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Impact of Malaria Parasitaemia on Haematologic Parameters in Pregnant Women at Booking in Ilorin, Nigeria

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Abstract: We determined the pattern of haematological parameters in malaria in pregnancy. Five hundred pregnant women who presented for booking in the antenatal clinic of the University of Ilorin Teaching Hospital, Ilorin, Nigeria were recruited. Their blood samples were analyzed for malaria parasites, MCV, MCHC and MCHC, WBC, absolute RBC count, platelet count, haemoglobin level and PCV. The prevalence of peripheral malaria parasitaemia at booking was 32.4%, out of which 46.9 and 53.1% had moderate and high parasite densities, respectively. *Plasmodium falciparum* was the only species identified. The MCHC was higher in malaria parasitaemia (34.28 g dL^{-1}±1.72, p = 0.0452) while RBC was higher without parasitaemia (10.46×10^{12} L^{-1}±1.30, p = 0.01). The WBC in women with high parasite density (7.02×10^{9} L^{-1}) was higher than the value in those with moderate parasite density (6.26×10^{9} L^{-1}) p = 0.03). Asymptomatic *Plasmodium falciparum* infection in pregnancy is associated with higher MCHC; WBC increases with rise in the parasite density in Ilorin, Nigeria. A rise in MCHC and WBC in pregnancy may indicate malaria.

Key words: Booking, haematological, malaria, Nigeria, parameters, pregnancy

INTRODUCTION

The blood is an important tissue in man. Haematological parameters are useful in making diagnosis of diseases and also help in the antenatal assessment of women in pregnancy. The physiological changes of pregnancy, due to the influence of hormones such as oestrogen, progesterone also affect haematological indices when compared with the non-pregnant state. The picture is further influenced by race, geographical location, age, environmental factors and the prevalence of infectious diseases such as malaria.

Studies have reported on the prevalence of peripheral and placental parasitaemia in areas of stable endemic malaria transmission in Africa. A review of studies from countries in Africa, gave a median prevalence of malaria infection in pregnancy as 27.8% (Steketee *et al*., 2001). On average, one in four pregnant women in areas of stable transmission in Africa has evidence of malaria infection at the time of delivery (Desai *et al*., 2007). This is based on the estimated prevalence of 26% of placental malaria (Guyatt and Snow, 2004).

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In Sub-Saharan Africa, *Plasmodium falciparum* infection of the placenta remains a major challenge among pregnant women (Uneke, 2008) and its prevalence is influenced by maternal age, gravidity, use of malarial prophylaxis, nutrition, host genetics, level of host's immunity, parasite genetics and transmission rates (Tako *et al*., 2005). The maternal and fetal effects of malaria are enormous in the tropics. These include miscarriages, stillbirths, preterm labour and deliveries, anaemia and severe malaria among others (Steketee *et al*., 2001).

Maternal anaemia is the commonest consequence of *Plasmodium falciparum* malarial infection (Uneke, 2008). In Sub-Saharan Africa, it is estimated that between 200,000 and 500,000 pregnant women develop severe anaemia as a result of malaria (Steketee *et al*., 2001). Anaemia is usually multifactorial in origin and although, malaria is an important contributor; nutritional deficiencies, hookworm, HIV infections and genetic red blood cell disorders (Sickle cell and thalassemias) are other important contributing factors (Shankar, 2000).

Excessive removal of non-parasitized erythrocytes, immune destruction of parasitized red cells and impaired erythropoiesis as a result of bone marrow dysfunction are few of the different mechanisms through which malaria may cause anaemia (Ekvall, 2003). The hypersplenism in malaria infection is associated with a reduction in all three blood series that is, causing not only anaemia, but also thrombocytopaenia and leucopaenia (Fleming, 1989a, b).

The severity and type of anaemia can be determined by the levels of haematological indices such as haemoglobin concentration, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH). Although, many studies have reported on anaemia as a complication of malarial infection in pregnancy, the pattern of these blood indices has not been documented. There is also dearth of information on the effect of pregnancy on other blood cells in the presence of *plasmodium falciparum* in Nigeria. Given the known complications of malaria in pregnancy even in the asymptomatic state there is need to determine the effect of malaria parasitaemia on various blood indices on pregnancy. Hence, this study examines the effect of malaria parasitaemia on haematological parameters in pregnancy.

MATERIALS AND METHODS

The study was conducted at the antenatal clinic of the University of Ilorin Teaching Hospital, Ilorin, Nigeria between March and June 2006. It attends to the population in the metropolis, surrounding towns and communities. It also serves as a referral centre for health facilities in the middle belt and parts of South-Western Nigeria. It is located in the North-Central geopolitical zone, an area of stable transmission of malaria all year round.

The Climate and Vegetation

The climate is tropical with guinea savanna vegetation. It has two distinct seasons, a characteristic rainy season between May and October with high rainfalls in June and August. The dry season is from December to February; completely devoid of rains.

The Study Population

Pregnant women who presented for booking for antenatal care were studied. Those who provided informed consent after counseling were enrolled into the study. Five hundred women were randomly recruited. Information on socio-demographic characteristics, obstetric and medical history and drug use were obtained by resident doctors in obstetrics and

gynecology, alongside one of the investigators. The height, weight and vital signs of the participants were measured and their complete medical and obstetric examinations were also done.

Sample Collection and Evaluation

Five milliliter of blood was collected by venepuncture using aseptic technique from each participant into two EDTA bottles. One part was used for the determination of haematological parameters while the other was used for the diagnosis of malaria.

Determination of Haematological Indices

Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCHC), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cell Count (WBC), absolute RBC count, platelet count, haemoglobin level and Packed Cell Volume (PCV) were determined using Sysmex KX 21 automated cell counter.

Diagnosis of Malaria

Thick and thin films were prepared and stained with Giemsa stain for parasite identification and quantification as described by Warhurst and Williams (1996). Parasite density was determined by counting the number of asexual parasites relative to at least 200 leucocytes in each thick blood film and assuming a mean leucocytes count of 800 μL^{-1} of blood (Trape, 1985). Parasitaemia was graded as low (parasite <1000 μ L^{-1}), moderate (>1000-9,999 μ L^{-1}) and high (>10,000 μ L^{-1}). This was done by a trained and experienced microscopist.

Data were entered into Microsoft excel data sheet. Statistical analysis were done using the Epi-info statistical software version 2005. Associations between variables were tested using Chi-square for discrete variables and the student t-test for continuous variable. Analysis of variance tests were used to compare means of the haematological parameters. Level of statistical significance was designated at p-values less than or equal to 0.05 ($p<0.05$).

RESULTS

A total of five hundred respondents were recruited into the study and their blood samples were analyzed. The mean age (SD) of the women was 28.5 (±5.19) years while 45.6% were 26-30 years old; pimiparous women constituted 59%.

Three hundred and thirty six respondents had tertiary education and 2.4% (12) had no formal education. A majority of the respondents (97%) were married, 293 women (58.6%) booked in the second trimester, while 56 (11.2%) and 151 (30.2%) booked in the first and third trimesters, respectively. The socio-demographic characteristics of these women are shown in Table 1.

One hundred and sixty two (32.4%) women had malaria parasitaemia, out of which 96.3% were not febrile. *Plasmodium falciparum* was the only malaria species found in this study. The levels of the malaria parasite density were categorized into low, moderate and high densities as shown in the methodology. Among the infected women, none had low parasite density, while 46.9 and 53.1% had moderate and high parasite densities, respectively. Table 2 shows the values of the haematological parameters of the study population. The mean values of the haematological parameters were within reference levels expected in pregnancy, this is shown in Table 2.

Table 1: Socio demographic characteristics of the participants

Variables	Freq (%) N = 500
Age	
<19	19 (3.8)
20-25	107 (21.4)
26-30	228 (45.6)
31-35	97 (19.4)
36-40	40 (8)
>40	19 (3.8)
Parity	
Nulliparous	8 (1.6)
Para 1	295 (59)
Para 2	113 (22.6)
>Para 3	84 (16.8)
Level of education	
None	12 (2.4)
Primary	39 (7.8)
Secondary	110 (22)
Tertiary	336 (67.2)
Marital staus	
Single	15 (3)
Married	485 (97)
Gestational age	
First trimester	56 (11.2)
Second trimester	293 (58.6)
Third trimester	151 (30.2)

Table 2: Ranges and means of haematological parameters

Parameters	Range	Mean	SD
MCV	26-101	82.25fl	±8.21
MCHC	22-39	34.03 g L^{-1}	±1.93
MCH	20-38	28.67 pg	±2.7
WBC	1.8-17	6.78×10^{9} L^{-1}	±2.21
Platelets	4.8-386.0	179.61×10^{9} L^{-1}	±69.98
Hb conc.	3.3-20	10.53 g L^{-1}	±1.80
RBC	2-37	4.19×10^{12} L^{-1}	±2.60

Table 3: Comparisons of the mean values of parameters with malaria parasitaemia

Parameters	Means± SD	t-statistics	p-value
MCV			
Parasitaemia + ve	82.57±8.84	0.37	0.5412
Parasitaemia - ve	82.09±7.90		
MCHC			
Parasitaemia +ve	34.28±1.72	4.03	0.0452
Parasitaemia - ve	33.91±2.02		
MCH			
Parasitaemia +ve	28.93±2.63	2.32	0.1282
Parasitaemia - ve	28.54±2.89		
WBC			
Parasitaemia +ve	6.64±2.22	0.81	0.3687
Parasitaemia - ve	6.83±2.21		
Platelets			
Parasitaemia +ve	172.44±72.46	2.52	0.1134
Parasitaemia - ve	183. 05±68.61		
Hb			
Parasitaemia +ve	10.69±2.00	1.78	0.1824
Parasitaemia - ve	10.46±1.70		
RBC			
Parasitaemia + ve	4.61±4.14		
Parasitaemia - ve	10.46±1.30	6.10	0.01

Table 3 shows the comparisons between the means of the haematological parameters and peripheral malarial parasitaemia in the pregnant women. The MCHC was higher in the

Table 4: The relationship between degree of parasite density and the mean values of the parameters

Parameters	Parasite density	Mean±SD	t-statistics	p-value
MCV	Moderate	82.67±8.78		
	High	82.47±9.3	0.02	0.8887
MCHC	Moderate	34.28±2.24		
	High	34.20±1.98	0.06	0.8092
MCH	Moderate	29.22±2.38		
	High	28.80±2.72	1.08	0.2999
WBC	Moderate	6.26±2.10		
	High	7.02±2.28	4.81	0.03
Platelet	Moderate	173.50±68.87		
	High	183.98±65.65	0.98	0.3232
Hb	Moderate	10.98±1.79		
	High	10.60±2.04	1.57	0.2119
PCV	Moderate	31.93±4.43		
	High	31.45±4.45	0.47	0.4929
RBC	Moderate	4.00±0.99		
	High	5.14±5.56	3.1	0.08

parasitaemic group than the non- parasitaemic group and the difference was statistically significant ($p = 0.0452$). There was also a significant correlation between RBC and parasitaemia ($p = 0.01$). The mean values of other hemoglobin and red cell indices were higher in the parasitaemic group than the non- parasitaemic group. Conversely, platelet and WBC counts were higher in those without malaria parasites than the infected women.

The levels of parasite density and its effects on the haematological parameters are shown in Table 4.

The mean value of WBC of $7.02\times10^{9}\ L^{-1}$ in women with high parasite density was higher than the value of $6.26\times10^{9}\ L^{-1}$ in the moderate and the difference was statistically significant ($p = 0.03$).

DISCUSSION

The pattern of peripheral malaria infection in this study agrees with findings from other studies on malaria in the Sub-Saharan region, where *Plasmodium falciparum* is the commonest species of the parasite (Uneke, 2008). Also, *Plasmodium falciparum* infection during pregnancy is usually asymptomatic and remains undetected and untreated despite the presence of the parasite in the placenta (Steketee *et al*., 2001). In this study, 3.7% of the women were symptomatic. The MCHC was significantly lower in pregnant women without peripheral parasitaemia than those with the parasite even though both groups had mean values within normal limits. The MCHC is an index of haemoglobin concentration per red cell, indicating the oxygen carrying capacity of each red cell. The inverse relationship between MCHC and malaria parasitaemia is unexpected knowing that anemia is the commonest complication of *Plasmodium falciparum* infection, nevertheless, the significant lower absolute count of RBC in the infected women was in consonance (Uneke, 2008). Probably, the higher MCHC in the parasitaemic women is compensatory for the presence of the parasite at the red cell level and seemingly lower value in the non-infected women was the expected norm from the haemodilutional effect of pregnancy. Both groups had mean values that demonstrated normochromia. Thus, a rise in MCHC in pregnancy may be indicative of malaria infection. More studies are needed on the effect of *Plasmodium falciparum* on MCHC to generate a definite conclusion on this finding.

The mean values of WBC count in both infected and non infected women were within normal reference range probably because the participants were evaluated at booking.

Whereas during labour and puerperium, WBC may be more markedly elevated (Onwukeme, 1992). Nevertheless, this compared favorably well with the findings of other workers in Jos and Ibadan, Nigeria (Onwukeme and Uguru, 1990; Akingbola *et al*., 2006) although, they did not control for malarial infection but rather compared findings in pregnant and non pregnant women.

We found a significant increase in the mean values of WBC as the level of peripheral malaria parasitaemia increased. This was at variance with an expected decrease in the WBC count and possibly leucopaenia, as a result of depressed cellular immunity from the combined effect of malaria and pregnancy. In addition, Mckenzie *et al*. (2005) demonstrated that WBC count was lower in *Plasmodium falciparum* infection than *Plasmodium vivax* infection and non-infected individuals. Without particular reference to pregnancy, wickramasinghe and others showed that abnormalities in the blood changes in malaria are dependent on immunity, age, chronicity and the type of plasmodium infection (Wickramasinghe and Abdalla, 2000). This may explain the finding in this study.

Severe malaria caused by *Plasmodium falciparum* can cause thrombocytopaenia and rarely DIC (Abdalla, 2004) pregnancy is also associated with profound alterations in the fibrinolytic and coagulation systems. *Plasmodium falciparum* caused a lower platelet count in pregnant women in this study, when compared with those without peripheral parasitaemia. The difference was not statistically significant and the mean values were within normal range. In area of unstable malaria, platelet counts were significantly lower in patients with an episode of *falciparum* or *vivax* malaria compared to healthy pregnant women and symptomatic women infected by *P. falciparum* also had thrombocytopaenia more commonly (Tan *et al*., 2008). The reduced platelet count in malaria is said to be due to platelet activation, splenic pooling and a decreased platelet life span (Abdalla, 2004; Beale *et al*., 1972).

In conclusion, this study describes the pattern of blood parameters in asymptomatic *plasmodium falciparum* infection of the peripheral blood in pregnancy. There was normochromia with or without the infection but MCHC increased with peripheral parasitaemia. Similarly, asymptomatic *P. falciparum* was associated with normal WBC count in pregnant women at booking and total white cell count increased with parasite density.

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