**Plasmodium falciparum** and HIV-1/2 Co-infection among children presenting at the Out-patient clinic of Oni Memorial Children Hospital in Ibadan, Southwestern Nigeria

Okonko IO¹, Adejuwon AO², Okerentungba PO¹, Frank-Peterside N¹

¹Medical Microbiology Unit, Department of Microbiology, University of Port Harcourt, East-West Road, P.M.B. 5323, Choba, Port Harcourt, Rivers State, Nigeria; 
²Department of Microbiology, Lead City University, Ibadan, Oyo State, Nigeria; 
mac2finney@yahoo.com, iheanyi.okonko@uniport.edu.ng; Tel.: +234 803 538 0891

**ABSTRACT:** This study was carried out to detect circulating *Plasmodium falciparum* and HIV-1/2 as co-infection among attendees of Oni Memorial Children Hospital in Ibadan, Southwestern Nigeria. A total of 217 blood samples were collected from consenting subjects (ages 3 days to 15 years) from April to September, 2011. The detection of circulating *P. falciparum* malaria was carried out using Malaria *Plasmodium falciparum* Rapid Test Device. Microscopic examination of thick and thin films techniques was also employed as standard while the screening for HIV-1/2 antibodies was carried out using Chembio HIV-1/2 Stat-Pak® and Abbott Determine HIV-1/2® test kit. All tests were done according to the manufacturers’ specifications. Overall prevalence rate of *P. falciparum* was 35.5%. Of the 35.5% *P. falciparum* positive subjects; 9.1% had HIV-1/2 as co-infection. Similarly, 64.5% were *P. falciparum* negative and 3.6% of them were positive for HIV-1/2 antibodies. The study showed no significant difference between age groups for either malaria infection (29.2% vs. 30.1%, P >0.05) or HIV co-infection (8.7% vs. 9.7%, P > 0.05). Also, no significant difference existed between sexes for either malaria infection (37.3% vs. 33.6%, P>0.05) or HIV co-infection (4.9% vs. 13.9%, P > 0.05) and no significant difference in HIV between groups with & without history of vaccination (7.0% vs. 15.0%; P>0.05). The study however, showed that subjects with no history of vaccination had a significantly higher prevalence of malaria (44% vs. 27%; P=0.001). Thus, it further confirmed the presence of *Plasmodium falciparum* and HIV co-infection among children and teenagers in Oyo State, Nigeria. General surveillance and public health education to stop the spread of the infection among children in Ibadan and indeed the whole society is advocated.

**Keywords:** Antibody, children, HIV -1 & -2, seropositivity, risk factors, mosquito eradication, malaria, malaria interventions, *Plasmodium falciparum*

1. **INTRODUCTION**

Malaria and Human immunodeficiency virus (HIV) are coendemic in many developing countries, with anemia being the most common pediatric hematological manifestation of each disease (Davenport et al., 2010). Malaria and HIV are two of the most common global health challenges today and the two infections commonly overlap in distribution in most countries especially in sub-Saharan Africa (Abu-Raddad et al., 2006). Studies have demonstrated interaction between these two infections with the majority of studies conducted in adults (Cohen et al., 2005; Patnaik et al., 2005; Kamya et al., 2006; Hewitt et al., 2006). HIV-1 infection has been found to be associated with severe forms of malaria and particularly cerebral malaria in adults but there is still a paucity of information on the interaction between the two infections in children (Chalwe et al., 2009).

Different reports have it that incidence of malaria is not common in HIV-infected individuals ( Muller et al., 1990; Dayachi et al., 1991), while others have reported uncommon incidence of malaria in HIV-infected individuals in malaria endemic areas (Grimwade et al., 2003, 2004; Cohen et al., 2005; Abu-Raddad et al., 2006), although malaria transmission is unstable throughout the year in these reports. Most HIV infection of children is acquired from infected mothers, particularly during breast-feeding and at the time of delivery (Newton et al., 2005). In sub-Saharan Africa, the distributions of malaria and HIV widely overlap (Muema et al., 2011).

The diagnosis of malaria *P. falciparum* infection using *P. falciparum* antigen has been widely accepted as a rapid antigen test for *P. falciparum* malaria (Onyenekwe et al., 2007). Its accuracy has also been put at 86–99% compared with microscopic detection of malaria parasites in smears (Mens et al., 2007) as with very high specificity. It has been recommended for use where microscopic detection of malaria parasites in smears is not possible. However, one of the
limitations is that the malaria antigen may still be detected after treatment has been effected with successful clearance of parasites from blood (Onyenekwe et al., 2007).

Mass treatment as a means to reducing *P. falciparum* transmission was used during the first global malaria eradication campaign and is increasingly being considered for current control programmes (Okell et al., 2011). Thus, it may be important to investigate the prevalence of malaria and HIV co-infections in children residing in a malaria endemic area with stable transmission throughout the year. This will help to know if pattern and burden of malaria and HIV amongst children are similar in all endemic areas irrespective of stable or unstable transmission throughout the year. This present study reports the prevalence of circulating *Plasmodium falciparum* and HIV-1/2 co-infection among children presenting with some form of illness at the outpatient clinic of the Oni Memorial Children Hospital in Ibadan, the capital city of Oyo State located in the forest zone of southwestern Nigeria.

### 2. MATERIAL AND METHODS

#### 2.1. STUDY AREA

The study area is the Oni Memorial Children Hospital, located at the municipal area of Ibadan, which is made up of five local government areas. Ibadan is the capital city of Oyo State located in the forest zone of southwestern Nigeria. Ibadan city lies on the longitude 3°5' East of Greenwich meridian and latitude 7°23' North of the Equator. Besides being the largest indigenous city in Africa south of Sahara, the city is an important trade and educational centre. It also houses one of the largest and foremost teaching hospitals in Africa. However, the city is characterized by low level of environmental sanitation, poor housing, and lack of potable water and improper management of wastes especially in the indigenous core areas characterized by high density and low income populations.

#### 2.2. STUDY POPULATION

Blood samples were collected from two hundred and seventeen (217) attendees of the Oni Memorial Children Hospital, Ibadan, South-Western, Nigeria after informed consent was sought. The study was approved and followed the ethical considerations and guidelines. The study was carried out between April and September, 2011.

#### 2.3. DEMOGRAPHIC INFORMATION

The study groups were also stratified by age, sex and history of childhood vaccinations. The ages of the subjects ranges from 3 days to 15 years. All the subjects used for this study were children presenting with some form of illness at the outpatient clinic of the hospital. Table 1 summarizes the characteristics of subjects used in this study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. Tested</th>
<th>No. males (%)</th>
<th>No. females (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Groups (Year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5 (Children under 5 years)</td>
<td>114(52.5)</td>
<td>72(63.2)</td>
<td>42(36.8)</td>
</tr>
<tr>
<td>5-15 (School-aged children)</td>
<td>103(47.5)</td>
<td>38(36.9)</td>
<td>65(63.1)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>110(50.7)</td>
<td>100(100.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Females</td>
<td>107(49.3)</td>
<td>0(0.0)</td>
<td>107(100.0)</td>
</tr>
<tr>
<td>History of vaccinations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>171(78.8)</td>
<td>86(50.3)</td>
<td>84(49.7)</td>
</tr>
<tr>
<td>No</td>
<td>46(21.2)</td>
<td>24(52.2)</td>
<td>22(47.8)</td>
</tr>
<tr>
<td>Total</td>
<td>217(100.0)</td>
<td>110(50.7)</td>
<td>107(49.3)</td>
</tr>
</tbody>
</table>

#### 2.4. SAMPLE COLLECTION

The method of sample collection employed was venepuncture technique (Cheesbrough, 2006). When sufficient blood had been collected, it was transferred into an EDTA bottle. This was also centrifuged and the plasma was then pipetted into sterile ependorf tubes and stored at -20°C until ready for use.

#### 2.5. DETECTION OF PLASMODIUM FALCIPARUM

**2.5.1. Malaria *Plasmodium falciparum* Rapid Test Device**

Whole blood was used for the diagnosis of *P. falciparum* malaria using parallel Malaria *Plasmodium falciparum* Rapid Test Device (manufactured by Global device, USA and IND® Diagnostica, USA). The malaria P.f. Rapid Test Device (Whole Blood) is a qualitative, membrane based immunoassay for the detection of P.f antigen in whole blood. The principle 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. After 15 min 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* infection while the presence of only one pink line in the control region signifies absence of *P. falciparum*. The Malaria P.F. Rapid Test Device (Whole Blood) has tested with thin or thick blood smears on clinical samples. The results show that the sensitivity of the Malaria P.f rapid test Device (Whole Blood) is >99.0% relative to blood smears. The malaria P.f Rapid test Device (Whole Blood) uses an antibody that is highly specific for malaria P.f. antigen in Whole Blood. The results show that the specificity of the Malaria P.f. Rapid Test device Whole Blood is >99.0% relative to blood malaria.

2.5.2. Microscopic Examination of Thick and thin blood films

The collected blood samples were analyzed within 1 to 2 h of collection. Thick and thin blood films were prepared according to the technique outlined by Cheesebrough (2006). A drop of each blood sample was placed in the center of a greasefree clean glass slide. Thereafter, the reverse side of the slide was cleaned with cotton wool and kept for air-drying and staining with field’s stain A (eosin) for 5 s. It was washed off gently in clean water and then dipped in field’s stain B (methyl azure) for 5 s and washed again in clean water. The back of the slide was cleaned with cotton wool and kept in the draining rack to air-dry for eventual examination under the microscope, using oil immersion at 100X magnification to observe for *Plasmodium* parasites. Presence of ring forms of *Plasmodium* and Trophozoites of *Plasmodium* indicate positive results. A blood smear was considered negative if no parasite seen after 10 min of search or examination under 100 high power fields of microscope.

2.5.3. Identification

Positive specimens were identified on the basis of microscopy. Using standard methods (CDC, 2007), the malaria blood slides were interpreted. Prevalence of *Plasmodium* was calculated as the proportion of sampled persons with a positive result divided by the number of persons who provided blood samples. All point estimates were weighted, with empirically estimated standard errors used to account for prevalence.

2.6. DETECTION OF ANTIBODIES TO HIV-1/2 IN HUMAN PLASMA

Two different methods were used namely Chembio HIV-1/2 Stat-Pak® by CHEMBIO DIAGNOSTICS SYSTEMS, INC. Medford, New York 11763 USA; sensitivity 100% (129/129) and specificity 100% (207/207)] and Abbott Determine™ HIV-1/2 test kit [by ABBOTT JAPAN CO.,LTD. Minto-Ku, Tokyo, Japan; sensitivity 100% and specificity 100%]. These are an *in vitro* visually read immunoassay and immunochromatographic test for the qualitative detection of antibodies to HIV-1/2 in human plasma. For the Chembio HIV-1/2 Stat-Pak® and Abbott determineTM HIV-1/2, the procedure as described by the manufacturers was used for the analysis. The results of the tests were read at zero minute after the addition of the running buffer. This method has inherent quality control that validates the results. The presence of two pink/purple lines in the region of test sample and control indicates HIV seropositive reaction while a single pink/purple line at the control region indicates HIV seronegative reaction. HIV seropositive results’ using these two methods was used to classify participants as presenting with HIV infection.

2.7. DATA ANALYSIS

The prevalence for malaria *Plasmodium falciparum* and possible co-infection with HIV was calculated by using patients with positive samples as numerator and the total numbers of patients enrolled in this study as denominator. The data generated from this study were presented using descriptive statistics. The data was subjected to Fisher’s Exact Test for comparison of proportions to determine any significant relationship between infection rate, age, gender and history of childhood vaccinations. Confidence level was set at p= 0.05

3. RESULTS ANALYSIS

A total of 217 subjects from different households in different communities and locations were enrolled in the study. Rapid test at the Medical Microbiology & Virology Unit, Department of Microbiology, Lead City University Ibadan detected 77 (35.5%) *Plasmodium falciparum* infections among the 217 (100.0%) subjects who had provided a whole blood sample. Of the 77 (35.5%) *P. falciparum* positive subjects; 7(9.1%) had HIV-1/2 antibodies as co-infection while the remaining 70(90.9%) had no HIV-1/2 antibodies as co-infection. Similarly, 140(64.5%) were *P. falciparum* negative. Of the 140 *P. falciparum*
negative subjects, 5(3.6%) were positive for HIV-1/2 antibodies, only among children under 5 years (7.4%) as shown in Table 2.

3.1. Prevalence rates of *Plasmodium falciparum* and HIV/malaria co-infection among subjects in relation to age

Table 2 shows the prevalence of *P. falciparum* in relation to the ages of the subjects in Ibadan, Nigeria. This study showed that there was no significant difference between age groups for either malaria infection (29.2% vs. 30.1%, \( P > 0.05 \)) or HIV co-infection (8.7% vs. 9.7%, \( P > 0.05 \)) among the population studied (Table 2).

Table 2: Prevalence rates of *Plasmodium falciparum* and HIV/malaria co-infection in relation to age

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>No. Tested (%)</th>
<th>No. Positive (%)</th>
<th>Co-infection with HIV (%)</th>
<th>No. Negative (%)</th>
<th>No. positive for HIV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children under 5</td>
<td>114(52.5)</td>
<td>46(29.2)*</td>
<td>4(8.7)*</td>
<td>68(59.6)</td>
<td>5(7.4)</td>
</tr>
<tr>
<td>5-15</td>
<td>103(47.5)</td>
<td>31(30.1)*</td>
<td>3(9.7)*</td>
<td>72(69.9)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>217(100.0)</td>
<td>77(35.5)</td>
<td>7(9.1)</td>
<td>140(64.5)</td>
<td>5(3.6)</td>
</tr>
</tbody>
</table>

Key: * = Not Significant (\( P > 0.05 \))

3.2. Prevalence rates of *Plasmodium falciparum* and HIV/malaria as co-infection in relation to sex

Sex distribution of *P. falciparum* and HIV/malaria co-infection indicated that there was no significant difference between sexes for either malaria infection (37.3% vs. 33.6%, \( P > 0.05 \)) or HIV co-infection (4.9% vs. 13.9%, \( P > 0.05 \)) among the population studied (Table 3).

Table 3: Prevalence rates of *Plasmodium falciparum* and HIV/malaria co-infection in relation to sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. Tested (%)</th>
<th>No. Positive (%)</th>
<th>Co-infection with HIV (%)</th>
<th>No. Negative (%)</th>
<th>No. positive for HIV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>110(50.7)</td>
<td>41(37.3)*</td>
<td>2(4.9)*</td>
<td>69(66.7)</td>
<td>4(5.8)</td>
</tr>
<tr>
<td>Females</td>
<td>107(49.3)</td>
<td>36(33.6)*</td>
<td>5(13.9)*</td>
<td>71(66.4)</td>
<td>1(1.4)</td>
</tr>
<tr>
<td>Total</td>
<td>217(100.0)</td>
<td>77(35.5)</td>
<td>7(9.1)</td>
<td>140(64.5)</td>
<td>5(3.6)</td>
</tr>
</tbody>
</table>

Key: * = Not Significant (\( P > 0.05 \))

3.3. Prevalence rates of *Plasmodium falciparum* and HIV/malaria co-infection in relation to history of vaccinations

Table 4 shows the prevalence rates of *Plasmodium falciparum* and HIV/malaria co-infection in relation to history of vaccinations. There was no significant difference in HIV co-infection between groups with & without history of vaccination (7.0% vs. 15.0%; \( P > 0.05 \)). Children with no history of vaccination had a significantly higher prevalence of malaria (43.5% vs. 33.3%; \( P = 0.001 \)) as shown in Table 4.

Table 4: Prevalence rates of *Plasmodium falciparum* and HIV/malaria co-infection in relation to history of vaccinations

<table>
<thead>
<tr>
<th>History of vaccination</th>
<th>No. Tested (%)</th>
<th>No. Positive (%)</th>
<th>Co-infection with HIV (%)</th>
<th>No. Negative (%)</th>
<th>No. positive for HIV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>171(78.8)</td>
<td>57(33.3)**</td>
<td>4(7.0)*</td>
<td>114(66.7)</td>
<td>4(3.5)</td>
</tr>
<tr>
<td>No</td>
<td>46(21.2)</td>
<td>20(43.5)**</td>
<td>3(15.0)*</td>
<td>26(56.5)</td>
<td>2(7.7)</td>
</tr>
<tr>
<td>Total</td>
<td>217(100.0)</td>
<td>77(35.5)</td>
<td>7(9.1)</td>
<td>140(64.5)</td>
<td>5(3.6)</td>
</tr>
</tbody>
</table>

Key: * = Not Significant (\( P > 0.05 \)); ** = Significant (\( P = 0.001 \))
4. DISCUSSION
The aim of this study is to determine the prevalence of circulating *Plasmodium falciparum* and HIV-1/2 as co-infection among attendees of Oni Memorial Children Hospital in Ibadan, Nigeria. The overall prevalence of *P. falciparum* amongst the subjects was 35.5%. The prevalence of *P. falciparum* in these subjects is a reflection of the prevalence of this species of malaria in the population. Similar prevalence has been reported previously in Nigeria (Okonko et al., 2010). The 35.5% overall prevalence of *Plasmodium falciparum* infection in the study population is lower compared to the 59.9% reported by Ojo and Mafiana (2005) among children less than 15 years in Abeokuta, Southwestern Nigeria. It is also lower than 45.0% prevalence reported for placental malaria (PM) among HIV infected mothers in rural Rwanda (Bulterys et al., 2011). It is however higher than the 9.34% reported by Greenberg et al. (1991) among children in Kinshasa, Zaire. It is also higher than the 24.3% reported by Kuadzi et al. (2011) among children in Ghana.

Amongst the *P. falciparum* malaria-positive subjects, the prevalence rate of HIV as a co-infection was 9.1%. This value is higher than the 1.0% and 1.1% relative rates of occurrence reported by Kalyesuula et al. (1997) among children in Kampala, Uganda. It is comparable to the 11.8% and 10.6% reported by Onyenekwe et al. (2007). HIV increases the burden of malaria by increasing susceptibility to infection and decreasing the response to malarial treatment (Imani et al., 2011). HIV has also been found to suppress the immune system and predispose to severe forms of malaria in adults (Imani et al., 2011). Recurrent or persistent co-infections may increase HIV viral load and, consequently, risk of HIV transmission, thus increasing HIV incidence (Barnabas et al., 2011). Co-infections may increase HIV viral load in populations where they are prevalent, thereby facilitating HIV transmission (Barnabas et al., 2011). The most common parasitic infections in a study by Thigpen et al. (2011) among pregnant women in Malawi was malaria (37.6%) and 14.2% of these women were HIV-infected (Thigpen et al., 2011).

Our findings are in agreement with studies conducted in KwaZulu Natal, South Africa, in Kenya and in Uganda where researchers in those countries also found similar association with HIV infection and malaria co-infection in children (Grimwade et al., 2003, 2004; Berkley et al., 2009; Imani et al., 2011). In South Africa, the prevalence of HIV among children with severe malaria was 17.0% compared to 7.5% in children with uncomplicated malaria while the study in Kenya, found a prevalence of 12.0% among children admitted with severe malaria. In Uganda, HIV was present in 9.0% of children with cerebral malaria compared to 2.3% in uncomplicated malaria and 2.5% in children with no malaria. In both studies, the forms of severe malaria which the children presented with and HIV prevalence in cerebral malaria was not reported (Imani et al., 2011).

Malaria can affect all the age humans groups and both male and female sexes. This present findings showed no significant difference between sexes for either malaria infection (37.3% vs. 33.6%, P>0.05) or HIV co-infection (4.9% vs. 13.9%, P > 0.05). A predominance of malaria infections in male patients has been documented in some cases, but there is no scientific evidence to prove the higher prevalence being related to gender as susceptibility to malaria infection is not influenced by gender (Abdullahi et al., 2009).

Generally, with regards to the age distribution of the subjects, this present findings showed no significant difference between age groups for either malaria infection (29.2% vs. 30.1%, P >0.05) or HIV co-infection (8.7% vs. 9.7%, P > 0.05). Our findings deviates from what was previous reported in some studies. It was previously reported that HIV is associated with admission to hospital in rural Kenya with severe malaria among children, except in infancy (Muema et al., 2011). HIV-infected children with severe malaria were older, had higher parasite density and increased mortality, raising a hypothesis that HIV interferes with naturally acquired immunity to malaria, hence with little effect at younger ages (a shorter history of exposure) (Muema et al., 2011).

Our present findings showed no significant difference in HIV between groups with and without history of vaccination (7.0% vs. 15.0%; P>0.05). However, children with no history of vaccination had a significantly higher prevalence of malaria (43.5% vs. 33.5%; P = 0.001). Generally, there is slow acquisition of active immunity to malaria (Abdullahi et al., 2009). The high prevalence of malaria infections in children with no history of vaccination suggests that these persons have lost some degree of immunity as a result of poor living conditions other than lifelong exposure. Also, children born to immune mothers are protected against malaria during their first half year of life by maternal antibodies. As they grow older, after continued exposure from multiple infections with malaria parasites over time, they build up an acquired immunity and become relatively protected against disease and
blood stage parasites hence lower prevalence of malaria among the older age groups (Abdullahi et al., 2009).

One of the limitations of this study is that the study was conducted using one secondary health centre, though located in the strategic location of the township; these results may reflect what is happening in other health centers in the metropolis. The second limitation is that a PCR study was not carried out in younger children. However, the results obtained are within limits compared to similar researches (Abdullahi et al., 2009; Berkley et al., 2009; Imani et al., 2011; Kuadzi et al., 2011) and also within the limits of the malaria prevalence rate reports in Nigeria (Ojo and Mafiana, 2011) and also within the limits of the malaria epidemic trajectories and positive prevention: a systematic review and meta-analysis. AIDS. 2006; 21(13): 2405–2416. doi: 10.1097/QAD.0b013e3283491e3e

5. CONCLUSION
The study showed no significant difference between age groups and sexes for either malaria infection or HIV co-infection. It showed no significant difference in HIV between groups with and without history of vaccination. The study however, showed that children with no history of vaccination had a significantly higher prevalence of malaria. The high prevalence of malaria infection observed in this study is worrisome because high-density urban African populations are not often considered particularly vulnerable to malaria infection. The findings from this study add to the existing knowledge of interaction between HIV infection and P. falciparum malaria in children/teenagers which are of both clinical and public health importance. Therefore, irrespective of site or location in a malaria endemic area, the problem of HIV and malaria infections calls for concern as both could lead to high mortality rate. Due to high mortality rates associated with malaria infection in an endemic area, it may be necessary that routine malaria screening be adopted as part of the management policy to check the co-infection. Other malaria preventive measures such as use of insecticide-treated mosquito nets should also be emphasized during counseling sessions (Imani et al., 2011). Further studies could be undertaken to investigate other epidemiological parameters and is needed on methods to rapidly estimate needs (incidence) and coverage and on strategies to efficiently expand treatment access.

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CORRESPONDENCE TO:
Iheanyi O. Okonko;
Department of Microbiology,
University of Port Harcourt,
P.M.B 5323, Uniport Post Office, Choba,
East/West Road, Port Harcourt, Rivers State,
Nigeria;
E-Mail: mac2finney@yahoo.com;
iheanyi.okonko@yahoo.com;
Tel: +234-080-3538-0891

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