incubated at room temperature for 5 min, after which the absorbance of the sample blank was read at 560 nm against distilled water while the absorbance of the sample tests and standards were read at 560 nm against reagent blank. Subsequently, the concentration of serum iron was calculated.

Statistical analysis

The variables were expressed as mean (\pm SD). The independent student t-test was used to assess significant mean differences. Significant level was considered at <0.05 .

RESULTS

No significant difference was observed in PCV in Symptomatic and Asymptomatic HIV-malaria infected (0.30 ± 0.07 , 0.35 ± 0.08) groups when compared to the malaria uninfected (0.31 ± 0.04 , 0.34 ± 0.04) counterparts ($P>0.1$, $P>0.1$) respectively. Also no significant difference was observed between the HIV/AIDS malaria infected and uninfected subjects ($P>0.1$).

The mean serum iron concentration ($\mu\text{g}/\text{dl}$) in symptomatic and asymptomatic HIV-Malaria infected (97.30 ± 56.40 ,

81.20 ± 36.70) groups were significantly reduced than in the corresponding HIV-malaria uninfected (131.7 ± 48.6 , 133.4 ± 53.3) counterparts ($p < 0.01$, $p < 0.05$). The malaria parasite density (/ μl) for symptomatic HIV-malaria subjects was 604 ± 440 . Similarly, the malaria parasite density for asymptomatic HIV-malaria infected subjects was 803 ± 526 . In addition, the malaria parasite density for malaria infected HIV/AIDS subjects on ART was 690 ± 480 . Furthermore, the malaria parasite density for malaria infected control subjects was 890 ± 380 . (Table 1)

The mean PCV (l/l) were significantly reduced in both Symptomatic and Asymptomatic HIV-malaria infected (0.30 ± 0.07 , 0.35 ± 0.08) and uninfected (0.31 ± 0.04 , 0.34 ± 0.04) subjects when compared to the corresponding control (0.39 ± 0.08 , 0.40 ± 0.06) groups ($p < 0.01$, < 0.05 , < 0.05 , < 0.05). There was also significant reduction in mean PCV in HIV/AIDS malaria infected (0.32 ± 0.07) and uninfected (0.32 ± 0.04) subjects on ART when compared to control malaria-infected (0.39 ± 0.08) and uninfected (0.40 ± 0.06) subjects ($p < 0.01$) in each case.

The mean serum iron concentration in symptomatic and asymptomatic HIV-malaria infected groups was significantly lower than that of the control malaria infected (158.7 ± 69.70) group ($p < 0.05$). On the contrary, no significant difference was observed in mean serum iron of symptomatic HIV, asymptomatic HIV-malaria uninfected subjects when compared with the corresponding control (165.9 ± 88.9) subjects ($p > 0.1$) in each case.

Also the mean serum iron concentration in HIV/AIDS malaria infected and uninfected (81.1 ± 27.1 , 95.9 ± 38.9) subjects on ART were significantly lower than the control counterparts (158.7 ± 96.2 , 165.9 ± 88.9) $p < 0.05$, $p < 0.05$ respectively.

However, there was no significant difference in malaria parasite densities amongst all the groups studied ($p > 0.1$) (Table 2).

DISCUSSION

The present study shows that the packed cell volume (PCV) of malaria infected symptomatic HIV subjects on ART was not significantly different from the corresponding control. For the malaria uninfected symptomatic HIV subjects on ART, the PCV was significantly lower than the value observed in the corresponding control group. The PCV is synonymous with haemoglobin concentration (Hb), which is greatly reduced in malaria infection due to excessive haemolysis of parasitized red blood cells (rbc). Anaemia (low PCV) is one of the commonest complications of malaria in children and pregnant women (Eliades et al., 2004; Murphy and Breman 2001; Cheesbrough Monica, 1998). HIV infection complicates the situation by making malaria attacks more frequent and severe (Whitwort et al., 2000; Laufer et al., 2006; Onyenekwe et al., 2008) and inducing loss of appetite which makes it difficult for the subjects to obtain the necessary nutrients required for erythropoiesis under normal conditions. The infiltration of the bone marrow by HIV virus (Moses et al., 1996), coupled with the suppressive effects of antiretroviral drugs will all act to reduce erythropoiesis and hence low PCV in malaria infected HIV subjects. The use of ART may improve the PCV by reducing viral load and encouraging erythropoiesis. It may also encourage nutritional stability by improving appetite. Furthermore, antiretroviral therapy (ART) improves immunity thereby reducing the incidence of clinical malaria hence Hb haemolysis is reduced. All these will lead to improved PCV and haemoglobin concentration. The study further showed that the mean serum concentration of iron was significantly reduced in malaria infected and uninfected symptomatic HIV group on ART when compared with the corresponding control group. The reduction was more marked in symptomatic HIV group than in

Table 1: Packed Cell Volume (l/l), serum iron ($\mu\text{g}/\text{dl}$) and malaria parasite density ($/\mu\text{l}$) of malaria infected and uninfected symptomatic, asymptomatic HIV, HIV/AIDS subjects on ART and control subjects.

Group	PCV (l/l)	Iron ($\mu\text{g}/\text{dl}$)	Parasite density $/\mu\text{l}$
Symptomatic HIV malaria infected (n=28)	0.30 ± 0.07	97.30 ± 56.40	604 ± 440
Symptomatic HIV malaria uninfected (n=25)	0.31 ± 0.04	131.7 ± 48.6	-
P value	$>0.1(\text{ns})$	<0.05	
Asymptomatic HIV malaria infected (n=35)	0.35 ± 0.08	81.20 ± 36.70	803 ± 526
Asymptomatic HIV malaria uninfected (n=32)	0.35 ± 0.08	133.4 ± 53.3	-
P value	$>0.1(\text{ns})$	<0.01	
HIV/AIDS malaria infected (n=28)	0.32 ± 0.07	81.10 ± 27.10	690 ± 480
HIV/AIDS malaria uninfected (n=19)	0.32 ± 0.04	95.90 ± 38.90	-
P value	$>0.1(\text{ns})$	$>0.1(\text{ns})$	
Control malaria infected (n=20)	0.39 ± 0.08	158.7 ± 96.20	890 ± 380
Control malaria uninfected (n=20)	0.40 ± 0.08	165.90 ± 88.90	-
P value	$>0.1(\text{ns})$	$>0.1(\text{ns})$	

Tabulated values are Mean \pm SD. ns = not significant; PCV = Packed Cell Volume.**Table 2:** Comparison of Packed Cell Volume (l/l), serum iron ($\mu\text{g}/\text{dl}$) and malaria parasite density ($/\mu\text{l}$) in malaria infected and uninfected symptomatic, asymptomatic HIV, HIV/AIDS subjects on ART and control subjects.

Group	PCV (l/l)	Iron ($\mu\text{g}/\text{dl}$)	Parasite density $/\mu\text{l}$
Symptomatic HIV malaria infected	0.30 ± 0.07	97.30 ± 56.40	604 ± 440
Control malaria infected	0.39 ± 0.08	158.7 ± 96.20	890 ± 380
P value	<0.01	<0.05	>0.1
Symptomatic HIV malaria uninfected	0.31 ± 0.04	131.7 ± 48.6	-
Control malaria uninfected	0.40 ± 0.08	165.90 ± 88.90	-
P value	<0.05	$>0.1(\text{ns})$	
Asymptomatic HIV malaria infected	0.35 ± 0.08	81.20 ± 36.70	803 ± 526
Control malaria infected	0.39 ± 0.08	158.7 ± 96.20	890 ± 380
P value	<0.05	<0.05	$>0.1(\text{ns})$
Asymptomatic HIV malaria uninfected	0.35 ± 0.08	133.4 ± 53.3	-
Control malaria uninfected	0.40 ± 0.08	165.90 ± 88.90	-
P value	<0.05	$>0.1(\text{ns})$	
HIV/AIDS malaria infected	0.32 ± 0.07	81.10 ± 27.10	690 ± 480
Control malaria infected	0.39 ± 0.08	158.7 ± 96.20	890 ± 380
P value	<0.01	<0.05	$>0.1(\text{ns})$
HIV/AIDS malaria uninfected	0.32 ± 0.04	95.90 ± 38.90	-
Control malaria uninfected	0.40 ± 0.08	165.90 ± 88.90	-
P value	<0.01	<0.05	

Tabulated values are Mean \pm SD. ns = not significant; PCV = Packed Cell Volume.

those without malaria infection. This suggests a significant impact of malaria on serum iron concentration, which has been previously reported (Onyenekwe et al., 2008). The implication of this observation is that there is iron deficiency anaemia in malaria infected HIV subjects. The prevalence of iron deficiency anaemia in HIV infected children in Uganda was found to be 44.5% (Dana et al., 1999). The fact that serum iron was reduced in malaria infected HIV positive subjects suggests that serum iron could be the most sensitive indicator of possible impact of malaria infection in HIV infected subjects. Study reports elsewhere suggest the existence of significant relationship between iron status and HIV disease (Boelaert et al., 1996; Savarino et al., 1999). Serum iron has been associated with progression of HIV disease (Boelaert et al., 1996). It is believed that iron increased the oxidative stress, which enhances HIV replication in affected subjects (Jacobus et al., 1999; De Monye et al., 1999). Furthermore, iron loading and its subsequent deposition in tissues is said to be directly cytotoxic or harmful to immune effector cells including macrophages thereby increasing HIV-1 replication (Boelaert et al., 1996; Savarino et al., 1999). Other reports contradict any relationship between iron and HIV infection (Vanden Broek et al., 1998). Reports of iron deficiency anaemia in HIV positive pregnant women have been documented in developing countries (Asobayire et al., 2001). Onyenekwe et al., (2008) have reported a negative association between serum iron and PCV in Nigeria. Studies elsewhere have reported reduced haemoglobin concentrations, and transferrin, raised ferritin and transferrin saturation in malaria infected asymptomatic HIV subjects but similar serum iron concentration in malaria infected and uninfected control groups (Odunukwe et al., 2000). The findings in this study and the ones cited above could suggest possible depletion of iron stores in HIV infected subjects thereby making more iron available in transport. This is said to be detrimental to HIV infected subjects. Onyenekwe et al. (2008) believed

that this process might be blocked and reversed by malaria infection especially in endemic areas thus favouring reduced iron in transport with consequent increase in storage.

In conclusion, the present study shows that malaria-HIV co-infection has a negative effect on affected subjects in the study area one of which is anaemia. Concerted efforts should therefore be made at the various levels of government to bring malaria and HIV infection under control so as to reduce the high mortality rates attributed to these two diseases.

ACKNOWLEDGMENTS

We sincerely wish to acknowledge the staff of the VCT unit, HIV laboratory unit of Nnamdi Azikiwe University Teaching Hospital Nnewi and the staff and management of Awka South Local Government Area, Anambra State, Nigeria.

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