FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 7 No. 3, June (Special Issue), 2023, pp 283 - 287
DOI: https://doi.org/10.33003/fjs-2023-0703-1870

HAEMATOLOGICAL CHANGES IN MALARIA INFECTION AMONG PREGNANT WOMEN IN SOKOTO METROPOLIS NIGERIA

[*1]Garba, I., [1]Umar, A. I., [2]Tijjani, M. B., [2]Aliyu, M. S., [2]Muhammad, H. I. D., [3]Raji, M. I. O., [1]Fana, S. A., and [1]Thompson, J.

[1]Department of Medical Microbiology, School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria
[2]Department of Microbiology, Faculty of Life Science, Ahmadu Bello University, Zaria, Nigeria
[3]Department of Pharmaceutics and Pharm. Microbiology, Usmanu Danfodiyo University, Sokoto, Nigeria

*Corresponding authors' email: ibrahimzurmi@yahoo.com Phone: +2348065378378

ABSTRACT

One of the most fatal illnesses that affects humans is malaria. This study was aimed at determining the haematological changes in pregnant malaria-infected women. One hundred blood samples were taken from pregnant women between the ages of 15 and 45. Samples were screened using standard methods. Out of 100 pregnant women examined, 28% had parasites whereas 72% did not. Based on age, women between the ages of 36 and 40 had the highest prevalence of malaria during pregnancy (33.3%). The haematological parameters of pregnant women with malaria infection and those who were not infected did not differ significantly, according to a Student T-test. Pregnant women's mean Packed cell volume was 28.43% compared to pregnant women who weren't sick of malaria. The mean value of Red blood cell in infected pregnant women $3.45x10^{12}$/l was lower than the non parasitaemic pregnant women $3.53x10^{12}$/l, the mean value of White blood cell $6.89x10^{9}$/l was in the normal range for both infected and non-infected pregnant women, Red cell indices (MCH, MCHC) were low 27.00pg, 33.80pg in malaria infected pregnant women than non-infected pregnant women 28.23pg, 34.35pg, the mean value of MCV(82.02fl) was higher in pregnant women than the non-infected pregnant women (81.83fl), the mean Platelet value ($217.1x10^{9}$/l) was high in infected pregnant women than non-malaria pregnant women ($213.1x10^{9}$/l). Neutrophil and Lymphocyte were (60.92%, 27.49%) low in malaria infected pregnant women than the non parasitaemic (61.69%, 28.73%). Screening for malaria and haematological alterations in pregnancy will help in reducing the scourge of malaria infection.

Keywords: Haematological, Changes, Malaria infection, Pregnant women, Sokoto

INTRODUCTION

One of the worst diseases in the world, malaria primarily affects people in tropical and subtropical areas of the globe. It continues to be the most intricate and daunting health issue that humanity is currently confronting (Ekwunife *et al*., 2011). Obligate intracellular protozoa of the genus *Plasmodium* are responsible for causing malaria. An important public health issue, malaria infection during pregnancy poses serious hazards to the unborn child, the pregnant woman, and her fetus (WHO, 2016). The widely used and practically applicable approach for determining the density of malaria parasites is microscopic examination using thick blood films stained with Giemsa, which continues to be the reference standard for parasite identification. Usually, it varies according to the species (Boyce *et al*., 2015).

Haematological abnormalities are among the most frequent malaria sequelae and they significantly contribute to the disease's pathogenesis (UNICEF, 2009). These alterations affect the main cell types, including thrombocytes, leucocytes, and red blood cells (Ovakporaye, 2011; Imoru *et al*., 2013).

Malaria is an infectious disease that is transmitted to humans by the bites of infected female Anopheles mosquitoes, sometimes known as "malaria vectors," and is brought on by a eukaryotic Protista of the genus Plasmodium. The two most dangerous parasite species, *P. falciparum and P. vivax*, are among the five that cause malaria in humans (WHO, 2019). Nigeria's population 97% is at risk for malaria, with the remaining 3% living in the country's malaria-free highlands (WHO, 2012). Malaria is endemic across Nigeria, with seasonal variations in the various regions of the nation. Beyond its effects on children and pregnant women, malaria impacts the entire population, with more than 90% of the population at risk and at least 50% experiencing at least one episode annually (WHO, 2012).

One of the most typical malarial consequences is haematological change, and plays a significant part in the pathogenesis of malaria. Major cell types such as Red Blood Cells, leucocytes, and thrombocytes are affected by these modifications (Maina *et al.,* 2010). Patients with malaria typically had significantly lower platelets, WBCs, lymphocytes, Eosinophils, RBCs, and Hb levels than patients without malaria, although their monocyte and neutrophil counts were significantly higher (Bakhubaira, 2013). People who live in malaria-endemic areas may find it simple and helpful to obtain these haematological abnormalities for diagnostic purposes (Manas *et al.,* 2014).

MATERIALS AND METHODS

Study design

This is a cross sectional study that was carried out on one hundred and nine (109) pregnant women for the determination of haematological changes associated with malaria in Sokoto metropolis.

Sample size

The sample size was determined using the formula:

$$n=\frac{Z^2pq}{d^2}$$

Where
n = number of samples (sample size)
z = standard normal deviate at 95% confidence interval=1.96
p = previous prevalence 7.7% (Agomo *et al*., 2009) =0.077
d = degree of confidence at 0.05

q = 1-p =1-0.077=0.923
Therefore n =3.8416x0.077x0.923/0.0025=109

Inclusion criteria
Pregnant women within the age 15-45 years having malaria infection attending ante-natal clinic in Specialist Hospital and Maryam Abacha Hospital and who consented to the study were included.

Exclusion criteria
Pregnant women with no clinical symptoms of malaria or are on antimalarial drugs and those who gave no consent were excluded.

Ethical consideration
Ethical approval for this study was obtained from institutional Ethical review committees of the selected hospitals.

Blood Sample Collection
For venous blood collection, a sterile five milliliter (ml) syringe was used to collect five (5ml) of blood, dispensed into an EDTA container, and properly mixed as described by Cheesbrough, (2006).

Preparation of thin blood film
On a labelled, spotless glass slide that had no oil, a drop of thoroughly mixed blood was inserted. A clean spreader with a smooth edge was used; it was brought back to touch the blood and allowed to extend along the spreader's edge. Before staining, the blood film is allowed to air dry while the spreader is held at an angle of 45°. The spreader is then softly but forcefully moved along the horizontal slide to form a thin smear.

Preparation of thick blood film
After cleaning the finger's lobe with 70% v/v alcohol and allowing it to air dry, a sterile lancet was used to prick the finger and squeeze it gently to obtain a significant amount of blood. A large drop of blood was spread to make a thick smear that measured roughly 15x15mm on a labelled clean grease-free glass slide. The blood was then allowed to air dry before being stained.

Staining procedure for thick blood film
Giemsa stain (3%) was made and applied to the thick blood smear, allowing it for 30 minutes on a staining rack. The stain was then removed off the film using buffered distilled water (pH 7.2), and the slide's underside was cleaned before being placed on a rack to dry naturally.

Staining procedure for thin blood film
The thin blood film that had been air-dried was fixed in methanol. After letting it dry, it was then flooded with a 10% Giemsa solution, stained for 30 minutes before being rinsed with water and allowed to air dry.

Microscopic examination of blood film
The stained thick and thin blood films were screened at a low magnification (x10 and x40 objective lens) to detect suitable field with even distribution of the white blood cells. The smear was then examined using x100 objective with a drop of oil immersion.

Estimation of Parasite Density
Malaria parasites were calculated using assumed white blood cell count of 8000/µl, which has been accepted as reasonably accurate in estimating malaria parasite densities by the WHO.

Method for estimating WBC
WBC count x parasites counted against 100 WBC /100

Determination of haematological parameters
The blood sample was analysed using an automated Mythic 22 CT analyser, the automated machine has two channels for cell counting, in one channel red blood cell and platelet are analysed and in the other white blood cell are analysed. Extra channels are used for the differential cell counting and reticulocyte count. The parasite density was determined using (Number of parasites counted/WBC counted) × WBC count/µl of participant.

Statistical analysis
Statistical analysis was performed using SPSS version 20.0. Data are presented as mean ±SD and percentage. Statistical comparison between groups were carried out using independent sample T test and p-value ≤ 0.05 was taken as statistically significant.

RESULTS AND DISCUSSION

Results
Table 1 shows the prevalence of malaria parasite among pregnant women. Pregnant women between the ages of 36 and 40 had the highest prevalence (33.3%) of malaria parasite, whereas pregnant women between the ages of 31 and 35 had the lowest prevalence (11.1%). Malaria infection was prevalent in pregnant women aged 15 to 25 by 32.7%, but not in those aged 41 to 45, where it was 0.00%. Based on age, the chi square test indicates that there is no significant difference in the prevalence of malaria infection among pregnant women ($p > 0.05$).
Table 2 shows prevalence of malaria among pregnant women based on gravidity. The highest prevalence of 40.3% was observed among pregnant women that have had multiple child birth while the lowest prevalence of 33.3% was observed among pregnant women having their first child.
Table 3 shows mean malaria parasite density among pregnant women based on gravidity. The highest mean malaria parasite density of 379parasite/µl was observed among pregnant women that have had multiple child birth while the lowest mean malaria parasite density of 336parasite/µl was observed among pregnant women having their first child.
Table 4. shows prevalence of malaria among pregnant women based on trimesters. The highest prevalence of 33.3% was observed among pregnant women in their first trimester while the lowest prevalence of 16.7% was observed among pregnant women in their third trimester. The prevalence of 32.8% was observed among pregnant in their second trimester.
Table 5 shows the mean malaria parasite density among pregnant women based on Trimesters. The highest mean malaria parasite density of 380parasite/µl was observed among pregnant women in their third trimester while the lowest mean malaria parasite density of 240parasite/µl was observed among pregnant women in their second trimester was 330parasite/µl.
The haematological indices of malaria positive pregnant women compared with malaria negative pregnant women in Sokoto metropolis was determined. The mean ±SD of haematological parameters (HCT, PLT, RBC, WBC, MCV, MCH, MCHC, NEUTRO and LYMPH) of malaria positive and negative pregnant women are shown in table 6.

Discussion
In sub-Saharan Africa, malaria is still a serious public health concern, and the use of malaria preventative measures may have an effect on the incidence of the disease during

pregnancy (Tongo *et al*., 2011). According to Boel *et al*. (2012), pregnant women are more vulnerable to malaria than non-pregnant ones. The results of this study showed that 28% of pregnant women had malaria. This may be because of the unsanitary conditions in the study area, which put communities at risk for stagnant water bodies that serve as mosquito vector breeding grounds. Repeated exposure to the vector can also be a source of malaria transmission in pregnancy.

Due to their repeated births, which may have compromised their immune systems and exposed them to ongoing malaria parasite attacks, pregnant women aged 36 to 40 are more likely to contract the parasite than other age groups. The results of this study are in contrast to those of studies conducted in Ebonyi, Nigeria, by Alo *et al.* (2014), who found that pregnant women between the ages of 26 and 30 had a high prevalence of the malaria parasite. The difference in malaria prevalence may be due to variations in methods of analysis, geographical regions and the physiological and or immunological states of the pregnant women examined.

In this study, pregnant mothers who have given birth to several children have the highest malaria prevalence. This may be related to delayed prenatal care at the end of the first trimester or the beginning of the second trimester, antimalarial chemoprophylaxis due to concern that the drug may not have any effect on the fetus, and seasonal variance. In contrast, Saidu *et al.* (2015) reported a malaria prevalence of 38.3% in Sokoto State in their study.

In the first trimester the highest prevalence of malaria was observed with a mean malaria parasite density of 240parasite/µl, in second trimester 32.8% was observed with a mean malaria parasite density of 330parasite/µl while 16.7% was observed in the third trimester with a mean malaria parasite density of 380parasite/µl, It is possible that pregnancy-related immune suppression, which may be brought on by the loss of acquired immunities, is to blame for the high prevalence of malaria in these trimesters which is in contrast with the findings by Saidu *et al*. (2015), who reported high prevalence in first trimester followed by second trimester in Sokoto.

Contrary to findings by Fana *et al.* (2015), who reported high prevalence between pregnant women that are Primigravidae followed by Multigravidae in Kebbi State, Nigeria, in this study, the highest prevalence of 40.3% was observed in pregnant women that are multigravidae with a mean parasite density of 379parasite/l, and 33.3% was observed in pregnant women in primigravidity with a mean malaria parasite density of 336parasite/l. This may be attributed to pregnancy-related immune suppression, which may be brought on by the loss of acquired immunities.

There was no statistically significant difference ($p > 0.05$) in the mean value of HCT, RBC, WBC, PLT, MCV, MCH, MCHC, NEUTROPHIL, and LYMPHOCYTE. This can be attributed to the pregnant women having access to high-quality medical care, supplementation of their diet, and effective management of their blood profile.

Pregnant women with malaria had lower mean red blood cell counts (3.45SD) than pregnant women without malaria (3.53SD). This may be due to anemia, a decrease in the formation of red blood cells in the bone marrow, mechanical destruction of red blood cells that have been parasitized, or the nutritional status of pregnant women who have the infection, which was in agreement with the findings by Garba *et al.* (2015) in Sokoto State, Nigeria, who reported that pregnant women with malaria had lower PCV than those who do not.

In contrast to Osonuga *et al.* (2011), who reported that WBC was elevated possibly due to pregnancy, the mean total white blood cell count in this study was in the normal range with the malaria positive and negative pregnant women. This may be because the white blood cell is responsible for body defense during pregnancy as a result of the body building immunity for the fetus, immune response against malaria infection, and immunomodulation in the presence of microbial immunity.

The mean value of haematocrit or packed cell volume of malaria positive pregnant women was slightly lower than those of negative pregnant women, the drop in PCV values in malaria positive subjects may have resulted from the mechanical destruction of parasitized red blood cells, reduction in red blood cell production in the bone marrow, phagocytosis of uninfected red blood cells, autoimmune destruction of red blood cells and nutritional status of infected individuals, this is in agreement with findings by Ozougwu *et al*.(2015), Who reported that malaria parasite decreased packed cell volume, red blood cell count and haemoglobin in pregnancy.

In contrast to the findings of Ozougwu *et al*. (2015), who observed a drop in WBC and platelet count in pregnant malaria infected women, this study found an increase in platelet of infected pregnant women compared to non-infected pregnant women.

The most significant leukocytic changes related to malaria infection are those in neutrophil and lymphocyte counts. A decrease in lymphocyte counts may be caused by redistribution and sequestration in the spleen, whereas a decrease in neutrophil counts may be due to activated neutrophil production or excessive production from the bone marrow.

In contrast to findings by Adesina *et al.* (2009) in Ilorin, Nigeria, who reported high red cell indices in malaria-infected pregnant women, the mean cell haemoglobin concentration and mean cell volume in this study were lower in infected pregnant women than in non-infected pregnant women. This may be because anaemia causes low hemoglobin concentration.

REFERENCE

Adesina, K.T, Balogun, O.R., Babatunde, A.S. and Sani, M.A. (2009). Impact of malaria parasitaemia on haematological parameters in pregnant women. *Trends in Medical Research* 4 (4);84-90

Agomo, C.O., Oyibo,W.A., Anorlu, R.I., Agomo, P.U. (2009). Prevalence of malaria in pregnant women in Lagos, South-west Nigeria. *Korean Journal of Parasitology*,47 (2):179-83.

Alo, M. N., Okonkwo, E. C., Ayim, C. and Uchenna, I, U. (2014). Haematological profile of pregnant women infected with malaria parasite at Federal Teaching Hospital Abakaliki, Ebonyi State. *Science Publication* 11-17

Bakhubaira, S. (2013). Hematological parameters in severe complicated *Plasmodium falciparum* malaria among adults in Aden. *Turkey Journal of Haematology*, 30: 394-399.

Boel, M.E., Rijken, M.J., Brabin, B.J., Nosten, F. and McGready, R. (2012). The epidemiology of postpartum malaria. *Malaria Journal*, 11: 1-7

Boyce, R.M., Muiru, A., Reyes, R., Ntaro, M., Mulogo, E., Matte, M and Siedner, M. J. (2015). Impact of rapid diagnostic test for the diagnosis and treatment of malaria at a

peripheral health facility in western Uganda: An interrupted time series analysis. *Malaria Journal*, 14:886-936.

Cheesbrough. M. (2006) district laboratory practice in tropical countries part 2, Cambridge University press, international United Kingdom pp 239-249.

Ekwunife, C.A., Ozumba, N.A., Eneanya, C.I. and Nwaorgu, O.C. (2011). Malaria infection among Blood Donors in Onitsha Urban, Southeast Nigeria. *Sierra Leone Journal of Biomedical Research*, **3**(1):21-26.

Fana, S. A., Mohammed, D., Abubakar, B., Sule, A. A., Asiya, U. I. and Shehu, U. N. (2015). Prevalence and risk factors associated with malaria infection among pregnant women in a semi urban community of North Western Nigeria, *Infectious Disease of Poverty*.10:1186.

Garba, N., Danladi, S.B. and Muhammed, A. (2015). Determination of some Haematological parameters in malaria infected subjects in Usmanu Danfodiyo University Teaching Hospital (UDUTH). Sokoto, Nigeria. *Bayero Journal of Pure and Applied Sciences*, **8**(1): 80-83

Imoru, M., Shehu, M.A., Ihesiulo, R U.G. and Kwaru, A.H. (2013). Haematological changes in malaria infected children in North West Nigeria. *Turkey Journal of Medical Science*, **43**:838-842.

Maina, R.N., Walsh, D., Gaddy, C. Hongo, G., Waitumbi, J., Otieno, L., Jones, D. and Ogutu, B. R (2010). Impact of *Plasmodium falciparum* infection on haematological parameters in children living in Western Kenya. *Malaria Journal,* **9** (Suppl 3), S4

Manas, K., Bhukdee, P., Nuoil, P., Chaowanee, C. and Suwit, D. (2014). Effect of malarial infection on haematological parameters in population near Thailand-Myanmar border, Malaria *Journal;* **13**:2-18

Osonuga, I. O., Osonuga., O. A., Onadeko., A. A., Osonuga., A. and Osonuga A. A. (2011). Hematological profile of pregnant women in southwest of Nigeria. *Asian Pacific Journal of Tropical Disease*, **1**(3):232–234.

Ovuakporaye, S.I. (2011). Effect of malaria parasite on some haematological parameters: red blood cell count, packed cell volume and haemoglobin concentration. *Journal of Medical and Applied Bioscience*, 3:45-51.

Ozougwu, J.C., Obimba, K.C., Obiukwu, C. E. and Elom,M.O. (2015). Studies of the Effects of Malaria Parasite on Haematological Profile of Pregnant Malarious Women. *World Journal of Medical Sciences* 12 (4): 383-386.

Saidu, A.Y, Sadiya, H., Dikwa, M.A., Abubaka, M.M., Fana, S.A. and Nuraddeen, M.B. (2015). Detection of *Plasmodium* specie among pregnant women attending ante natal care. *Journal of Dental and Medical Sciences*, 14:61-66

Tongo, O.O., Orimadegun, A. E. and Akinyinka, O. O. (2011). Utilisation of malaria preventive measures during pregnancy and birth outcomes in Ibadan, Nigeria. *BMC Pregnancy and Childbirth*, 11: 1-7.

UNICEF. (2009). Partner to Roll Back Malaria in Nigeria's Bauchi state at a glance. Abuja, Nigeria: Nigeria united Nation's children fund

World Health Organization (2012). Indoor residual spraying: use of indoor residual spraying for scaling up global malaria control and elimination, 3:49

World Health Organization (2016). World malaria report Fact Sheet, www.who.int/mediacentre/factsheets/fs094/en/(accessed on 10 October 2017)

World Health Organization (2019). Malaria Fact Sheet, https://www.who.int/news-room/fact-sheets/detail/malaria accessed on 13th September 2019)

Table 1: Prevalence of Malaria Parasite in Pregnant Women Based on Age

Age group (years)	No. of sample examined	No. of positive samples	% prevalence
15-25	55	18	32.7
26-30	28	7	25
31-35	9	1	11.1
36-40	6	2	33.3
41-45	2	0	0.00
Total	100	28	102.1

χ^2=3.637, p-value=0.457

Table 2: Prevalence of Malaria among Pregnant Women Based on Gravidity

Gravidity	No. of samples examined	No. of positive samples	% positive
Primigravida	15	5	33.3
Multigravida	57	23	40.3
Total	100	28	53.8

Table 3: Mean malaria parasite density among pregnant women based on gravidity

Gravidity	No. of samples examined	No. of positive samples	% positive	MPD (parasite/µl
Primigravida	20	5	25	336
Multigravida	80	23	28.75	379

KEY: MPD: mean parasite density, %: percentage of positive sample.

Table 4: Prevalence of Malaria among Pregnant Women Based on Trimesters.

Trimester	No. of samples examined	No. of positive samples	% positive
First	3	1	33.3
Second	67	22	32.8
Third	30	5	16.7

Table 5: Mean malaria parasite density among pregnant women based on trimesters.

Trimester	No. of samples examined	No. of positive samples	% positive	MPD(parasite/µl)
First	3	1	33.3	240
Second	67	22	32.8	330
Third	30	5	16.6	384

KEY: MPD: mean parasite density, %: percentage of positive sample

Table 6: Haematological Indices of Malaria Positive and Negative Pregnant Women in Sokoto

Parameters	Malaria negative (Mean±SD)	Malaria positive (Mean±SD)	p-value	T-test	Reference range
HCT/PCV	28.80±2.72	28.43±3.25	0.654	0.453	37- 47 l/l
PLT	213.1±88.20	217.1±75.98	0.852	0.188	150 – 400x109/l
WBC	6.89±1.47	6.89±2.81	1.000	0.000	4.0 – 11.0x109/l
RBC	3.53±0.474	3.45±0.319	0.444	0.777	3.8 – 5.8x1012/l
MCV	81.83±5.06	82.02±8.56	0.913	0.111	80 – 100 fl
MCH	28.23±2.25	27.00±6.73	0.370	0.912	27 – 32 pg
MCHC	34.35±1.45	33.80±5.43	0.608	0.519	300 – 500g/dl
NEUTRO	61.69±13.31	60.92±17.18	0.823	0.226	50 – 60%
LYMPH	28.73±7.25	27.49±8.67	0.569	0.576	20 – 50 %

KEY: HCT: haematocrit, PLT: platelet, WBC: white blood cell, RBC: red blood cell, MCV: mean cell volume, MCH: mean cell haemoglobin, MCHC: mean cell haemoglobin concentration, NEUTRO: neutrophil, LYMPH: lymphocyte, fl: femtolitre, pg: picogram.
P-value ≤ 0.05 was considered statistically significant.