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CD4 count, viral load and parasite density of HIV positive individuals undergoing malaria treatment with dihydroartemisinin in Benin City, Edo state, Nigeria

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Abstract

Background & objectives: A prospective study on 72 HIV infected and 33 HIV negative individuals undergoing malaria treatment with dihydroartemisinin (Cotecxin) was undertaken to compare CD4 cells count, viral load and parasite density at two time-points, a baseline visit and a 9-day post-treatment visit.

Methods: CD4 count and viral load of the subjects were estimated using Dynabeads T4–T8 Quantification Protocol (Dyneal Biotech, Norway) and Amplicor HIV-1 Monitor Test respectively (Roche, United Kingdom).

Results: There was a significant decrease in CD4 count at 9-day post-treatment when compared with baseline value ($p < 0.05$) in HIV infected individuals with CD4 $\leq$200 cells/µl. Also, the 9-day post-treatment viral load value was statistically higher than the baseline value ($p < 0.05$). In HIV positive patients with CD4 > 200 cells/µl, a marked significant increase was obtained when the mean viral load at baseline was compared to the 9-day post-treatment visit value ($p < 0.05$). The mean parasite density in HIV positive subjects was statistically higher when compared to that of HIV negative individuals at baseline and 9-day post-treatment ($p < 0.05$).

Interpretation & conclusion: The study as such may not confirm the impact of malaria infection on progression to AIDS, incorporating effective malaria control in HIV management programmes may improve tremendously the quality of life of HIV infected individuals.

Key words Dihydroartemisinin – clearance CD4 – *Plasmodium* – viral load

Introduction

As the world enters the third decade of the AIDS epidemic, the evidence of its impact is obvious. Wherever the epidemic has spread unchecked, it is robbing countries of the resources and capacities on which human security and development depend. The association between HIV and malaria has important implications. Malaria and HIV are two of the commonest infections in sub-Saharan Africa and, to a lesser extent, in other developing countries. It is estimated that 29.4 million Africans are infected with HIV[1] whereas at least 500 million suffer from malaria each year[2]. Therefore, any interaction between these two infections will be of major public health significance.

The HIV/AIDS pandemic in areas where *Plasmodium falciparum* is endemic has generated concern about potential interactions between the two infections, especially in sub-Saharan Africa[3]. Studies have shown increased HIV replication both in blood mononuclear cells exposed to malaria antigens *in vitro*[4] and in transgenic mice-infected with *P. chabaudi*[5]. This significant increase occurs especially when the individuals have a parasites density more than 2000 parasites per microlitre and baseline CD4 of 300 cells per microlitre[6].

HIV-1 RNA concentrations and CD4 cell counts are moderately but inconsistently associated with parasitaemia. A high parasite density with fever is associated with HIV-1 seropositivity and low CD4 cell count[7]. Infection with HIV causes progressive cellular immunosuppression, and any impairment in immune response resulting to malaria might be associated with failure to prevent infection or to suppress parasitaemia and clinical disease. However, laboratory-based studies have found that some components of the human immune response to *P. falciparum* are modified by HIV-1, but that others are unaffected[4]. It has also been shown to increase the potential reservoir for HIV in the placenta by increasing the number of CCR5-positive macrophages[8]. However, a study from Malawi showed that HIV-1 plasma viral loads are significantly higher in patients with malaria than in those without, and these levels remain higher for at least four weeks after treatment[9]. On the other hand, Kublin *et al*[6] in their study in the same country revealed that increased HIV virus concentration was reversible within eight to nine weeks in individuals who had been treated for malaria and the viral load reached almost the baseline level.

Presently, the co-existence of HIV and malaria is gaining a major focus in the management of HIV infection and malaria control. This study aimed at determining the prevalence of malaria in HIV-infected individuals and also evaluate the outcome of malaria treatment with dihydroartemisinin on CD4, viral load and parasitaemia level of such individuals in Benin City.

Material & Methods

Study design: Eighty-one confirmed HIV-infected individuals and 52 HIV negative individuals of which 72 and 33 respectively with uncomplicated malaria being treated for malaria with dihydroartemisinin (Cotecxin) at the Lahor Medical Centre between the months of December 2004 and February 2005 were enrolled for this study. The month of December to February in Edo State is characterised by irregular rains as a result of which malaria transmission is relatively reduced.

The patients were selected irrespective of their age, sex and occupation. Patients who have earlier taken any antimalarial drugs or antiretroviral therapy (ARVs) before reporting to the hospital were excluded. Control subjects were HIV negative individuals who have been diagnosed of suffering from uncomplicated malaria and had not embarked on any antimalarial therapy. The subjects were reporting to the hospital for the first time and had not started any antiretroviral therapy. Baseline investigations (CD4, viral load, liver function tests, renal function test, acid fast bacilli test for those with and without chest X-ray) before administration of antiretroviral therapy (ARVs) was in process while they started malaria treatment after diagnosis.

Sample processing: Five millilitre of venous blood was collected from the subjects before Cotecxin administration and at exactly 9-day post-treatment visit, thick and thin blood films were made and stained with Giemsa for identification of parasites and count. An aliquot of the whole blood sample and plasma obtained from the remainder blood sample were used for CD4 count and viral load respectively. The CD4 count and viral load of the subjects were assessed using Dynabeads T4–T8 Quantification Protocol (Dyneal Biotech, Norway) and Amplicor HIV-1 Monitor Test respectively (Roche,United Kingdom)

following the manufacturer's instructions. The data obtained from this study were analysed using the Analysis of Variance (ANOVA) method.

Results

Of the 81 HIV positive patients studied, 69 (85.1%) were infected with *P. falciparum,* 3 (3.7%) suffered from *P. malariae* infection while 9 (11.2%) were malaria parasites free. On the other hand, of the 52 HIV negative patients, 32 (61.5%) were infected with *P. falciparum* while 1 (1.9%) suffered from *P. malariae* infection and 19 (36.6%) had no parasitaemia (Table 1).

The age and sex distribution of malaria revealed that children between the age of 0 and10 yr suffered malaria infection more than individuals in other age groups irrespective of their HIV status. About 16 out of 16 HIV positive children within this age bracket had malaria as against 25 (67.5%) out of 37 HIV negative children in the same age bracket. There was a statistical increase in the prevalence of malaria among HIV positive and HIV negative children ($p < 0.05$). Of the 81 HIV positive subjects studied, 37 were males, of which 31 (83.7%) were infected with malaria while 18 (54.5%) out of 33 HIV negative males had malaria. Of the 44 HIV positive females, 41 (93.1%) suffered malaria infection while 15 (51.7%) out of 29 HIV negative female had the infection. Generally there was a marked statistical increase in the prevalence of malaria among HIV positive when compared to HIV negative individuals ($p < 0.05$) (Table 2).

A two time-point assessment of the CD4 cells and viral load (baseline and exactly nine days after) revealed that the mean CD4 count of HIV positive patients with CD4 ≤200 cells/µl at 9-day post-treatment visit was significantly lower than the baseline value (a decrease from 180 cells/µl to 120 cells/µl). Also, the 9-day post-treatment viral load value was statistically higher than the baseline value (an increase from 3.05 HIV-RNA log copies/µl to 4.08 HIV-RNA logcopies/µl). In HIV positive patients with CD4 >200 cells/µl, a marked significant increase was obtained when mean viral load at baseline (2.65

Table 1. Prevalence and distribution of *Plasmodium* sp among HIV positive or negative group

Cases	No. examined	*P. falciparum*	*P. malariae*	Total
HIV (+)ve	81	69 (85.1)	3 (3.7)	72 (88.8)
HIV (–)ve	52	32 (61.5)	1 (1.9)	33 (63.4)
Total	133	101 (75.9%)	4 (3.0%)	105 (78.9%)

$\chi^2 = 3.25$; $p < 0.05$. Figures in parentheses indicate percentage.

Table 2. Age and sex distribution of malaria among HIV positive or negative patients

Age group	Male				Female			
	HIV positive		HIV negative		HIV positive		HIV negative	
	No. examined	No. infected (%)	No. examined	No. infected (%)	No. examined	No. infected (%)	No. examined	No. infected (%)
0–10	10	10 (100)	19	13 (68.4)	6	6 (100)	18	12 (66.6)
11–20	4	3 (75)	5	1 (20)	10	9 (90)	3	1 (33.3)
21–30	10	8 (80)	3	0 (0)	16	14 (87.5)	3	1 (33.3)
31–40	6	4 (66.6)	3	2 (66.6)	9	9 (100)	2	0 (0)
41–50	4	3 (75)	2	1 (50)	3	3 (100)	2	0 (0)
>50	3	3 (100)	1	1 (100)	0	0 (0.0)	1	1 (100)
Total	37	31 (83.7)	33	18 (54.5)	44	41 (93.1)	29	15 (51.7)

Table 3. Mean CD4 count, viral load and parasite density of HIV-positive or negative patients before and after therapy

Parameters	Before treatment (Baseline)	Post-treatment	Difference	p-value
HIV (+)ve (CD4 ≤200)				
CD4 (cells/µl)	180	110	70	<0.05
HIV-RNA (logcopies/µl)	3.05	4.08	1.03	<0.05
Parasites density (Parasites/µl)	17,500	1,500	16,000	<0.05
HIV (+)ve (CD4>200)				
CD4 (cells/µl)	280	300	20	>0.05
HIV-RNA (logcopies/µl)	2.65	2.85	0.20	<0.05
Parasite density (Parasites/µl)	8,500	500	8,000	<0.05
HIV (–)ve				
CD4 (cells/µl)	820	870	20	>0.05
Parasite density (Parasites/µl)	1,250	100	1,150	<0.05

HIV-RNA log copies/µl) was compared to 9-day post-treatment visit value (3.85 HIV-logcopies/µl). However, there was no statistical difference in the CD4 cells/µl at baseline when compared to values at 9-day post-treatment (despite an increase from 280 cells/µl to 300 cells/µl). There was a significant decrease between the parasite density of HIV (+ve) and that of HIV (–ve) individuals before and after therapy ($p < 0.05$) (Table 3).

Discussion

HIV infection increases the incidence and severity of clinical malaria. HIV infection has been found to roughly double the risk of malaria parasitaemia in clinical malaria. *P. falciparum* was found to be the most prevalent species in the study, Cheesbrough[10] reported that of *P. falciparum* assumes the leading role in the causation of malaria in west Africa. The prevalence of malaria was higher (88.8%) in HIV-infected individuals compared to HIV negative individuals (63.4%) ($p < 0.05$). This finding is supported by that of Patnaik *et al*[7] who reported that HIV-infected adults in malaria-endemic areas are at increased risk for malaria. The immunosuppression in this group of individuals may contribute immensely to the high prevalence of malaria in them.

The age and sex-wise distribution of malaria among HIV (+ve) and HIV (–ve) individuals showed that children within the ages of 0 and 5 yrs were more affected by malaria irrespective of their HIV status as reported by WHO-RBM[2].

Opportunistic infections are extrinsic factors that stimulate viral replication. With an active viral replication, the rate of CD4 cells destruction might outweigh the rate of production of newer cells. Therefore, this could justify why this decrease was observed in patients with CD4 ≤200 cells/µl. According to UNAIDS[11], patients with CD4 cells count above 200 cells/µl are not regarded as AIDS patients; they still possess immunological competent which able to mount an effective immunological response against some of these opportunistic infections and HIV itself.

This study revealed that patients with CD4 count ≤200 cells/µl experienced a decrease in their CD4 count after therapy but not statistically significant. A statisticaly significant increase in the viral load of patients in this group was recorded after therapy, this finding was also reported by Hoffman *et al*[9] in their study in Malawi which showed that HIV-1 plasma viral loads are significantly higher in patients with malaria than in those without, and these levels remain

higher for at least four weeks after treatment. Reason could be that infection with *P. falciparum* has been shown to stimulate HIV-1 replication through the production of cytokines (Interleukin-6 and tumor necrosis factor-alpha) by activated lymphocytes. Also, they recorded a higher parasitaemia compared to those with CD4 higher than 200 cells/µl, this possibly could be due to the fact that the CD4 cells which play a central role in the immune defence against many pathogens have been depleted by the HIV virus. Tatfeng *et al*[12] reported that CD4 cells depletion could weaken the immune response paving way to pathogens including opportunistic infections to establish. It was also observed that parasitaemia in the generality of patients could not be completely resolved after therapy leaving the patients with a possibility of recrudescence. This may also suggest the emergence of some resistant strains to cotecxin.

Though this study may not confirm the contributions of malaria infection in the progression towards AIDS, it points out the high prevalence of malaria in these individuals. This may increase the medical burden and their regular or repeated visit to health facilities. In the light of these, concise malaria control programme should be incorporated in HIV control schemes instituted in our health institutions across the continent. Finally, the need for enacting and implementing health policies aiming at subsidising and distributing effective antimalarials can never be overemphasised otherwise the Roll Back Malaria vision would only remain a mirage.

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