

**EFFECTS OF MALARIA INFECTION ON THE HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF PREGNANT WOMEN IN ONITSHA, ANAMBRA STATE, NIGERIA**

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**Abstract**

**Background:** Malaria remains a major public health problem in sub-Saharan Africa especially among pregnant women and unborn infants.

**Aim:** The effects of malaria infection on the haematological and biochemical parameters of pregnant women were investigated in Onitsha, Anambra State, Nigeria.

**Materials and Methods:** Venous blood samples were collected from 1500 pregnant women who attended antenatal clinic, in Onitsha, Anambra State, Nigeria. The haematological and biochemical parameters of the malaria positive samples were analysed using automated analysers and Giemsa stained blood films. Data was analysed using Chi-square analysis at level of significance of  $p < 0.05$ .

**Results:** A total of 423 were positive for malaria. Haemoglobin and White blood cell (total and differential) counts were significantly associated with malaria infection ( $P < 0.05$ ). A total of 28 pregnant women (6.6%) who had malaria were found to be anaemic with highest prevalence of anaemia observed: among those less than 20years old (43.3%;  $p < 0.05$ ), in first trimester (20.0%;  $p < 0.05$ ), and among Secundigravidae (7.8%;  $p < 0.05$ ). Case wise, there were 68.3% leucocytosis and 1.4% leucopenia. Decreased platelet count was observed among malaria infected women, with 16(3.8%) having thrombocytosis while 24(5.7%) had thrombocytopenia. The biochemical parameters showed a significant difference between pregnant women with normal values and those with abnormal values ( $p < 0.05$ ). A total of 24(5.7%) of them had elevated ALT, 19(4.5%) had elevated AST, 15(3.6%) had elevated ALP and 32(7.6%) had elevated total bilirubin. Pregnant women less than twenty years old had the highest prevalence 9(30.0%) of elevated

ALT, 7(23.3%) of elevated AST, 6(20%) of elevated ALP and 11(36.7%) of elevated total bilirubin.

**Conclusion:** Malaria in pregnancy is a major public health problem affecting women. Pregnant women below 20 years of age are particularly vulnerable to the disease and its associated morbidities including decreased haemoglobin (Hb), platelets counts, full blood counts, resulting in increased risk for anaemia.

**Keywords:** *Placental malaria, liver function test, low birth weight, Plasmodium falciparum*

### **Introduction**

Malaria remains a major public health problem in sub-Saharan Africa especially among pregnant women<sup>1</sup>. The World Health Organization (WHO) reported 241 million cases and 627 thousand deaths from malaria in 2020<sup>2</sup>. Malaria in pregnancy has severe consequences for the woman and her unborn infant. An infant born to a mother with malaria is more likely to have low birth weight (LBW), which is the single greatest risk factor for death during the first few months of life. The risk of maternal death also increases considerably as a pregnant woman suffering from malaria is likely to develop severe maternal anaemia<sup>3,4</sup>. Parasites in peripheral blood without symptoms are commonly seen in hyper endemic areas and usually associated with Placental sequestration and chronic anaemia<sup>5</sup>. Approximately one in four pregnant women present with malaria infection during delivery and this has been evidenced by findings from clinical studies, suggesting that placental malaria is common<sup>6</sup>. There is increased susceptibility to malaria and other infections during pregnancy due to the suppression of the immune system<sup>7</sup>.

Haematological changes are common complications of malaria in pregnancy. These changes involve the major cell lines such as red blood cells, leucocytes and thrombocytes. Haematological abnormalities such as anaemia, thrombocytopenia and

leukocytosis or leucopenia have been observed in patients with malaria<sup>8</sup>. The biochemical parameters of pregnant women are also impacted especially if the woman is having malaria. In pregnancy, liver synthesis and metabolic functions are affected by increased serum estrogen and progesterone levels. Increased metabolic and physiological stress of pregnancy, could cause previously been subclinical liver disorders to become symptomatic<sup>9</sup>. Women who are infected with malaria parasite during pregnancy are at risk of miscarriage, still birth, low birth weight of the foetus, maternal anaemia and intra-uterine growth retardation<sup>4</sup>. It therefore became imperative that regular malaria parasite test be included in the routine tests for pregnant women, and liver function and haematological parameters are assayed for pregnant women who test positive for malaria, to enable better management of the condition and prevent adverse consequences. This study was hence carried out to ascertain the impact of malaria infection on haematological and liver function parameters of pregnant women in Onitsha metropolis.

### **Materials And Methods**

#### **Study Area**

Onitsha is an urban city, a commercial, educational and religious centre as well as a river port on the Eastern bank of the River Niger in Anambra State, Southeastern

Nigeria. It has an estimated population of 1,003,000 people<sup>10</sup>. Onitsha lies between the latitudes 6°7'N and 6°47'E and longitudes 6°17'N and 6°78'E in the rainforest belt of Nigeria. Onitsha town is situated about 5 miles (8km) Northeast of Asaba and 25 miles (40km) Southeast of Awka, the capital city of Anambra State. Onitsha experiences two distinct seasons - a wet season which begins in March/April and ends in October or early November with annual rainfall range of 2000mm to 3000mm and about four/five months of dry season which lasts from November to February/March. The daily temperature ranges from 22°C to 36°C. The indigenous people of Onitsha are Igbo and they speak Igbo language. The occupational groups in Onitsha include traders, civil servants and artisans.

#### **Study Population and Sample size**

A total of 1500 pregnant women attending clinic at Holy Rosary Specialist Hospital and Maternity, Onitsha, and General Hospital, Onitsha both in Anambra State volunteered and participated in the study. Sample size formula  $n = N/1+N(e^2)$  was used to determine sample size for the study<sup>11</sup>.

#### **Study Design**

The study design was a cross sectional study of pregnant women that attended clinic at Holy Rosary Specialist Hospital and Maternity Onitsha and General Hospital Onitsha within a period of 12 months.

#### **Ethical Consideration**

Ethical approval for the study was obtained from the ethical committee of Anambra State Hospitals Management Board (SHMB/AD196/VOL.V/124) and the ethical committee of NnamdiAzikiweUniversityTeachingHospital Nnewi NAUTH/CS/66/VOL.14/VER.3/294/2021/067). The informed consent of the pregnant mothers was obtained through careful

explanation of the project intentions and advantages.

#### **Sample Collection**

A total of 1500 blood samples were collected for the study. From each participant, 5ml of blood was withdrawn. 2ml of blood was dispensed into an Ethylenediaminetetra-aceticacid (EDTA) container; the remaining 3ml was transferred to a clean dry test tube. The sample in EDTA container was used for thick and thin blood film preparation, and full blood count test. The samples in the clean dry test tube were used for Liver function test (LFT).

#### **Preparation of Thick Blood Films, Staining and Examination**

Thick blood films were prepared, stained and examined following standard haematological techniques<sup>12</sup>.

#### **Haematological assay**

Automated haematology analyzer by Mindray BC-5300 was used to analyse haematological parameters of haemoglobin concentration, platelet count, total white blood cell and differential count.

#### **Biochemical Assays (Liver Function Test)**

The liver function tests were determined using automated mindray chemistry analyzer (BS-230).

#### **Analysis of Data**

Chi-square analysis was used to compare the association among the different groups for significant difference. 95% level of confidence was used. Questionnaire was also used for other relevant information.

### Results

A total of 28(6.6%) pregnant women who had malaria were found to be anaemic. The highest prevalence 13(43.3%) of anaemia was observed among those <20 years. No case 0(0.0%) of anaemia was observed among those who are 41+ years as shown in Table 1. Prevalence of anaemia in relation to age was statistically different among the age groups ( $P<0.05$ ). The highest prevalence 15(20.0%) of anaemia was observed in pregnant women in their first trimester while the least 7(2.8%) was observed in pregnant women in their third trimester. There was a statistical difference in the prevalence of anaemia in relation to trimester ( $P<0.05$ ). The highest prevalence 8(7.8%) of anaemia was observed in secundigravidae while the least 15(6.1%) was observed in the primigravidae. Prevalence in relation to gravidity was not statistically different ( $P>0.05$ ).

Malaria infection in pregnant subjects affected their Total WBC counts (TWBC) (Table 2). Of the 423 infected subjects, 128(30.3%) had TWBC within the normal range, while the remaining 295 subjects recorded 289(68.3%) leukocytosis and 6(1.4%) leucopenia. The difference between normal and abnormal TWBC were statistically significant ( $P<0.05$ ) across the age groups. Age group <20 recorded highest percentage 24(80.0%) of abnormal count while age group 41+ recorded the least 5(62.5%).

A total of 383(90.5%) had normal count of thrombocyte while 40(9.5%) had abnormal

thrombocyte count. Malaria-infected pregnant women that were 41+ years and above had the highest cases of abnormal thrombocytes 5(62.5%) while the age group 21 - 30 had the least cases of abnormal thrombocytes 12(5.2%) as shown in (Table 3). Statistical analysis showed a significant difference in the platelet count of malaria-infected pregnant women studied across the age groups. ( $P<0.05$ ).

Table 4 showed a total of 399(94.3%), 404(95.5%) and 408(96.4%) had normal ALT, AST and ALP values respectively, while 24(5.7%), 19(4.5%) and 15(3.6%) had abnormal ALT, AST and ALP values respectively. A total of 391(92.4%) and 395(93.4%) had normal value of total bilirubin and direct bilirubin respectively, while 32(7.6%) and 28(6.6%) had abnormal total bilirubin and direct bilirubin values respectively. Age group (<20years) recorded the highest percentage abnormality for all the liver enzymes assayed; ALT - 9(30.0%), AST - 7(23.3%), ALP - 6(20.0%), Total Bilirubin - 11(36.7%), Direct Bilirubin - 10(33.3%), while the least percentage abnormality, 0 (0.00%) for ALT, AST and ALP were recorded in age group (41+years), but for Total and Direct bilirubin age group (21-30years) recorded least abnormality 11(4.7%) and 9(3.8%) respectively. The differences in the liver function parameters across the age groups in malaria infected pregnant women were statistically significant at ( $p<0.05$ ).

**Table 1: Prevalence of anaemia in relation to age, trimester and gravidity of the malaria infected pregnant women studied.**

<i>Variables</i>	<i>Number examined</i>	<i>Number positive (%)</i>	<i>Number anaemic (%)</i>	<i>Number not anaemic (%)</i>
<b>Age (years)</b>				
<20	48	30 (62.5)	13(43.3)	17(56.7)
21-30	774	234(30.2)	10(4.3)	224(95.7)
31-40	600	151(25.2)	5(3.3)	146(96.7)
41+	78	8(10.3)	0(0.0)	8(100.0)
(P<0.05, $\chi^2$ =172.99, df = 3, P-value=0.000)				
<b>Trimester</b>				
First	300	75(25.0)	15(20.0)	60(80.0)
Second	429	99(23.1)	6(6.1)	93(93.9)
Third	771	249(32.3)	7(2.8)	242(97.2)
(P<0.05, $\chi^2$ =20.46, df =2, P-value=0.000)				
<b>Gravidity</b>				
Primigravidae	630	246(39.0)	15(6.1)	231(93.9)
Secundigravidae	330	102(30.9)	8(7.8)	94(92.2)
Multigravidae	540	75(13.9)	5(6.7)	70(93.3)
(P>0.05, $\chi^2$ =4.08, df=2, P-value=0.130)				
<b>Total</b>	<b>1500</b>	<b>423(28.2)</b>	<b>28(6.6)</b>	<b>395(93.4)</b>

**Table 2: Total WBC count (TWBC) of malaria infected pregnant women in relation to age**

Age (years)	No. Inf. (%)	No. with Normal range of TWBC (Leucocyte) (%)	No with abnormal TWBC count (%)	No. normal TWBC (Leucocytosis) (%)	No. below normal range TWBC (Leucocytopenia) (%)
<20	30(62.5)	6(20.0)	24(80.0)	21(70.0)	3(10.0)
21-30	234(30.2)	65(27.8)	169(72.2)	168(71.8)	1(0.4)
31-40	151(25.2)	54(35.8)	97(64.30)	96(63.6)	1(0.7)
41+	8(10.3)	3(37.5)	5(62.5)	4(50.0)	1(12.5)
<b>Total</b>	<b>423(28.2)</b>	<b>128(30.3)</b>	<b>295(69.7)</b>	<b>289(68.3)</b>	<b>6(1.4)</b>

(p<0.05,  $\chi^2$  =29.21,df=3, p-value=0.000).

**Table 3: Platelet count of malaria infected pregnant women in relation to age**

Age (years)	No. (%)	Inf.	No. with Normal range of Platelets (%)	No. with abnormal platelet count (%)	No. above normal range of platelets (Thrombocytosis) (%)	No. below the normal range of platelets (Thrombocytopenia) (%)
<20	30(62.5)		16(53.3)	14(46.6)	4(13.3)	10(33.3)
21-30	234(30.2)		222(94.9)	12(5.2)	6(2.6)	6(2.6)
31-40	151(25.2)		142(94.0)	9(6.0)	3(2.0)	6(4.0)
41+	8(10.3)		3(37.5)	5(62.5)	3(37.5)	2(25.0)
<b>Total</b>	<b>423(28.2)</b>		<b>383(90.5)</b>	<b>40(9.5)</b>	<b>16(3.8)</b>	<b>24(5.7)</b>

( $p < 0.05$ ,  $\chi^2 = 91.72$ ,  $df = 6$ ,  $p\text{-value} = 0.000$ ).

**Table 4: Liver function parameters of malaria infected pregnant women in relation to age**

Age (years)	No examined	No. inf. (%)	No. with normal range of ALT (5-35 IU/L) (%)	No with abnormal range of ALT (%)	No. with normal range of AST (0-35 IU/L) (%)	No with abnormal range of AST (%)	No. with normal range of ALP (41-133 IU/L)	No with abnormal range of ALP (%)	No. with normal range of Total Bilirubin (2-12µmol/L)	No with abnormal range of total bilirubin	No. with normal range of Direct Bilirubin (<12µmol/L)	No with abnormal range of direct bilirubin
<20	48	30(62.5)	<b>21(70.0)</b>	<b>9(30.0)</b>	23(76.7)	7(23.3)	24(80.0)	6(20.0)	19 (63.3)	11(36.7)	20(66.7)	10(33.3)
21-30	774	234(30.2)	224(95.7)	10(4.3)	227(97.0)	7(3.0)	229(97.9)	5(2.1)	223(97.9)	11(4.7)	225(96.2)	9(3.8)
31-40	600	151(25.2)	146(96.7)	5(3.3)	146(96.7)	5(3.3)	147(97.4)	4(2.6)	142(94.0)	9(6.0)	144(95.4)	7(4.6)
41+	78	8(10.3)	8(100.0)	0(0.0)	8(100.0)	0(0.0)	8(100.0)	0(0.0)	7(87.5)	1(12.5)	6(75.0)	2(25.0)
<b>Total</b>	<b>1500</b>	<b>423(28.2)</b>	<b>399(94.3)</b>	<b>24(5.7)</b>	<b>404(95.5)</b>	<b>19(4.5)</b>	<b>408(96.4)</b>	<b>15(3.6)</b>	<b>391(92.4)</b>	<b>32(7.6)</b>	<b>395(93.4)</b>	<b>28(6.6)</b>

### Discussion

Various hematological abnormalities have been associated with the prevalence of malaria. They include anemia, leucopenia, leucocytosis, thrombocytosis and thrombocytopenia<sup>13,14</sup>. The results of this study showed that the presence of malaria infection in the subjects studied brought about some increases/ decreases on some hematological parameters evaluated.

This study showed that haemoglobin (Hb) was significantly lower among younger age groups in the malaria-infected women. This could be attributed to destruction of both

parasitized and unparasitized erythrocytes that take place in the spleen during malaria infection<sup>15</sup>. Haemoglobin is the major biomolecule that is metabolized by the malaria parasite. This results in destruction of red blood cells, a condition known as anaemia. This finding is in line with the reports of which indicated that infected patients tended to have significantly lower haemoglobin and red blood cell count<sup>13,16</sup>. The World Health Organization has stated that anaemia is present in pregnancies when Haemoglobin concentration is less than 11 g/dL<sup>17</sup>.



The overall prevalence of anaemia among pregnant women in this study is lower than that reported in other studies<sup>18,19</sup>. The factors strongly associated with anaemia in this study such as age, trimester and gravidity; are similar to those reported in many other studies in different geographical locations<sup>19</sup>. Pregnant women less than 20 years of age were observed from this study to have the highest prevalence of anaemia than women in the other age bracket although pregnant women that were 41+ years and above had no cases of anaemia. This may be because teenage pregnancy is usually unintended, unplanned, sometimes outside wedlock in our setting with associated poor nutrition, poor health-seeking behaviour and poor antenatal care. Compared with similar studies, a decrease in haemoglobin of pregnant women in relation to trimester was shown in this study<sup>16,20</sup>. Pregnant women in their first trimester were found to be more anaemic than others in their second trimester and third trimesters.

This is in agreement with findings from similar studies which reported that pregnant women in their first trimester were more anaemic than those in their second and third trimesters.<sup>21</sup> Nevertheless, Wogu et al.,<sup>22</sup> reported progressive decline in haemoglobin (Hb) concentration from the first to third trimester, has been reported and attributed it to an increase demand for iron as pregnancy progresses. More iron is required to meet the expansion of maternal Hb mass and the needs of fetal growth. In relation to gravidity, it was observed in this study that secundigravids have a higher prevalence of anaemia than the primigravids and multigravids. This finding is in line with Akinbami et al.,<sup>23</sup> who reported that women with at least one previous birth or pregnancy were more likely to have anaemia than women who have not given birth. On the contrary it has also been observed that prevalence of anaemia was highest among

primigravidae and decreased with subsequent pregnancies and hence complication rates were higher in primigravidae as compared to multigravidae patients<sup>24</sup>.

Other haematological abnormalities / changes were linked to malaria prevalence in pregnancy and include leucocytosis and leucopenia. In this study leucocytosis was found higher than leucocytopenia among malaria infected pregnant women. However, Obebe et al.,<sup>25</sup> reported an elevation of leucocytes in malaria parasitized pregnant women in agreement with the findings in this study. Nlinwe et al.,<sup>26</sup> affirmed that leucocytosis occurring during pregnancy may be due to the physiologic stress induced by the pregnant state. However, contrary to this, some researchers observed leukopenia in their various studies<sup>27</sup>. This study showed low percentage of thrombocytopenia as one of the haematological changes in malaria parasitaemia. This is in line with observations of a significant reduction in the platelet counts of more than half of their study population<sup>26</sup>. Similar findings of increased thrombocytopenia among malaria subjects were reported in Kenya<sup>8</sup>. The plausible reason could partly be due to haemodilution and partly due to increased platelet activation and accelerated clearance.

This study showed an elevated value of the liver enzymes (biochemical parameter) in some of the malaria infected pregnant women especially the <20 years old. Malaria infection may cause liver disease of varying severity evident in the levels of serum aminotransferases at some stages of the infection<sup>27</sup>.

This increased activities of liver enzymes observed among malaria infected ones in this study could be as a result of leakage of transaminases and alkaline phosphatase from parenchymal and hepatocytes



membrane respectively into the circulatory system or exchanged cord blood between the mother and the child as reported by Bawah et al.<sup>28</sup>.

### **Conclusion**

Malaria in pregnancy is a major public health problem affecting women in Onitsha, Anambra State, Nigeria. Pregnant women below 20 years of age are particularly vulnerable to the malaria infection and its associated morbidities including decreased haemoglobin (Hb), resulting in increased risk for anaemia. Therefore, there is need to conduct malaria parasite test, full blood count test and liver function test on pregnant women attending antenatal to ensure prompt management and prevention of adverse consequences.

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**Competing Interest:** Authors declare that there exists no conflict of interests.

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