

## Prevalence of HIV and Malaria parasites co-infection in pregnant mothers and their babies post delivery

Adeoti O. M.<sup>1,2\*</sup>, Anumudu C.I.<sup>2</sup>, Nwuba R.I.<sup>2</sup>, Awobode H.I.<sup>3</sup>, Olaniyan M.F.<sup>4</sup>, Olayiwola O<sup>4</sup>, Fagbade O<sup>4</sup>.

1. Cellular Parasitology Programme, Cell Biology and Genetics unit, Department of Zoology, University of Ibadan, Nigeria, Biological Sciences Unit, Department of Science Laboratory Technology, P.M.B 021, the Polytechnic Ibadan, Saki Campus
2. Cellular Parasitology Programme, Cell Biology and Genetics unit, Department of Zoology, University of Ibadan, Nigeria
3. Parasitology Unit, Department of Zoology, University of Ibadan, Nigeria
4. AIDS preventive Initiatives Laboratory, Baptist Medical Centre, Saki

\* E-mail of the corresponding author: \* [txy23m@yahoo.com](mailto:txy23m@yahoo.com), +234-806-7285-175

### Abstract

Worsened perinatal outcomes and increased rates of maternal morbidity are consequences of co-infection of HIV and *Plasmodium falciparum* in pregnant women. This study was designed to ascertain the proportion of co-infection of both diseases in pregnant mothers and babies born to HIV-infected mothers.

A total of 149 pregnant mothers and 30 babies of HIV-infected mothers were engaged in a longitudinal study for 18 months in the endemic area of Saki and Ibadan. Only babies born to HIV infected mothers were enrolled and systematically followed-up for six months post delivery. Determine<sup>(R)</sup> and Unigold rapid diagnostic tests kits were used for HIV test in mothers whereas HIV screening was conducted on the babies using polymerase chain reaction at six months post delivery. Giemsa stained thick blood smear was used to determine the presence of asexual stages of *Plasmodium falciparum*. Descriptive statistics was used to determine the percentage of infections status. Chi-square and student t-test was used to compare maternal data and babies six months after birth.

The results showed that 85/149(57.0%) mothers and 11/30(36.7%) babies had microscopically detectable malaria parasites whereas the seroprevalence were 64(33.0%) and 19(10.7%) for mothers and infants respectively. In mothers, 19(12.8%) had HIV alone, 51/149(34.2%) malaria only, 34/149 (22.8%) were co-Infected and 45/149(30.2%) had neither HIV nor malaria. In infants, 9/30 (30.0%), 10/30(33.3) had HIV only, 2/30(6.7%) had malaria only whereas 9/30(30.0%) had neither malaria nor HIV. Parasitemia ranged between 251.5 of cells/ $\mu$ L in mothers and 205.7 of cells/ $\mu$ L in babies born to HIV infected mothers.

**Keywords:** *Perinatal, Plasmodium falciparum*, Seroprevalence, co-infected, Parasitemia.

### 1. Introduction

Malaria and HIV are among the two leading global health problems of our time. Together, they cause more than four million deaths per year (WHO, 2011). By 2006, it was estimated that, the interaction of malaria and HIV in one Kenyan district alone had caused 980, 000 excess malaria episodes and 8 500 excess HIV infections since HIV's emergence in 1980s (Abu-Raddad *et al.*, 2006). Today, approximately 50% of the world's population, or 3.3 billion people, are at varying degree of risk of malaria. Malaria parasitemia varies in instances of presumed reported symptoms of malaria and clinical malaria. The combined effects of one on the other, both HIV and malaria one another accounted for more deaths in 2007 (WHO, 2008, UNAIDS 2007) and more deaths are still likely. Malaria is endemic in many areas of India and some other continents with repeated infections with *Plasmodium falciparum* and *P. vivax* (Shankarkumar *et al.*, 2011). There are two types of HIV: HIV-1 and HIV-2- with a number of related viruses in non-human primates. Both HIV-1 and HIV-2 have been described in Nigeria and other parts of West Africa. Like all viruses, HIV-1 must usurp the cellular machinery at multiple steps to complete a productive cycle. The virus enters cells by fusing with the cellular membrane, taking advantage of receptor and co-receptor host proteins which otherwise play important roles in immunity and inflammation (16). The world malaria report of 2010, 225 million cases of malaria and an estimated 781 000 deaths occurred in 2009. Most deaths occurred among children living in Africa where a child dies every 45 seconds for malaria and this disease accounts for approximately 20% of all childhood deaths. It is estimated that each year about 24 million pregnant women are infected by *P. falciparum*, especially in sub-Saharan Africa (27) and about 1 million per year are co-infected with HIV (31).

#### 1.1 Research Questions

1. Does malaria in pregnancy increase the risk of vertical transmission of HIV?
2. What is the proportion of mono infected and co-infected pregnant women in the study area?

##### 1.1.1 Objective

To document the prevalence of all categories of infection status in individual pregnant mothers and babies born to HIV infected mothers

#### 1.1.2 Scope of the study

The present study is aimed at relating peripheral cellular responses from HIV positive and HIV negative pregnant women, both with and without malaria infection in the endemic area of Saki and Ibadan, Oyo State south-western Nigeria.

#### 1.1.3 Justification of the study

Many studies have documented inconsistent data concerning the overlap. Two thirds of HIV infections were in sub-Saharan Africa (WHO, 2011). However, data on co-infection of malaria and HIV in Nigeria is very scanty. This study was designed to ascertain the proportion of co-infection of both diseases in pregnant mothers and babies born to HIV-infected mothers.

### 1.4 Methodology

#### 1.4.1 Study area

Saki is a border town bounded by Kwara State in the north and to the west by Cotonou (Republic of Benin) and Lome (Togo). It comprises of three local government areas, viz: Saki west, Saki East and ATISBO. The selected Hospitals are located in region mesoendemic to *P. falciparum* infection a typical of what is generally obtainable in sub-Saharan Africa, where quality health facilities are virtually nonexistent. Saki is located in 8° 26' and 9° 5' north of the equator and longitudes 2° 45' and 3° 37' East of the meridian. This location confers on the town the equatorial climatic conditions. There are two distinct seasons namely wet and dry seasons. The wet season is the period for rainfall, which is between mid-April and October characterized by double maxima distribution in the Southern part, as a result of Western monsoon wind on the atmosphere. The dry season lasts between November and March and it is characterized by moderate weather. The rainfall pattern is remarkably constant ranging between 1,211 mm at Kisi (East of Saki). The mean temperature is 33°C while the sunshine hours per day range from 3.4 hours in August to 11 hours in February. Rice and roots crops are the main agricultural products. Indigo is also grown in the surrounding area, making Saki an important center for distribution of dyed products. According to the 2006 national census figures Saki is the third largest in Oyo state with a population density of 189,700. The town is heterogeneous comprising the main people of the Yoruba ethnic group who speak the Yoruba language and in the minority; other ethnic groups which include the Fulani, Togolese, Beninous, and the Tangitas. Like other Yorubas, Saki indigenes claimed descent from Oduduwa. The study is a longitudinal study covering an 18- month's period (August, 2009-February, 2011) in five contiguous sites in Saki, south-west; Nigeria and PEPFAR clinic in University Teaching Hospital, Ibadan. The selected hospitals were: Baptist Medical Centre, General Hospital, Tunmise maternity Clinic, and Isale-Taba, maternity and Muslim Hospital. The sample size was determined by using the technique as described elsewhere (Phil, 2004) and the data of the previous study (Adeoti, 2008 unpublished) conducted in the antenatal clinics of the selected hospitals.

At enrolment, after informed consent was sought from each respondent, 5µL of blood was collected from each patient, out of which 2µL were, dispensed each into sterile EDTA and vacutainer tubes bottles which were commercially available in diagnostic laboratories with EDTA as anti-coagulant. The remaining 1 µL of blood was used to prepare both thin and thick blood smear for malaria parasitemia, PCV and a drop on Whatmann filter paper. This longitudinal study was carried out among counseled and consented pregnant women who reported for routine antenatal check up in the selected hospitals. As delivery of the neonate, the umbilical cord was cut and clamped about 3 cm from the abdomen. Cord blood was collected by releasing the clamp and compression and used for parasitological studies. A standardized questionnaire was administered to all the volunteers to obtain information on age, use of IPT since gestation number of previous pregnancies (gravidity), gestation, history, income status, educational status, history of previous pregnancies e.t.c. all subjects were encouraged to deliver at the selected hospital.

#### Inclusion criteria

Pregnant women whose pregnancy was between 20-24 weeks were selected using a stratified-random sampling technique.

#### Exclusion criteria:

1. History of or active presence of a major or life threatening opportunistic infection
2. Active substance abuse which, might prevent compliance with the study's requirements
3. Non residents in the study area

4. Those that are not willing to comply with the study requirements

#### **1.4.2 Follow-up procedures**

Until delivery, each woman enrolled had a systemic monthly follow-up even until few months after delivery. At each antenatal and post partum visit, information on occurrence of symptoms, illness, and treatment taken since the last visit to the project was collected on standardized questionnaires and structured interview session. All women recruited received Insecticide Treated Mosquito Nets (LLINs) and free SP (IPT) according to the WHO recommendation.

#### **1.4.3 Laboratory techniques**

##### **Microscopy**

A rapid staining protocol using 30% Giemsa stain for thick smear for 15 minutes and Leishman stain was used for thin smear. Parasite counts standardized per 200 leukocytes was obtained from thick/thin blood films.

The number of parasites per microliter of blood was calculated by assuming an average white blood cell count of 8,000/ $\mu$ L. The preparation of both thick and thin blood films for confirmation of parasitemia followed the methods described by Cheesbrough (5).

A drop of immersion oil was placed on the stained thin film and another drop on thick film. They were systematically examined using X 100 objectives (oil immersion).

##### **HIV screening**

All subjects who consented to participate were systematically screened for the presence of HIV antibody by using Determine rapid HIV rapid test kits and/or UNI-Gold Rapid Test grouped into reactive and non-reactive samples according to (30).

#### **1.4.4 Ethics of the study**

The study was approved by the Joint Ethical Committee UI/UCH and the concerned hospitals. Other ethical considerations were obeyed as contained in the National code of Health research ethics (10). Each respondent was pre and post-counseled after informed consent was given.

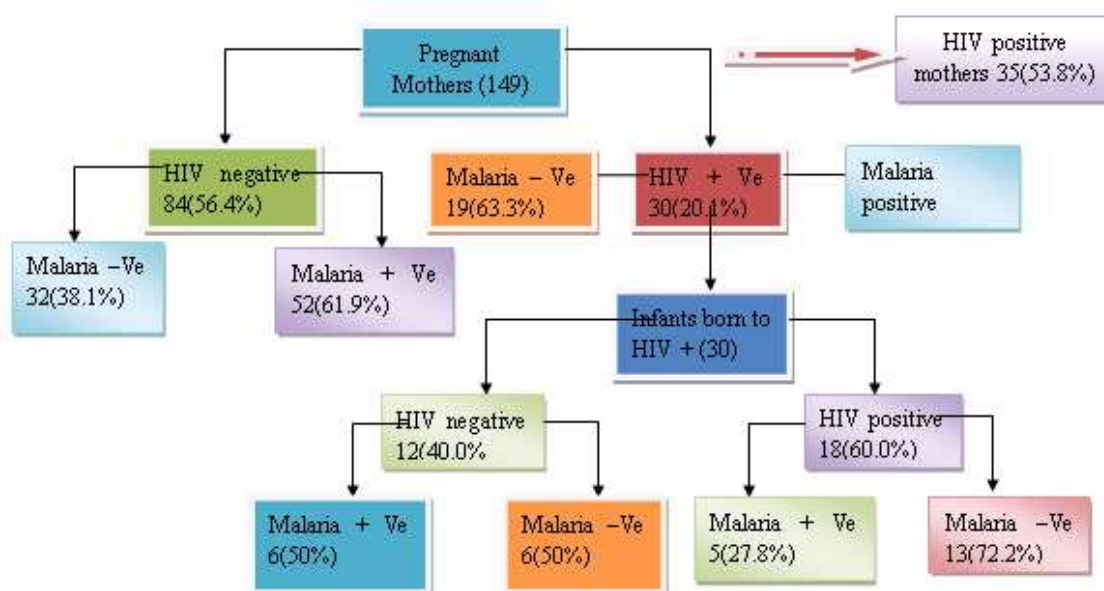
## **2.0 Results**

### **2.1 General characteristics of the study participants**

Most participants in the study were Yoruba 145(97.3%) other nationalities were 3(2.0%). The majority had Primary education 53(35%), 9(19.5%) Secondary while 3(26.2%) had no formal education, 49.0% belong to others category of employers while 12.8% were government employee, 29.5% were self employed whereas 2.0% were unemployed. The household size of the respondents ranged from 78.2% with less than 5 members and 21.3% who had more than 5 members. Among the respondents in this study, majority were of no salaried income status whereas the lowest percentage of 2.9% belong to high income status ( $>$ ₦20, 000 per month). Of the (149) mothers recruited, 32.9% (49/149) were primigravidae who had only one previous pregnancy, 27.5% (41/149) had more than one previous pregnancy (secungravidae). The remaining 39.6% (59/149) had three or more previous term pregnancies. Among the pregnant women recruited, 11.4% (17/149) were in their first three months (first trimester), mothers whose gestation age falls between 13 -24 weeks were 47.0% (70/149) while the remaining 42.6% (62/149) were in their last trimester. At delivery, 30 infants from HIV infected mother were enrolled and systematically followed up over a six-month period. For the purpose of data analysis of this study, all the 30 selected infants that fulfilled the inclusion criteria of at least one post delivery show up at the study site for sample collection and infant physical examination were recruited. At presentation, the maternal presumed symptoms were correlated with their infection status. Out of the 149 respondents who were interviewed, 16.4% (24/149) had fever, 16.4% (24/149) had chills 22.6% (33/149) had headache, 24.0% (35/149) complained of general body weakness whereas 14.4% (21/149) and 16.0% (23/149) were reported to experience restlessness within the last 24 hours of presentation. Other symptoms included nausea 14 (21/149), Coke-like urine 11.6% (17/149), other symptom 34.7 (41/149). Also, the study observed that 46.8% had received one dose of IPT (Laridox), 23.4% received the two recommended doses of Sulfadoxine – Pyrimethamine while the remaining 29.8% had not received IPT, majority of this class claimed ignorant of compulsory WHO recommended IPT policy for pregnant mothers.

We observed inadequate usage of other preventive measures in pregnancy because, an unacceptable 5% respondents slept under ITN whereas 25% claimed they had not seen LLINS before (Data not shown). From figure 1, Out of all respondents, 47.0% (70/149) mothers and 40.0% (12/30) infants were mono-infected. Of these, 12.8% (19/149) mothers and 33.3% (10/30) infants were infected with HIV only whereas 34.2% (51/149) mothers and 6.7% (2/30) infants had malaria only respectively. Out of 43 (24.0%) of the participants that were co-infected with malaria and HIV, 22.8% (24/149) and 30.0% (9/30) were co-infected mothers and infants respectively. Out of the 179 participants, 30.2% (54/179) had neither HIV nor malaria. Among the non-infected group 30.2% (45/149) were mother while 30.0% (9/30) were infants. Figure 1 clearly shows the recruitment profile of the study to document the prevalence of infections in mothers and their infants. Babies born to thirty-five of the HIV-positive were not included in the study for their unwillingness to comply with the requirements of the study.

Figure 1:



Schematic flowcharts showing the prevalence of all categories of infection status of all subjects

### 3.0 Discussion

This study reported high parasitemia in the study population. The prevalence of malaria parasites in HIV negative mothers 61.9% (52/34) corroborates several studies that have document malaria as a major public health problem affecting between 300-500 million people annually (31, 20,) and a major cause of maternal and infant morbidity and mortality in Sub-Saharan Africa (11, 24). This study agrees with the data on the burden of malaria in pregnancy, in particular from areas of low transmission per-inborn site of Kampala by Namusoke and others. Although their study obtained blood smear from three measure, namely peripheral, placental smear and placental histology. Also, the prevalence of malaria parasite in infants recruited from this study 36.6% (11/30) corroborates early studies that suggest that each year between 100,000 to 300,000 infant deaths may be attributable to malaria during pregnancy (13,19). This study also document 50% (6/12) prevalence of *falciparum* malaria in HIV negative infants is critical to health policy for effective management and control of malaria during pregnancy so as to prevent poor birth outcome and mother-to-child from transmission of malaria. The prevalence of HIV infection in this study in both pregnant mothers 43.6% (65/149) and infant 60.0% (18/30) suggest mother-to-child transmission of HIV. This observation was corroborated by other workers from different parts of the country, which suggest increased HIV infection in Nigeria since early 1990s (14, 7). The first national surveillance of HIV infection among pregnant women attending clinics was in 1991 (7, 8, 9). This study corroborate earlier studies by (22, 3) who documented the prevalent rate of HIV in Ibadan and Saki. The median rate of infection among the antenatal attendees in different parts of the country

increased from 1.8% in 1991, to 4.5% in 1995, 5.4% in 1999 and 5.8% in 2001. This study reported a median rate of HIV transmission to validate earlier studies. The high 60% (18/30) prevalence of HIV in babies born to HIV infected mother in this study however validate existing data on rates and timing of mother-to-child transmission of HIV (14). In the work of (16) who presented that HIV can be transmitted in inters, intrapartum and during breast feeding (26). The study predicts mother-to-child from transmission is possible through breast feeding and intra-partum. This study reported 22.8 (34/149) of co-infection rate in mothers and 30.0% (9/149) in infants. This validate the result of several studies the established overlap of malaria and HIV in pregnant mothers (15, 2, 27, 28). In particular, Perrault *et al*; 2009 reported mean HIV prevalence in pregnant women attending antenatal clinics in south Africa where he notes prevalence rate of 15% to 40% in south Africa but a lesser prevalence between 5% to 10% in East Africa (29). This study however could not validate or confirm perceived low rate of Co-infection of the two diseases as reported by Chandramohan and Greenwood (5), who did not demonstrate any evidence of an interaction between malaria and HIV (5). Later, it became progressively obvious that such interactions existed in areas where malaria was endemic.

Evidenced from this study was the impaired ability of HIV-infected pregnant women to control *Plasmodium falciparum* in agreement with the observation of (27). Compared with HIV negative mothers, this study observed a lower prevalence in HIV negative mothers than in HIV-positive mothers. This study did not document effect of Co-infection on anemia and poor birth outcome but this result of this study reported that infants born to Co-infected women seen to be at greater risk of developing anemia, intra-uterine growth retardation Low birth weight as reported by (21,23,12). This study proceeded to investigate the prevalence of HIV and malaria among different age group. This study observed a progressive decrease in parasite density in a month-by-month analysis of neonates, this however suggest active recruitment innate immune responses of clear parasitemia, few months after delivery as argued by (4). The infant mortality rate 16.7% (5/30) and 4/149 maternal reported in this study further corroborate a prospective study of antimalarial prophylaxis in over 4,000 mothers in malaria where the maternal mortality rate was 370 per 100,000 women and risk rate between 6 weeks to 1 year post partum was 341 per 100,000 per births (18). This study did not report any relationship between malaria in pregnancy and associated illness. The study could not confirm a study conducted in Cameroon who found no correlation between frequency of malaria in pregnancy between infants born to positive mothers (46.5%) and infant born to negative mothers (38.5%) during the first two years of life. In conformity study with earth by Namusoke (20), he documented general characteristics of pregnant mothers and related some selected characteristics with the prevalence of malaria during pregnancy. This study establishes no statistical by significant relationship between socioeconomic indices and transmission (prevalence) of both HIV and malaria in pregnancy. Although most studies that reported significant association between HIV transmission and socio economic status examined such relationship among young girls (non-pregnant). The study population consist 47(31.6%) primigravidae, 52(34.9%) secungravidae and 59(39.5%) multigravidae.

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