Original Article

Plasmodium falciparum Infection in HIV-Infected Patients on Highly Active Antiretroviral Therapy (HAART) In Benin City, Nigeria

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ABSTRACT

Background: Plasmodium falciparum infection is endemic in most tropical countries and will definitely infect Human Immunodeficiency Virus (HIV) positive patients living in this region at one time or the other during the course of their infection. This study was conducted to determine the prevalence of malaria infection in HIV-infected patients on highly active antiretroviral therapy in Benin City, Nigeria.

Methods: A total of 285 (84 males and 201 females) adults attending the hospital were enrolled in this study between July 2010 and June 2011. Blood specimens were collected from each participant and processed for CD4 counts, P. falciparum detection and hemoglobin concentration using standard procedures. The software INSTAT (GraphPad Software Inc., La Jolla, CA, USA) was used for all statistical analyses.

Results: A total of 6 (2.11%) out of 285 HIV infected patients on Highly Active Antiretroviral therapy (HAART) treatment had malaria and anemia. CD4 count <200 cells/µl was significantly associated with P. falciparum infection with odd ratio estimate of 11.61 (95% CI: 2.06, 65.48; P<0.004). Anemia was significantly associated with asymptomatic malaria infection among HIV patients on HAART with an odd ratio of 16.47 (95% CI: 0.919, 295.5; P=0.021).

Conclusion: The study reveals a low prevalence of asymptomatic malaria among HIV patients on HAART. Measures to reduce malaria infection and anemia among HIV patients on HAART are advocated.

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Introduction

Human Immunodeficiency Virus (HIV) is a public health problem with socio-economic burden as well as a threat for development1. Recent research indicates an estimated 33 million persons living with HIV, 94% of whom live in low income countries2. Malaria is prevalent in poor countries, occasioned by poverty, caused by inadequate sewage treatment, poor hygiene, and substandard housing3. Co-infection with HIV and malaria is very common in sub-Saharan Africa4. Malaria and HIV are among the two most important global health problems of our time, together they cause more than four million deaths per year5. Plasmodium falciparum infection is endemic in most tropical countries and will definitely infect HIV patients living in this region at one time or the other during the course of their infection6. Highly active antiretroviral therapy (HAART) has significantly reduced morbidity and mortality induced by AIDS. The introduction of HAART, and artemisinin-based
combination therapy (ACT) could control HIV infection, improve the immune status, manage malaria infection in HIV-infected patients and reduce prevalence rate of malaria. A prevalence of 46% of malaria infection among HIV positive patients not on HAART was observed in Benin City.

There is lack of information on the prevalence of *P. falciparum* infection in HIV-infected persons on HAART in Benin City. Accordingly, this study was conducted to determine the prevalence of malaria in HIV-infected patients on HAART in Benin City, Nigeria.

**Methods**

**Study population**

The cross-sectional study was carried out from July 2010 to June 2011 at the University of Benin Teaching Hospital, Benin City, - a referral teaching hospital and a center for the United States President's Emergency Plan for AIDS Relief (PEPFAR). Within the period of the study about 600 HIV patients on HAART fulfilled the inclusion criteria for this study. However, only 285 patients gave consent to participate in the study given a rejection rate of 52.5%. No previous study within our environment had reported asymptomatic malaria on HIV patients. However, we have observed a prevalence of 0.85% (unpublished) in our institution. Assuming a prevalence of 0.85%, we need a sample size of approximately 285 adults (84 males and 201 females) at 5% significance level and 0.04 as permissible error. Patients with signs and symptom of malaria, AIDS defining conditions and those not on HAART were excluded from this study. These patients had no sign and symptom of malaria, and were on HAART regimen as well as prophylactic antimalaria therapy (Sulfadoxine, pyrimethamine and dihydroartemisinine). The agents used in the HAART regimen for HIV-infected patients consist of zidovudine, stavudine and nevirapine. The age of the participants ranged from 20 to 66 years. Informed consent was obtained from all study participants. The study was approved by the Ethics Committee of the University of Benin Teaching Hospital, Benin City, Nigeria.

**Specimen collection and processing**

Five milliliter of venous blood was obtained aseptically using sterile needle and syringe and dispensed into EDTA bottles. CD4 counts were evaluated using the flow cytometry (Partec, Gmbh, Germany). Briefly, 20 μl of whole blood was placed in a Partec tube, and 20 μl of CD4+ T cell monoclonal antibodies was added. The mixture was then incubated in the dark for 15 minutes at room temperature after which 800μl of buffer was added. The tube was then placed in the flow cytometer for counting and the CD4+ T cells value obtained from a programmed computer connected to the instrument. The blood sample was further analyzed for hemoglobin (Hb) concentration using an auto-analyser-sysmex kx-21 (Sysmex Corporation, Kobe, Japan). Anemia was defined according to WHO criteria (Hb <13 g/dL for males and Hb <12 g/dl for females).

The preparation of both thick and thin blood films for detection and confirmation of parasitemia were carried out using an earlier described method.

The stained films were examined for malaria parasites by microscopy using an x100 oil immersion objective lens. A total of 200 fields per film were examined.

**Data analysis**

The data obtained were analyzed by using Chi-squared ($\chi^2$) test to compare the frequency data. The odd ratio (OR) was calculated for each potential risk factor at 0.05 significance level. The software INSTAT (GraphPad Software Inc., La Jolla, CA, USA) was used for all statistical analyses.

**Results**

A total of 6 (2.11%) out of 285 HIV-infected patients on HAART treatment had malaria and anemia. CD4 count <200 cells/μl was significantly associated with *P. falciparum* infection with an odd ratio of 11.61 (95% CI: 2.06, 65.48; $P<0.004$). Of the 285 recruited patients, 129 (45.26%) had anemia. All six patients that had malaria parasitemia were anemic (4.65%) (Table 1).
Table 1: Effect of risk factors on the prevalence of malaria parasitemia in HIV-infected patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Malaria Parasitemia</th>
<th>Absent (%)</th>
<th>Present (%)</th>
<th>Odd Ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 counts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥200 cells/µl</td>
<td>238 (99.17)</td>
<td>2 (0.83)</td>
<td></td>
<td>11.61</td>
<td>2.06, 65.48</td>
<td>0.005</td>
</tr>
<tr>
<td>&lt;200 cells/µl</td>
<td>41 (91.11)</td>
<td>4 (8.89)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>156 (100.00)</td>
<td>0 (0.00)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>123 (95.35)</td>
<td>6 (4.65)</td>
<td></td>
<td>16.47</td>
<td>0.919, 295.3</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Anemia was significantly associated with asymptomatic malaria infection among HIV patients on HAART with an odd ratio of 16.47 (95% CI: 0.919, 295.3; P=0.021).

Discussion

Malaria and HIV are among the two most important global health problems of our time. Together they cause more than four million deaths per year. HIV-infected patients are at higher risk for malaria because of their weakened immune systems. It is suggested that malaria may be helping the spread of HIV infection. Both malaria and HIV can cause anemia independently. Use of HAART regimen has been reported to improve immunity and hemoglobin concentration in HIV-infected patients. It is therefore possible that with improved immunity and use of anti-malaria treatment, the prevalence of malaria and anemia would reduce significantly among HIV-infected patients. However, no such data are available in our center.

An overall prevalence of 2.11% of asymptomatic malaria was observed in this study. This is lower than the 4% and 75% observed in Ilorin (Nigeria) and Uganda, respectively. The high prevalence observed in Ilorin may be because their patients were symptomatic for malaria. The difference in the findings of this study and that of the Uganda study may be due to the type of anti-malaria regimen (Cotrimoxazole) used for the patients.

Among HIV patients on HAART, CD4 count <200 cells/µl was significantly associated with malaria parasitemia. This may indicate that patients on HAART whose immunity may not have fully recovered are still susceptible to malaria infection. However, it is important to note that viral load has been reported as better predictor of HIV replication and immune status in HIV patients. Perhaps, a study on viral load and asymptomatic malaria parasitemia may give a better understanding of this issue.

The prevalence of anemia observed in this study was 45.26%. This is lower than the 51.15% previously reported among HIV patients on HAART. The use of zidovudine in HAART regimen, antibodies to HAART agents, and infections such as parasitic have been reported to be associated with anemia. Ziduvidine is one of the HAART agents used by our patients and may explain the finding in this study. Indeed malaria parasitemia was significantly associated with anemia. Therefore, malaria is still an important cause of anemia among HIV-infected patients on HAART.

Conclusion

The study reveals a low prevalence of asymptomatic malaria among HIV patients on HAART. Measures to reduce malaria infection and anemia among HIV patients on HAART are advocated.

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Conflict of interest statement

The authors declare that they have no competing interests.
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