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## Placental Malaria histological features and the burden of congenital malaria among HIV/Malaria Co-infected Mothers in Benin City, Edo State

DOI:<http://dx.doi.org/10.4314/njp.v48i1.3>

Accepted: 16th April 2020

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**Abstract:** *Background:* It is well documented that sub-Saharan Africa bears the highest burden of both malaria and HIV. Co-infection with both diseases is also well documented. Malaria parasites infecting the placenta lead to inflammation, intervillous fibrin deposition and infarction. This pathologic effect of malaria on the placental has led to the staging of placental malaria histology. These pathologic features may reflect different levels in the breach of the integrity of the placenta which may predispose to transmission of congenital malaria and possibly HIV. But few if any have examined the association of maternal placental malaria histology stages in HIV positive and negative mothers and the effects of these on their newborns (congenital malaria).

*Methods:* Subjects were 162 newborns of HIV/malaria co-infected mothers and Controls were 162 newborns of HIV negative malaria infected mothers. Blood film for malaria parasites was done on cord blood and peripheral blood on days 1, 3 and 7 in the newborns. Maternal peripheral blood film for malaria parasite was done

at delivery and placental tissue was obtained for confirmation of placental malaria by histology. Diagnosis of malaria in blood films was by light microscopy.

*Results:* The placental malaria histology in HIV positive mothers were predominantly the chronic type (51.9%) and past type (54.6%) in HIV negative mothers respectively. Congenital malaria was significantly more in chronic types of placental malaria histology irrespective of maternal HIV status ( $p=0.017$  in subjects and  $p=0.000$  in controls respectively)

*Conclusion:* Babies born to mothers are at increased risk for congenital malaria if their placental malaria histology is of the chronic type compared to the other types (active and past) irrespective of maternal HIV status. This risk (chronic type) is highest in mothers with HIV; therefore, all babies born to HIV positive mothers should be screened for congenital malaria and managed as appropriate.

**Key words:** Placental malaria histology; HIV; congenital malaria; HIV/malaria co-infected mothers; Benin-City; Nigeria

### Introduction

It is well documented that sub-Saharan Africa bears the highest burden of both malaria and HIV.<sup>1,2</sup> Co-infection with both diseases is also well documented.<sup>3,4</sup> It has also been reported that HIV and malaria tend to have synergistic effect with each other<sup>5</sup>

The risk of transmission of malaria from mother to child is increased when the placenta is parasitized with malaria.<sup>6</sup> The prevalence of placental malaria is 10-45% in malaria endemic areas with significant *P. falciparum* dominance.<sup>6-8</sup>

Congenital malaria (CM) is the occurrence of malaria confirmed by the presence of asexual forms of malaria

parasite (MP) in the peripheral blood or cord blood smear within the first seven days of life irrespective of presence of clinical symptoms.<sup>9</sup> Congenital malaria contributes to increased morbidity and mortality in newborns.<sup>10</sup> Congenital malaria is a consequence of maternofetal transmission of the malaria parasite. Maternal malaria parasitaemia and placental parasitaemia are known risk factors for congenital malaria.<sup>9,11-12</sup>

Nigeria has the second highest burden of HIV/AIDS in Africa after South Africa<sup>13</sup> and third in the whole world after South Africa and India.<sup>13</sup> HIV infection in pregnancy is associated with higher rates of clinical malaria, higher malaria parasite density and a higher risk of ma-

ternal anaemia and low birth weight (LBW) in the neonates.<sup>14</sup>

Malaria parasites infecting the placenta lead to inflammation, intervillous fibrin deposition and infarction.<sup>15</sup> The pathological changes in the placenta can indicate : uninfected (no parasites or pigment), active (parasites in intervillous spaces), chronic (parasites in maternal erythrocytes and pigment in fibrin or cells within fibrin and/or chorionic villous syncytiotrophoblast or stroma) and past (no parasites but pigment confined to fibrin or cells within fibrin monocytes or/and macrophages).<sup>16</sup>

These pathologic features may reflect different levels in the breach of the integrity of the placenta which may predispose to transmission of congenital malaria and possibly HIV. Placental malaria has also been documented to cause up regulation of CCR5 receptors to which HIV bind, predisposing to increased replication of HIV in the placental bed which may also predispose to viral transmission due to the high viral load.<sup>5</sup>

Previous studies have examined placental malaria parasitaemia and congenital malaria. There is paucity of research on the features of maternal placental malaria histology stages in HIV positive and negative mothers and the effects of these on their newborns (congenital malaria). This is the knowledge gap that this research intends to unravel.

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## Methods

This study is part of a larger prospective, cross-sectional analytic study on congenital malaria in infants of mothers who are co-infected with malaria/HIV and a control group of mothers who were HIV negative but had malaria in pregnancy.

HIV positive and negative pregnant women were recruited from ANC clinic at the third trimester (at 36 week gestation) over a six months period (from June through November, 2013). Written informed consent was obtained during this first contact. At delivery maternal peripheral blood samples and placental tissue were obtained for testing for malaria. Mothers who had either malaria parasitaemia or placental malaria and who had live births were enrolled for the study. Mothers (HIV positive and negative) who were negative for maternal parasitaemia and placental malaria, who had diabetes, sickle cell anaemia, those on treatments for malignancy, prophylactic or therapeutic cotrimoxazole were excluded.

At delivery, mother/baby pairs who met the inclusion criteria were enrolled consecutively from Central, UBTH and Stella Obasanjo Hospitals.

The study was carried out at the antenatal clinics (ANC), labour wards, Special Care Baby Units (SCBU) and maternity wards of the three public hospitals (University of Benin Teaching Hospital (UBTH), Central Hospital; and Stella Obasanjo Women and Children Hospital) providing medical care including antiretroviral therapy (ART) and prevention of mother to child transmission of HIV (PMTCT) services to pregnant women and their newborns in Benin City. Pregnant women routinely un-

dergo HIV testing during ANC visits using the national algorithm and for those found to be positive CD4 counts are determined at diagnosis then 3monthly.<sup>17</sup> Socio-economic classification was determined using Olusanyan et al<sup>18</sup>

**Upper (I &II):** Professional plus secondary or university top civil servant, Business , politicians

**Middle (III):** Skilled artisans, technicians, well to do traders, middle level bureaucrat plus secondary school

**Lower (IV &V):** Unskilled workers, minimum wage plus primary school

**Ethical** approval for the study was obtained from the Ethics and Research Committee of the UBTH.

### *Blood Sampling and Laboratory Procedure Malaria parasite*

Thin and Thick Films for malaria parasite determination were prepared using standard procedure.<sup>19</sup> Confirmation of malaria parasite infection was by microscopy using Giemsa staining technique. Parasite species was determined by examination of thin slide while parasite density was determined from thick blood smears.

### *Placental Malaria Histology*

Placental tissues were processed using standard procedure.<sup>16</sup> The presence of asexual forms of malaria parasites within the red blood cells in the intervillous spaces and/or malaria pigments either ingested by the macrophages and monocytes in the intervillous spaces or pigments within the fibrin (placental parenchyma) in the histology slides was considered positive. Placental malaria infections were classified based on the classification of Bulmer et al.<sup>16</sup> as: uninfected (no parasites or pigment), active (parasites in intervillous spaces), chronic (parasites in maternal erythrocytes and pigment in fibrin or cells within fibrin and/or chorionic villous syncytiotrophoblast or stroma) and past (no parasites but pigment confined to fibrin or cells within fibrin monocytes or/and macrophages).

### *Determination of HIV Antibodies*

The HIV status of each of the pregnant mothers was determined according to the WHO standardized serial testing algorithm and the national algorithm.<sup>17</sup>

### *Follow-Up*

Newborns whose malaria parasite tests were negative from umbilical cord blood analysis had further sample drawn on the first, third and seventh days of life. If an earlier sample is positive further sampling was discontinued. A baby was reported as negative for congenital malaria if all four blood samples (cord, days 1,3 and 7) were negative for malaria parasites.

All newborns that had positive malaria parasite test in any of the four blood samples (umbilical cord, days 1, 3 and 7 of life) were reported as having congenital ma-

laria. They were treated with oral quinine at a dose of 10mg/kg/dose given eight hourly for seven days. Subsequently, they were followed up routinely at the Well Baby clinics. Results of positive malaria parasite infection in mothers were made available to the attending obstetrician.

All babies born to HIV infected mothers were commenced on prevention of mother to child transmission protocol of Nevirapine and Zidovudine. However during the conduct of the study most babies received Nevirapine only because of Zidovudine was not available. Infant feeding counselling was also given and they were then referred to the UBTH Paediatric HIV clinic for DNA-PCR testing between the 6<sup>th</sup> and 8<sup>th</sup> week of life.

*Data analysis*

Data were entered into the SPSS spreadsheet. The data was analyzed using SPSS version 20.0 (SPSS for Window Inc; Chicago, LL, USA) Statistical software. Continuous data were summarized as mean ( $\pm$ SD), while categorical data was presented as proportions. Fisher's exact test or Pearson's chi-square was used to compare categorical data while student's t test was used to assess for differences between two means. The level of significance of each test was set at  $p < 0.05$  at 95% confident interval.

**Result**

Two hundred and eighty (280) HIV positive mothers were recruited during ANC. Of these, 252(90%) delivered in the three hospitals, 162(64%) were found to have malaria late in pregnancy. Ninety (90) women had no peripheral or placental malaria and were subsequently excluded. The infants of these 162 HIV/malaria co-infected mothers were recruited as subjects.

Four hundred and twenty five (425) HIV negative mothers were recruited during ANC. Of these, 361(85%) delivered in the three hospitals. 162(45%) were found to have malaria. One hundred and ninety nine (199) women were subsequently excluded. The infants of these 162 HIV negative mothers who had malaria in pregnancy were recruited as controls

The placental malaria histology stages for 162 HIV positive and negative mothers with the peripheral blood films of their newborns where analysed,

*Demographic and Clinical Characteristics of Study Participants*

There were 78(48.1%) male and 84(51.9%) female neonatal subjects; while controls (neonates) were 81(50.0%) males and 81(50%) females. Study participants' baseline characteristics are as shown in Table 1. The mean age of mothers of subjects of  $31.93 \pm 4.26$  years was significantly different from that of the controls (HIV negative mothers with malaria in pregnancy) of  $29.98 \pm 5.00$  years ( $p$ -value=0.000; Table 1).

The youngest mother was 18 years while the oldest was

43 years. Among the 162 mothers of subjects 101(63%) were in upper class, 15(9.3%) in middle class and 45 (27%) in the lower class and this socio-economic distribution was significantly different from what was observed among mothers of controls where 64(39.5%), 12 (7.4%) and 86(53.1%) were in upper, middle and lower classes respectively ( $p$ -value=0.000; Table 1).

There was also a significant difference in the distribution of marital status between mothers of subjects and of controls with mothers of subjects being more likely to co-habit. Among mothers of subjects 30(18.5%) were co-habiting, 132(81.5%) married and none was single as compared to mothers of controls where 14(8.6%), were co-habiting, 146(90.1%) married and 2(1.2%) single ( $p$ -value=0.010; Table 1).

Among mothers of the subjects 109(67.3%) had parities of 1-2 while 53(32.7%) had parities of three or more. This distribution in parity was significantly different from what was observed in controls where 99(56.2%) and 71(43.8%) had parities of 1-2 and 3 or more respectively ( $p$ -value=0.040<sup>a</sup>; Table 1).

**Table 1: Socio-demographic and clinical characteristics of study participants**

| Maternal characteristics       | Subjects n (%)    | Controls n (%)   | Test statistics | df | p-value             |
|--------------------------------|-------------------|------------------|-----------------|----|---------------------|
| <i>Marital status</i>          |                   |                  |                 |    |                     |
| Co-habiting                    | 30(18.5)          | 14(8.6)          | 8.156           | 2  | *0.010 <sup>a</sup> |
| Married                        | 132(81.5)         | 146 (90.1)       |                 |    |                     |
| Single                         | 0(0.0)            | 2(1.2)           |                 |    |                     |
| <i>Tribe</i>                   |                   |                  |                 |    |                     |
| Bini                           | 62(38.3)          | 71(43.8)         | 1.872           | 3  | 0.599 <sup>c</sup>  |
| Esan                           | 37(22.8)          | 38(23.5)         |                 |    |                     |
| Estako                         | 31(19.1)          | 23(14.2)         |                 |    |                     |
| Others                         | 32(19.8)          | 30(18.5)         |                 |    |                     |
| <i>Parity</i>                  |                   |                  |                 |    |                     |
| 1 – 2                          | 109(67.3)         | 99(56.2)         | 4.233           | 1  | *0.040 <sup>e</sup> |
| >3                             | 53(32.7)          | 71(43.8)         |                 |    |                     |
| <i>Religion</i>                |                   |                  |                 |    |                     |
| Christianity                   | 161(99.4)         | 160 (98.8)       | 0.336           | 1  | 1.000 <sup>a</sup>  |
| Muslim                         | 1(0.6%)           | 2(1.2%)          |                 |    |                     |
| <i>SES</i>                     |                   |                  |                 |    |                     |
| Upper class                    | 102(63.0)         | 64(39.5)         | 21.86           | 2  | *0.000 <sup>e</sup> |
| Middle Class                   | 15(9.3)           | 12(7.4)          |                 |    |                     |
| Lower Class                    | 45(27.8)          | 86(53.1)         |                 |    |                     |
| Maternal age                   | 31.93 $\pm$ 4.26  | 29.98 $\pm$ 5.00 | 3.841           | 32 | *0.000 <sup>b</sup> |
|                                | 6                 | 00               |                 | 2  |                     |
| <i>Newborn characteristics</i> |                   |                  |                 |    |                     |
| <i>Gender</i>                  |                   |                  |                 |    |                     |
| Male                           | 78(48.1)          | 81(50.0)         | 0.111           | 1  | 0.739 <sup>c</sup>  |
| Female                         | 84(51.9)          | 81(50.0)         |                 |    |                     |
| Gestational age                | 38.52 $\pm$ 1.191 | 38.70 $\pm$ 2.02 | 1.003           | 32 |                     |
| Birth weight (kg)              | 2.87 $\pm$ 0.41   | 3.109 $\pm$ 0.62 | 4.035           | 32 | *0.000              |
| Length (cm)                    | 48.63 $\pm$ 2.12  | 48.30 $\pm$ 3.74 | 0.951           | 32 | 0.343 <sup>b</sup>  |
| OFC (cm)                       | 34.54 $\pm$ 1.91  | 34.92 $\pm$ 3.24 | 1.388           | 32 |                     |
|                                | 1                 | 24               |                 | 2  | 0.166 <sup>b</sup>  |

<sup>a</sup>Fisher's exact, <sup>b</sup>Student's t-test; <sup>c</sup>Pearson chi-square; \*Significant at  $p < 0.05$

*Placental malaria histology staging for mothers of subjects and controls*

Of the 162 mothers with HIV infection (mothers of subjects) 46(28.4%) had the “active stage” of placental malaria histology. 84(51.9%) had the “chronicstage“, 32 (19.7%) had the “past stage”. Mothers who were HIV negative (mothers of controls) 25(15.5%), 48(29.6%) and 89(54.9%) had “active”, “chronic” and “past” stages respectively, p-value 0.000; Table 2

**Table 2:** Placental malaria histology staging for mothers of subjects and controls

|         | Subjects n(%) | Controls n(%) | Total n(%) |
|---------|---------------|---------------|------------|
| Active  | 46(28.4)      | 25(15.5)      | 71(21.9)   |
| Chronic | 84(51.9)      | 48(29.6)      | 132(40.7)  |
| Past    | 32(19.8)      | 89(54.9)      | 121(37.3)  |
| Total   | 162(100)      | 162(100)      | 324(100.0) |

Group  $\chi^2 = 42.88$ ;  $p = 0.000$

*Placental malaria histology stage and prevalence of congenital malaria*

Congenital malaria was observed in 22(39.3%), 26 (46.4%) and 8(14.3%) of subjects born to mothers with “active”, “chronic” and “past” histology stages of placental malaria.  $p=0.017$ ;Table 3. 8(22.2%), 19(52.8%) and 9(25.0%) of controls had “active”, “chronic” and “past” histology stages of placental malaria had congenital malaria.  $p$ -value=0.000; Table 3

**Table 3:** Placental malaria histology and prevalence of congenital malaria in the study groups

| Group   | Histo-logical staging | Congenital malaria |             | Total (%) | $\chi^2$ | df | p-value |
|---------|-----------------------|--------------------|-------------|-----------|----------|----|---------|
|         |                       | Present n(%)       | Absent n(%) |           |          |    |         |
| Subject | Active                | 22 (52.4)          | 20 (47.6)   | 42 (100)  |          |    |         |
|         | Chronic               | 26 (29.5)          | 62 (70.5)   | 88(100)   | 8.16     |    |         |
|         | Past                  | 8(25.0)            | (75.0)      | (100)     | 9        | 2  | 0.017   |
|         | Total                 | 56                 | 106         | (100)     |          |    |         |
| Control | Active                | 8(32.0)            | 17 (68.0)   | 25 (100)  |          |    |         |
|         | Chronic               | 19 (39.6)          | 29 (60.4)   | 48 (100)  |          |    |         |
|         | Past                  | 9(10.1)            | 80 (89.9)   | 89 (100)  | 17.3     | 2  | 0.000   |
|         | Total                 | 36                 | 126         | 162 (100) |          |    |         |

Pearson chi-square; Significant at  $p < 0.05$

*Prevalence of congenital malaria in cord blood and peripheral blood films at days 1,3 and 7 among subjects and controls*

The incidence of congenital malaria was significantly higher at the third day (day 3) of life among subjects

compared to controls ( $p=0.000$ ; Table 4). Overall, incidence of congenital malaria was lowest 2/92 (2.2%) on the seventh day of life (day 7). Incidence of congenital malaria in the cord blood and first day of life were different but the differences were not statistically significant ( $p > 0.05$ ; Table 4) in both subjects and controls.

*Anthropometry of newborns with and without congenital malaria*

The mean birth weight of  $2.89 \pm 0.40$ kg and mean length  $48.38 \pm 1.45$ cm observed in subjects with congenital malaria compared with  $3.26 \pm 0.62$ kg and  $47.61 \pm 5.33$ cm seen in controls with congenital malaria was not statistically significant, ( $p > 0.05$ ; Table 5). Moreover,  $3.07 \pm 0.62$ kg and  $48.50 \pm 3.14$ cm noted among controls without congenital malaria and  $2.86 \pm 0.43$ kg and  $48.75 \pm 2.40$ cm seen in subjects without congenital malaria was also not significant ( $p > 0.05$ ; Table 5). The mean OFC of  $34.64 \pm 2.89$ cm seen in subjects with congenital malaria and  $34.89 \pm 4.15$ cm noted in those without congenital malaria was also not statistically significant ( $p$ -value  $> 0.05$ ; Table 5).

*Use of insecticide treated nets (ITN) in pregnancy and congenital malaria amongst participants*

Congenital malaria amongst subjects whose mothers used ITN was 3(5.4%) and 53(94.6%) for those whose mothers did not use ITN but the difference was not statistically significant ( $\chi^2 1.96$ ;  $p=0.161$ ) Table 6. Similarly, amongst the controls whose mothers used ITN, congenital malaria was 5(13.9%) but this was not significantly different from 31(86.1%) for those whose mothers did not use ITN ( $\chi^2 = 1.86$ ;  $p=0.173$ ) Table 6. Use of intermittent preventive therapy (IPT-sp) in pregnancy and prevalence of congenital malaria amongst participants

Congenital malaria amongst subjects whose mothers used IPT-sp in pregnancy, 3(5.4%) was not significantly different from 53(94.6%) for those that did not use IPT-sp ( $\chi^2=1.551$ ;  $p=0.213$ ) Table 6. Similarly, congenital malaria amongst controls whose mothers used IPT-sp in pregnancy 12(33.3%) was also not statistically different from 24(66.7%) for those whose mothers did not use IPT-sp ( $\chi^2=0.190$ ;  $p = 0.663$ ) Table 6

*Mean peripheral blood malaria parasite density in subjects, controls and their mothers*

The mean malaria parasite density of subjects with congenital malaria of  $320.00 \pm 77.069/\text{mm}^3$  was significantly higher than the  $269.02 \pm 70.87/\text{mm}^3$  observed in controls with congenital malaria ( $t = 3.194$ ;  $p = 0.002$ ).

The mean peripheral blood malaria parasite density of HIV positive mothers (mothers of subjects) of  $1232.40 \pm 211.00/\text{mm}^3$  was significantly higher than that obtained in HIV negative mothers (mothers of controls) of  $1121.36 \pm 286.67/\text{mm}^3$  ( $t = 2.137$ ;  $p = 0.035$ )

**Table 4:** Prevalence of congenital malaria in cord blood and peripheral blood films at days 1, 3 and 7 among subjects and controls

| Type of blood sample | Subjects n(%) | Controls n(%) |
|----------------------|---------------|---------------|
| Cord blood           | 09(16.0)      | 10(27.8)      |
| Day 1                | 23(41.1)      | 21(58.3)      |
| Day 3                | 23(41.1)      | 04(11.1)      |
| Day 7                | 01(1.8)       | 01(2.8)       |
| Total                | 56 (100.0)    | 36 (100.0)    |

**Table 5:** Anthropometry of newborns with and without congenital malaria

| Anthropometric parameter | Congenital Malaria |            | t     | df  | p-value |
|--------------------------|--------------------|------------|-------|-----|---------|
|                          | Present            | Absent     |       |     |         |
| <b>Subjects</b>          |                    |            |       |     |         |
| Birth Weight(kg)         | 2.89±0.40          | 2.86±0.43  | 0.377 | 160 | 0.707   |
| Length(cm)               | 48.38±1.45         | 48.75±2.40 | 1.084 | 160 | 0.280   |
| OFC(cm)                  | 34.94±3.33         | 34.25±2.21 | 1.462 | 160 | 0.146   |
| <b>Controls</b>          |                    |            |       |     |         |
| Birth Weight(kg)         | 3.26±0.62          | 3.07±0.62  | 1.629 | 160 | 0.105   |
| Length(cm)               | 47.61±5.33         | 48.50±3.14 | 1.260 | 160 | 0.209   |
| OFC(cm)                  | 34.48±1.08         | 34.58±1.25 | 0.473 | 160 | 0.637   |

Student's t-test; Significant at p<0.05

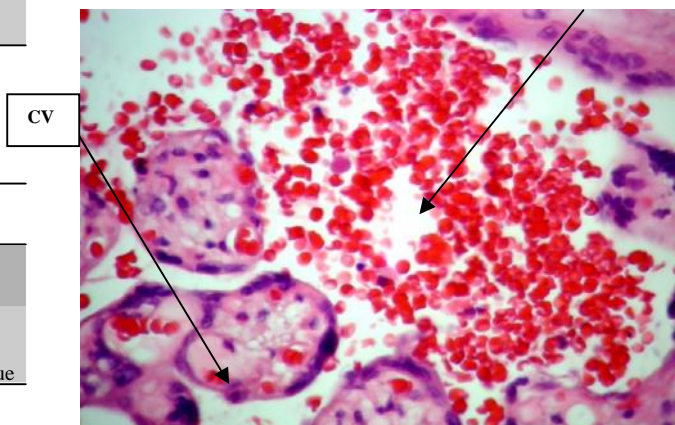
**Table 6:** Use of insecticide treated nets (ITN) in pregnancy and congenital malaria amongst participants

| Insecticide Treated Net (ITN) | Congenital malaria |              |            | c <sup>2</sup> | df | P value |
|-------------------------------|--------------------|--------------|------------|----------------|----|---------|
|                               | Present n (%)      | Absent n (%) | Total n(%) |                |    |         |
| <b>Subject</b>                |                    |              |            |                |    |         |
| Yes                           | 3(5.4)             | 13(12.3)     | 16(9.9)    | 1.96           | 1  | 0.161   |
| No                            | 53(94.6)           | 93(87.7)     | 146(90.1)  |                |    |         |
| Total                         | 56(100.0)          | 106(100.0)   | 162(100.0) |                |    |         |
| <b>Control</b>                |                    |              |            |                |    |         |
| Yes                           | 5(13.9)            | 31(24.6)     | 36(22.2)   | 1.86           | 1  | 0.173   |
| No                            | 31(86.1)           | 95(75.4)     | 126(77.8)  |                |    |         |
| Total                         | 36(100.0)          | 126(100.0)   | 162(100.0) |                |    |         |

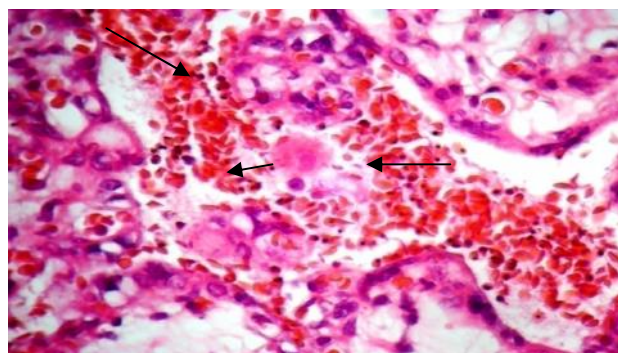
**Table 7:** Use of intermittent preventive therapy (IPT-sp) in pregnancy and prevalence of congenital malaria amongst participants

| Use of IPT-sp in pregnancy | Congenital malaria |             |            | c <sup>2</sup> | df | P-value |
|----------------------------|--------------------|-------------|------------|----------------|----|---------|
|                            | Present n(%)       | Absent n(%) | Total n(%) |                |    |         |
| <b>Subject</b>             |                    |             |            |                |    |         |
| Yes                        | 3(5.4)             | 12(11.3)    | 15(9.3)    | 1.551          | 1  | 0.213   |
| No                         | 53(94.6)           | 94(88.7)    | 147(90.7)  |                |    |         |
| Total                      | 56(100.0)          | 106(100.0)  | 162(100.0) |                |    |         |
| <b>Control</b>             |                    |             |            |                |    |         |
| Yes                        | 12(33.3)           | 47(37.3)    | 59(36.4)   | 0.190          | 1  | 0.663   |
| No                         | 24(66.7)           | 79(62.7)    | 103(63.6)  |                |    |         |
| Total                      | 36(100.0)          | 126(100.0)  | 162(100.0) |                |    |         |

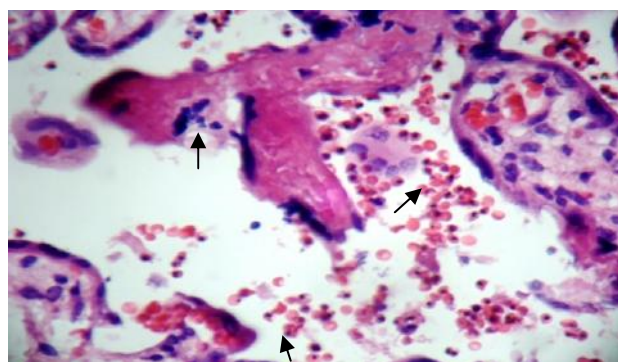
**Fig 1:** Normal (Uninfected) Placenta showing terminal chorionic villi (CV) and an intervillous space (IVS) packed with maternal erythrocytes.



**Fig 2:** active infection showing numerous parasitized erythrocytes in the intervillous space as indicated by the arrow

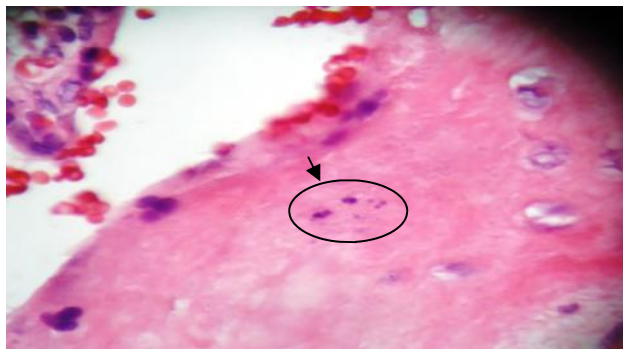


**Fig 3:** Chronic infection showing parasitized maternal erythrocytes within the intervillous space and haemozoin pigment in fibrin (arrow).

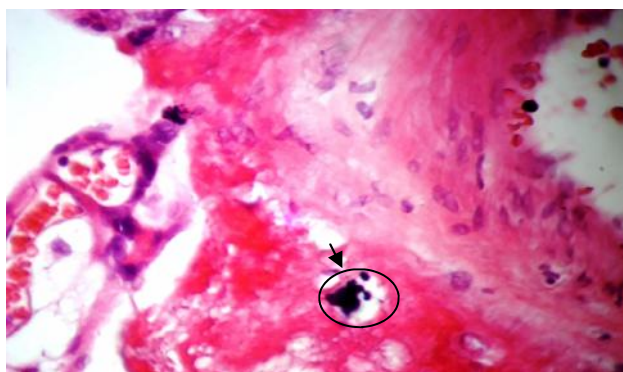




**Fig 4:** Past infection with haemozoin pigment confined to fibrin as shown in the encircled area above



**Fig 5:** False positive Placenta showing formalin artefact (encircled area above).



## Discussion

Placental malaria has been reported to be one of the risk factors associated with cord and congenital malaria. This study noted that the prevalence of congenital malaria was significantly more in the “chronic” stage of placental malaria histology in HIV exposed infants (subjects), compared to the other stages of placental malaria histology (“active” and “past”). Maternal placental malaria histology is an independent predictor of congenital malaria in newborns irrespective of maternal HIV status.<sup>20</sup> Most of the HIV positive women with malaria in pregnancy had the chronic histologic type of placental malaria. The babies of these mothers were also more likely to have congenital malaria. The researcher is not aware of any study that has evaluated this association.

The chronic stage of placental malaria histology represents the presence of asexual forms of *Plasmodium falciparum* infection of the erythrocytes within the intervillous spaces of the placenta and malaria pigments in the placental parenchyma indicative of continuous reinfection. The presence of these infected erythrocytes within the intervillous spaces of the placenta triggers influx of mononuclear cells, pro-inflammatory cells and cytokines into the intervillous spaces of the placenta. This increased congestion of the cells within the intervillous spaces of the placenta causes increased risk of placental injury and transmission of infected maternal blood to the foetus.<sup>21-22</sup>

The chronic histologic type of placental malaria is associated with more damage to the placenta than active and past types. The chronic histologic type of placental malaria is associated with a higher probability of transmission of malaria parasites to the baby.<sup>23-24</sup> These may explain the higher prevalence of congenital malaria in the HIV exposed infants (subjects) who had more chronic histologic type of placental malaria than HIV unexposed infants (controls) whose mothers had more of the past type.

Although placental malaria causes inflammatory changes by increased mononuclear cell infiltration in the intervillous space.<sup>25-26</sup> Placental malaria also causes a higher presence of CD4 presenting cells with increased replication of HIV-1 pro viruses in the intervillous space of the placenta. This may lead to further placental-tissue damage that could compromise placental perfusion. Moreover, in this study the mean malaria parasite was significantly higher in HIV positive mothers and their newborns when compared with the control group of HIV negative mothers and their newborns.

The exact mechanism responsible for this is not fully understood. Perhaps the continuous reinfection and chronic inflammation of the placental tissues following malaria and HIV infection have some roles to play especially in mothers with malaria and low CD4 cell counts. This study observed a significantly higher incidence of congenital malaria in the blood samples analyzed within the first three days of life while the least yield was at the seventh day of life for both subjects and controls. The reason for this is not fully understood but maybe because of the fact that some samples could be positive on day seven as observed in the study was the reason why congenital malaria is so defined<sup>9</sup>

This study also observed no significant differences in the prevalence of congenital malaria between male and female subjects and controls. This is in support of findings noted in previous studies that did not reveal any gender differential in cord malaria parasitaemia<sup>11, 14</sup> Malaria in pregnancy is known to be associated with some adverse pregnancy outcomes<sup>27</sup> such as intrauterine growth restriction. This could be made worse with HIV co-infection. In this study there was a significantly lower birth weight in subjects compared to controls and this may have been due to the additional effect of HIV infection.

Placental malaria causes inflammatory changes by mononuclear cell infiltration in the intervillous space<sup>21</sup> higher presence of HIV-1-presenting cells, leading to placental-tissue damage that may further compromise placental perfusion. This reduction in placental perfusion might eventually lead to reduced birth weight in the newborns delivered to HIV positive mothers co-infected with malaria. This is buttressed by the studies done by Ezechukwu et al.<sup>28</sup> and Sule-Odun et al.<sup>29</sup> who reported that malaria parasitaemia did not seem to affect the foetal birth weight. The observation of this study is similar to the study in 2013 by Cambrea et al.<sup>30</sup> who

reported that HIV in pregnancy causes reduction in newborn birth weight. The findings in this study suggest that subjects had significantly lower birth weight than controls. It was also noted in this study both subjects and controls with congenital malaria showed no significant difference to those without congenital malaria. This suggests that the significantly lower birth weight in HIV exposed compared to HIV unexposed infants is attributable to the maternal HIV status and not malaria.

This may be due to the effect of HIV in pregnancy as reported by Cambrea et al.<sup>30</sup> but the exact mechanism responsible is not fully understood. May be the chronic inflammation of the placental tissues following malaria and HIV infection have a role to play

The use of malaria preventive tools (ITN and IPT-sp) in this study was very low but there was no significant difference in the subjects and controls with congenital malaria whose mothers used any of the tools. Perhaps the use of these tools in this study is too low to make any significant inference.

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## Conclusion

HIV positive mothers have predominantly the chronic stage of placental malaria histology.

HIV negative mothers have significantly the past stage of placental malaria histology.

Congenital malaria is commoner in babies born to mothers with the chronic stage of placental malaria histology.

Newborns of mothers with HIV/Malaria co-infection have significant lower birth weight when compared to those of mothers who are HIV negative but had malaria in pregnancy.

The mean malaria parasite density is higher in mothers with HIV and their newborns with congenital malaria as compared to mothers who were HIV negative and their newborns with congenital malaria.

We recommend more research work on the association between placental malaria histology stage of mothers and the congenital malaria in the newborns in HIV positive and negative mothers.

## Acknowledgements

I wish to acknowledge Prof Kenneth Ekanem and Dr. V hriterhire Clement O. (Consultant Histopathologists) for their tutelage and assistance in reading the placental histology slides. I want to also acknowledge the contributions of the Histology laboratory scientist for training and helping me with placental tissue processing and placental histology slide preparation.

I am very grateful to Mrs Omijie Elizabeth (Chief Medical Laboratory Scientist, Microbiology laboratory) and Mr Oladipo, Head of the Research Laboratory of the Department of Child Health, University of Benin for their assistance in reading the slides and ensuring my active participation. I also appreciate my research assistants (Drs. Chison O, Dudu A, Osifo M, Oraifo S, Olubor A, Odili P) for their assistance in administering the questionnaires and helping with sample collection.

My special thanks go to the nurses and mid-wives in labour wards, ANC clinics, SCBU and Maternity wards at UBTH, Stella Obasanjo and Central hospitals respectively. To my colleagues in the Departments of Child Health and Obstetrics and Gynaecology I say thank you, and Mr. Nosa Micheal who assisted with data analysis.

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