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Morphological classification of Anaemia and Neutrophil Patterns in Pregnant Women with Asymptomatic Malaria Parasite Infection

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Abstract

Asymptomatic *Plasmodium* infection during pregnancy increases the risk of stillbirths, abortion, premature delivery, and low birth weight. It also hinders the control and prevention of malaria as infected hosts serve as silent reservoirs for transmission of *Plasmodium* species in the community. This study was conducted to assess the morphological classification of anaemia and neutrophil patterns in asymptomatic malaria parasite-infected pregnant women in Calabar, Nigeria. Fifty pregnant women attending Antenatal Clinic at the University of Calabar Teaching Hospital in Nigeria, and 50 age-matched non-pregnant women were recruited for the study. The red cell indices and neutrophil lymphocyte ratio (NLR) were determined using Mindray assay automated haematology analyser. while standard microscopy technique was used to detect malaria parasite. The MCV (79.93 ± 9.18fl) was significantly ($p < 0.05$) higher in pregnant women with asymptomatic malaria parasite infection compared to the non-pregnant women (73.04 ± 4.10fl) while their MCH (26.54 ±2.17pg) was seen to be significantly lower than the non-pregnant women (28.61± 1.67). The NLR value in this study was seen to be significantly (2.10 ±0.76) higher in pregnant women than non-pregnant women (1.22 ±0.51). The prevalence of anaemia amongst the pregnant women was 48% and normocytic normochromic anaemia (54.2%) was found to be the most prevalent form of anaemia in the pregnant women followed by microcytic hypochromic anaemia (41.7%) and then macrocytic normochromic anaemia (4.1%). Pregnant women were more susceptible to the parasite especially *Plasmodium falciparum* with mean parasite density of 692.16 ± 151.95 (parasite/µl). A strong negative correlation was seen between the malaria parasite density and the haematocrits of the pregnant women with asymptomatic malaria parasite infection. Malaria parasite density and NLR were seen to be significantly higher in pregnant women with asymptomatic malaria infection and normocytic normochromic anemia was the most prevalent form of anemia.

Keywords: Anaemia, Asymptomatic Malaria, Neutrophil-Lymphocyte Ratio, Morphological Classification of Anaemia

Introduction

Malaria is a significant public health concern in Nigeria; 97% of Nigerians are at risk of this disease, with substantial morbidity and mortality statistics across various age groups (Yaya *et al.,* 2018; WHO, 2020; Okafor *et al.,* 2023). Malaria impacts parturients and their neonates with complications and mortality (Okafor *et al.,* 2013). The World Health Organization (WHO) defines asymptomatic malaria as "a condition in which a person has malaria parasites in their blood but does not show any symptoms of the disease." (WHO, 2018). While the United States Agency for Infectious Disease (USAID) defines asymptomatic malaria as "a state of malaria infection where the patient does not show any clinical signs or symptoms of the disease, but malaria parasites are present in the bloodstream and can be detected by microscopic examination of a blood sample or by a rapid diagnostic test.

Also, USAID notes that people with asymptomatic malaria can still pass the infection on to others through the bite of an infected mosquito (USAID, 2018).

Pregnant women are particularly vulnerable to asymptomatic malaria, as they are more likely to have a higher parasite burden and a longer duration of infection (Okafor *et al.,* 2012). Pregnant women may also be more likely to have asymptomatic infections that progress to symptomatic disease, and they may have a higher risk of complications and poor pregnancy outcomes (Okafor *et al.,* 2012). Studies have found that the prevalence of asymptomatic malaria infection in pregnant women varies widely, depending on the geographic location, the time of year, and other factors (WHO, 2020). Asymptomatic malaria infection during pregnancy is associated with several risks and complications for both the mother and the baby. These risks and complications include; Anaemia in the mother, low birth weight of the baby, preterm birth, stillbirth, congenital malaria in the baby, increased risk of other complications such as eclampsia, and maternal death (WHO, 2019).

Anaemia, a common complication of asymptomatic malaria in pregnant women is thought to be caused by several factors such as destruction of red blood cells by the malaria parasite, an increased demand for iron by the mother and the baby, and an increased destruction of red blood cells due to the oxidative stress caused by the malaria infection. In addition to fatigue and weakness, anaemia during pregnancy can lead to a number of other complications like haemorrhage during labor and delivery, increased risk of postpartum infection, poor infant growth and development, and increased risk of death for both the mother and the baby (Okafor *et al.,* 2018). Despite being asymptomatic, pregnant women with malaria parasite infection may still experience anaemia and are unaware of the severity of damage it causes. However, there is limited understanding of the specific alterations in red blood cell indices, neutrophil/lymphocyte ratio, morphology classification of anaemia and malaria parasite density in this population, hence the need for this study.

Materials and Methods

Study area

This cross-sectional study was conducted in University of Calabar Teaching Hospital (UCTH), Calabar, Nigeria. A total of 100 individuals were enrolled for this study. Fifty (50) pregnant women positive for malaria parasites, but without any malaria-related symptoms attending Antenatal Clinic at the University of Calabar Teaching Hospital and Fifty (50) apparently healthy individuals were enrolled.

Inclusion criteria

Only pregnant women attending Antenatal Clinic at UCTH who were positive for malaria parasites upon microscopy, were confirmed to be without associated malaria symptoms, and gave informed consents were co-opted into the study.

Exclusion criteria

Those who did not meet the above-stated criteria were excluded from the study.

Ethical consideration/informed consent

Ethical clearance was obtained from Research and Ethical Committee of the University of Calabar Teaching Hospital (UCTH/HREC/33/VOL111/150). Detailed explanation of the purpose, objectives, risks, and benefits were given to participants, after which verbal consent was obtained. The respondents' right to refuse or withdraw from participating in the interview was fully maintained. Data was collected after obtaining informed consent and agreement from the patients under study. Sample collection was performed following ethical steps and procedures.

Administration of questionnaire

After a detailed explanation, the participants' consents were sought before being enrolled in the study. The responses of the participants were taken using structured questionnaire before blood sample collection. The questionnaire contained data on socio-demographic parameters. Each respondent's age group, marital status, educational level, occupation, previous malaria infection, time of last malaria episode, and 'use of bed net the previous night' were covered in the socio-demographic section.

Collection, handling, storage and analysis of samples

Four milliliters (4ml) of venous blood samples were drawn aseptically from each volunteer participants via venipuncture using a disposable plastic syringe into ethylenediamine tetra acetic acid (EDTA) anticoagulant (containing 6mg of the EDTA) and labeled with the name, gender, and unique identification number of each participant. Thick and thin blood films were made and labeled appropriately; the remaining blood was put into EDTA sample bottle for complete blood count. The samples were temporarily stored in a cold flask packed with ice prior to their transfer to the laboratory for analysis. The red cell indices, packed cell volume, neutrophil and lymphocyte count were determined using the automatic cell counter while neutrophil/lymphocyte ratio (NLR) was calculated from neutrophil and lymphocyte count. Standard microscopy technique was used to for detection of malaria parasite with thick and thin films stained with 3% Giemsa and examined microscopically with the x100 objective lens.

Quantification of Parasites

After detection of malaria parasite using thick blood film, the numbers of malaria parasites observed in the fields were quantified using this method:

$$\frac{\text{Number of parasites counted} \times 8000}{\text{Number of WBCs counted}} \quad \frac{}{1}$$

Unit= value gotten/µl of blood

Reporting: The parasite density was reported as the number of parasites per µL of blood.

Data analysis

Data obtained was presented in tables and compared using Student T-test and Pearson's correlation analysis. The Statistical Package for the Social Sciences (SPSS) version 22.0 software was employed for this study. A p-value ≤ 0.05 was considered significant.

Results

The socio-demographic characteristics of pregnant subjects attending Antenatal Clinic at the University of Calabar Teaching Hospital is shown on Table 1. Participants belonging to the age group of 35 – 39 years constituted the majority, 18(36.0%), followed by those belonging to the age group of 30 – 34 years (32.0%), while those belonging to the age groups of 20 – 24 years, 25 – 29 years, and >40 years constituted the remaining 4(8.0%), 8(16.0%), and 4(8.0%) respectively. Majority of the respondents were Christians 42(84.0%), while the remaining 2(4.0%) and 6(12.0%) were Muslims and traditional worshippers respectively. Nearly all of the respondents were married 47(94.0%) with only 3(6.0%) being singles. Participants with tertiary education constituted the majority 21(42.0%), while those with non- formal education, primary education, and secondary education constituted the remaining 5(10.0%), 12(24.0%), and 12(24.0%) respectively. Most of the respondents were business women 18(36.0%), while civil servants, hair dressers, and tailors made up the remaining 9(18.0%), 14(28.0%), and 9(18.0%) respectively. All of the respondents reported having previous malaria infections, while majority reported suffering from malaria infection within the last 6 months.

Table 2 shows the mean haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC) of pregnant women with asymptomatic malaria and non-pregnant controls respectively. Pregnant women with asymptomatic malaria had mean values of 32.51±5.71%, 79.93±9.18fl, 26.54±2.17pg, and 32.14±4.04 g/dl respectively, and the controls had mean values of 38.15±2.38%, 73.04±4.10fl, 28.61±1.67pg, and 32.72±0.77g/dl respectively. Significant variations were observed in the HCT, MCV, and MCH among the two groups studied: pregnant women and control group ($p \leq 0.05$). However, no significant variation was observed in the MCHC of the pregnant women and the non-pregnant controls ($p > 0.05$).

Table 3 shows the neutrophil count, lymphocyte count, and neutrophil to lymphocyte ratio (NLR) of pregnant women with asymptomatic malaria and non-pregnant controls. Pregnant women with asymptomatic malaria had mean values of 5.11±1.73 x 10^9/L, 2.62±0.81 x 10^9/L, and 2.10±0.76 respectively, while the non-pregnant controls had mean values of 2.52±1.26 x 10^9/L, 2.29±0.96 x 10^9/L, and 1.22±0.51 respectively.

Significant variations were observed in the neutrophil counts and NLR between the pregnant women and the non-pregnant controls ($p \leq 0.05$).

Table 4 shows the malaria parasite density (MPD) of pregnant women with asymptomatic malaria and non-pregnant controls. Pregnant women with asymptomatic malaria had mean values of 692.16±151.95 parasites/µl. Malaria parasite density (MPD) of the pregnant women was significantly higher ($p \leq 0.05$).

Prevalence of anaemia among pregnant women with asymptomatic malaria is summarized in Table 5 while table 6 shows the morphological classification of anaemia among pregnant women with asymptomatic malaria. Results obtained showed that 24(48.0%) of the pregnant women with asymptomatic malaria had anaemia, while the remaining 26(52.0%) were not anaemic. Of the 24 anaemic pregnant women, majority 13(54.2%) had normocytic normochromic anaemia, 10(41.7%) had microcytic hypochromic anaemia, while only 1(4.1%) had microcytic normochromic anaemia. Figure 1 shows a correlation plot of malaria parasite density (MPD) against haematocrit (HCT). A negative correlation ($r = -0.215$, $p = 0.133$) was observed between MPD and HCT in the pregnant women with asymptomatic malaria.

Table 1: Socio-demographic characteristics of pregnant women attending antenatal center at the University of Calabar Teaching Hospital.

Variables	Frequency (n = 50)	Percentage (%)
Age (Years):		
20 - 24	4	8.0
25 - 29	8	16.0
30 - 34	16	32.0
35 - 39	18	36.0
40 and above	4	8.0
Religion:		
Christianity	42	84.0
Islam	2	4.0
Traditional	6	12.0
Marital status:		
Married	47	94.0
Single	3	6.0
Education:		
No formal education	5	10.0
Primary education	12	24.0
Secondary education	12	24.0
Tertiary education	21	42.0
Occupation:		
Business	18	36.0
Civil servant	9	18.0
Hairdressing	14	28.0
Tailoring	9	18.0
On iron supplementation:		
Yes	47	94.0
No	3	6.0
Previous malaria infection:		
Yes	50	100.0
No	0	0.0
Period of last malaria infection:		
Within the last 6 months	44	88.0
About a year ago	4	8.0
More than a year ago	2	4.0
Use of bed net:		
Yes	48	96.0
No	2	4.0

Table 2: HCT, MCV, MCH, and MCHC of pregnant women with asymptomatic malaria and non-pregnant controls

	Groups			
Parameter	**Pregnant women with asymptomatic malaria n = 50**	**Non-pregnant controls n = 50**	**t**	**p –value**
HCT (%)	32.51±5.71	38.15±2.38	-6.446	0.001*
MCV (fL)	79.93±9.18	73.04±4.10	4.852	0.001*
MCH (pg)	26.54±2.17	28.61±1.67	-5.356	0.001*
MCHC (g/dL)	32.14±4.04	32.72±0.77	-1.001	0.319

Values are expressed as Mean±SD; HCT = Haematocrit; MCV = Mean Cell Volume; MCH = Mean Cell Haemoglobin; MCHC = Mean Cell Haemoglobin Concentration; * = Significant at $p \leq 0.05$

Table 3: Neutrophil count, Lymphocyte count and NLR of pregnant women with asymptomatic malaria and non-pregnant controls.

	Groups			
Parameter	**Pregnant women with asymptomatic malaria n = 50**	**Non-pregnant controls n = 50**	**t**	**p –value**
Neutrophil count ($x10^9$/L)	5.11±1.73	2.52±1.26	8.551	0.001*
Lymphocyte count ($x10^9$/L)	2.62±0.81	2.29±0.96	1.818	0.072
NLR	2.10±0.76	1.22±0.51	6.796	0.001*

Table 4: MPD of pregnant women with asymptomatic malaria and non-pregnant controls.

	Groups			
Parameter	**Pregnant women with asymptomatic malaria n = 50**	**Non-pregnant controls n = 50**	**t**	**p –value**
MPD (parasites/µl)	692.16±151.95	0.00±0.00	32.209	0.001*

Values are expressed as Mean±SD; MPD = Malaria Parasite Density; * = Significant at $p \leq 0.05$

Table 5: Prevalence of anaemia among pregnant women with asymptomatic malaria

Variable	Frequency	Percentage (%)
Number with anaemia	24	48.0
Number without anaemia	26	52.0
Total	50	100.0

Table 6: Morphological classification of anaemia among pregnant women with asymptomatic malaria

Morphologic type of anaemia	Frequency	Percentage (%)
Microcytic hypochromic anaemia (MCV <78fl, MCH <26pg and Hb <11g/dl)	10	41.7
Microcytic normochromic anaemia (MCV <78fl, MCH>26pg and Hb<11g/dl)	1	4.1
Normocytic normochromic anaemia (MCV >78fl but <95fl, MCH>26pg and Hb<11g/dl)	13	54.2
Macrocytic normochromic anaemia (MCV >95fl, MCH>26pg and Hb<11g/dl)	0	0.0
Total	**24**	**100.0**

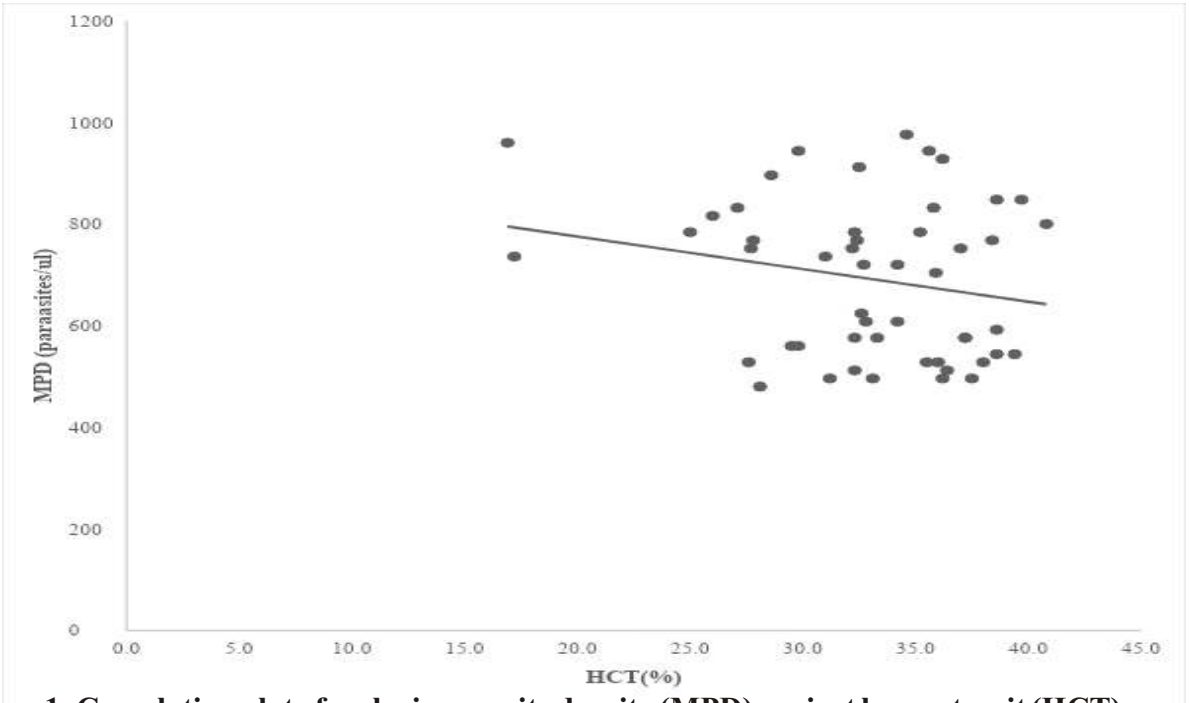

Figure 1: Correlation plot of malaria parasite density (MPD) against haematocrit (HCT)

Discussion

Malaria, a persistent and widespread infectious disease, casts a long shadow over tropical and subtropical regions, with pregnant women bearing the brunt of its burden. A silent form of the disease, "asymptomatic malaria," lurks unseen in these areas, harboring the parasite in the blood without triggering any symptoms (Okafor *et al.,* 2023). This study was conducted to assess the morphological classification of anaemia and neutrophil patterns in asymptomatic malaria parasite-infected pregnant women in Calabar, Nigeria.

The demographic table shows that 94% of the pregnant women take iron supplements which are essential for the proper formation of the foetus. On the other hand, 6% of the pregnant women were not on iron supplements. It's well established that iron deficiency during pregnancy is associated with a number of negative health outcomes for both the mother and the foetus (Okafor *et al.,* 2012). Low levels of iron during pregnancy can lead to anemia, which can cause fatigue, weakness, and other health problems (Okafor *et al.,* 2012). Additionally, low iron levels can lead to a decrease in foetal growth and an increased risk of premature birth and low birth weight. Taking iron supplements during pregnancy can help to prevent these negative outcomes and improve the health of both the mother and the baby. Daily iron and folic acid supplementation is currently recommended as part of antenatal care, to reduce the risk of maternal anaemia and iron deficiency (Okafor *et al.,* 2014; WHO, 2018). Ninety-six percent of them make use of insecticide- treated bed nets while 4% do not make use of insecticide- treated bed nets. Bed nets are an effective tool for preventing malaria, as they act as a physical barrier between the individual and the mosquitoes that carry the malaria parasite. This study showed that a higher number of the pregnant women enrolled make use of ITN, as the time of the year during which the data was gathered. People use mosquito nets when malaria transmission is at its highest, which typically coincides with wet/rainy seasons (Karema *et al.,* 2020). This emphasizes how important it is to maintain bed net availability, early procurement, and education/sensitization on mosquito bed net usage.

A significant variation in MCV, MCH and haematocrit were observed among pregnant women with asymptomatic malaria in this study. They had significant lower HCT values (32.51±5.71) when compared to the control groups. This finding agrees with the study by Obebe *et al.* (2018) who obtained 31.4g/dl in their work on "Impact of asymptomatic *Plasmodium falciparum* on haematological parameters of pregnant women at first antenatal visit in South-western Nigeria". The finding also agrees with the study by Erhabor *et al.* (2019) who reported low HCT values in pregnant women with asymptomatic malaria parasite infection. The possible reason for low HCT values in this study could be as a result of high density of malaria parasites. It has been shown that *Plasmodium falciparum* tends to accumulate in the placenta, causing damage to red blood cells and reducing their lifespan (Mutebi *et al.,* 2021; Okafor *et al.,* 2022). This process contributes to anaemia, impacting the overall haematocrit levels. It may also be attributed to the body's phenomenon of withholding iron during invasion of malaria parasite and other inflammatory conditions leading to dyserythropoiesis and ultimate reduction in haemotocrit (Okafor *et al.,* 2014). The MCV (79.93fl) values were significantly higher when compared to the non-pregnant women. This is slightly seen to be lower when compared with the study done by Obebe *et al.* (2018) who reported the MCV values of pregnant women with asymptomatic malaria to be 86.84fl. The low MCV values could possibly be as a result of the body's response to inflammation. Malaria can trigger an inflammatory response, and this, in turn reduces the MCV values as result of iron sequestration (Okafor *et al.,* 2014). MCH values (26.54pg) were seen to be significantly lower in pregnant women with asymptomatic malaria parasite infection and it agrees with study done by Obebe *et al.* (2018) as well. This study revealed that the low MCH values were as a result of destruction of the red blood cells by the malaria infection affecting the synthesis of haemoglobin. The diminished haemoglobin content in each red blood cell, coupled with smaller cell size (microcytosis), contributes to a reduction in MCH values.

The neutrophil and lymphocyte ratio (NLR) value in this study was seen to be significantly higher in pregnant women than non-pregnant women. This agrees with the work done by Kotepui *et al.* (2014). In a study conducted in Sudan involving children, they also obtained high NLR in malaria cases. The increase in NLR in malaria infection more than control may be due to an increase release of leukocytes at the

initial stage of infection to fight against the infection. This as well relates to the effective immune response to malaria in the endemic areas (Abdelsamea *et al.,* 2017). Similar studies showed an increase in neutrophil in *falciparum* malaria than control (Al-Salehy *et al.,* 2016; Kini & Chandra, 2016). This may also be due to the body's response to inflammation which triggers the production of antibodies to fight the malaria infection thus leading to increase in the neutrophil and lymphocyte counts. Although some studies have suggested that NLR is not a complete significant biomarker for malaria (Rakusan *et al.,* 2017), it can be used as a significant marker in pregnant women with asymptomatic malaria parasite infection as it becomes significantly higher due to its response to inflammation. The NLR indicates the balance between innate and adaptive immune responses and it is an excellent indicator of both inflammation and stress (Zakama *et al.,* 2019).The opposite changes in neutrophil and lymphocyte counts are a multifactorial dynamic process depending on fine tuning and regulation of various immunologic, neuroendocrine, humoral and biologic processes such as margination/ demargination, mobilization/redistribution, accelerated/delayed apoptosis, influence of stress hormones and sympathetic/parasympathetic imbalance of the vegetative nervous system.

Malaria parasite density value of the pregnant women with asymptomatic malaria was found to be significantly high. This agrees with the study conducted by Ogbu *et al.* (2015) and Emiasegen *et al.* (2019) who found the high prevalence of malaria in Nigeria. This value is also in line with those reported by a study in Ghana by Adu-Gyas *et al.* 2015). The high malaria density obtained in this study in spite of a high number of pregnant women who are using insecticide- treated mosquito nets in this study (96%), shows that the women may not be using insecticide- treated mosquito nets correctly, therefore, there is need for increased education and awareness.

The prevalence of anaemia among pregnant women with asymptomatic malaria in UCTH Calabar was found to be 48% in this study. This is found to be lower than 61.1% reported previously by Okafor *et al.* (2012) and slightly higher than 46% reported by Rouamba *et al.,* (2021). However, Olukosi and Afolabi (2018) in their study in Lagos, Nigeria had higher results of 81.4% prevalence of anaemia. According to Desai *et al*. (2007), malaria infection is highly associated with anaemia and poor pregnancy outcome at all levels of pregnancy, while White (2018) reiterated that malaria infection is associated with maternal anaemia. These authors stated that *P. falciparum* is directly linked with maternal death in a low transmission setting while in a high transmission setting, it is an indirect cause of mortality via maternal anaemia. The most common type of anaemia among pregnant and non-pregnant women in this study was normocytic normochromic type. This is likely due to malaria, as over 55% of the studied population tested positive for the parasite. This is because malaria typically causes this type of anaemia by destroying red blood cells and accelerating their removal from the body. However, pregnant women also had a higher rate of microcytic hypochromic possibly due to the increased demand for iron and folate during pregnancy. It may also possibly be due to the body's phenomenon of iron sequestration during invasion of microorganisms such as malaria parasite which subsequently leads to iron deficient erythropoiesis and ultimately microcytic hypochromic anaemia.

Negative correlation was observed between malaria parasite density and haemotocrit. During malaria infection, fluid loss due to sweating and diarrhoea can lead to hemoconcentration, which is an increase in the proportion of red blood cells in the blood. However, as the parasite density increases, the destruction of red blood cells outweighs the haemoconcentration, leading to a net decrease in HCT. Another factor is the suppression of erythropoiesis. Malaria parasites can directly or indirectly suppress the production of red blood cells in the bone marrow. This can lead to a decrease in the number of red blood cells and, consequently, a decrease in HCT. Haemolysis, also being another factor can be caused by the malaria parasites breaking down the red blood cells, causing a decrease in HCT.

In conclusion, this study has shown that asymptomatic malaria is still a cause for concern in

pregnancy as the prevalence of anaemia was found to be 48.0%. Normocytic normochromic anaemia was also found to be the most prevalent form of morphologic anaemia in these pregnant women, followed by microcytic hypochromic anaemia. This study also showed that NLR was significantly higher in pregnant women with asymptomatic malaria than in non-pregnant women. High malaria parasite density was observed among the pregnant women in spite of high usage of malaria treated net recorded among them. The study recommends that proper education should be given to the pregnant women on the appropriate use of Insecticide treated nets.

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References

Abdelsamea, M., Abakar, A.D., Ahmed, M.O., *et al.* (2017). Female fetus, a novel risk factor for placental malaria in pregnant Sudanese women. *Journal of Tropical Medicine;* **21(3)**: 14-20.

Adu-Gyasi, D., Asante, K., Newton, S., Amoako, S., Dosoo, D., Ankrah, L., Adjei, G., Amenga-Etego, S., Owusu-Agyei, S. (2015). Malaria Parasite Density Estimated with White Blood Cells Count Reference Value Agrees with Density Estimated with Absolute in Children Less Than 5 Years in Central Ghana. *Clinical Laboratory;* **61(2)**:317-322.

Al-Salehy, N., El-Rouby, R.H., Ahmed, Y., *et al.* (2016). Lower hemoglobin concentration at pregnancy booking and the relationship between trimester of delivery and birth weight in a low-prevalence area of malaria transmission. *International Journal of Gynecology & Obstetrics;* **136(1)**: 62-66.

Desai, M., ter Kulie, F.O., Nosten, F., McGready, R., Asamoa, K., Brabin, B., Newman, R.D. (2007). Epidemiology and burden of malaria in pregnancy. *Lancet Infectious* Diseases; **7(2)**: 93-104.

Emiasegen, S.E., Giwa, F.J., Ajumobi, O., Ajayi, I.O., Ahmed, S.A., Olayinka, A.T. (2018). Asymptomatic Plasmodium falciparum parasitaemia among pregnant women: a health facility-based survey in Nassarawa-Eggon, Nigeria. *Malaria World Journal*; **15(24)**:25–34.

Erhabor Osaro, Abdulrahaman, A., & Erhabor, T. (2019). Effects of Malaria Parasitaemia on Some Haematological Parameters of Pregnant Women of African Descent in Specialist Hospital Sokoto, North Western Nigeria. *JOJ Nurse Health Care; **10**(**4**)*: 555 -561.

Karema, C., Wen, S., Sidibe, A., Smith, J.L., Gosling, R., & Hakizimana, E. (2020). History of malaria control in Rwanda: implications for future elimination in Rwanda and other malaria-endemic countries. *Malaria Journal*;**19**:356.

Kini, R.G. & Chandra, S. (2016). Parasite and the Circulating Pool-Characterization of Leukocyte Number and Morphology in Malaria. *Journal of Clinical and Diagnostic Research; **10**(**15**)*: 44-48.

Kotepui, M., Phunphuech, B., Phiwklam, N., Chupeerach, C., Duangmano, S. (2014). Effect of malarial infection on haematological parameters in population near Thailand-Myanmar border. *Malar Journal*; **13**: 218.

Mutebi, J., Kabakyenga, C., Kakemboh, A., *et al.* (2021). Placental accumulation of Plasmodium falciparum causes anemia in pregnant women: evidence from immunofluorescent staining and anemia modeling in a Ugandan birth cohort study. *PloS One; **16**(**9**)*: e0255971.

Obebe, O.O., Falohun, O.O., Olajuyigbe, O.O., *et al* (2018). Impact of asymptomatic Plasmodium falciparum on haematological parameters of pregnant women at first antenatal visit in South-western Nigeria. *Tanzania Journal of Health Research; **20**(**2**)*: 31-42.

Ogbu, G.N., Otike-Odibi, B.I., Ezeokeke, C.I., Nwokeke, C.I. & Ogbu, C.I. (2015). Prevalence of asymptomatic malaria among pregnant women attending antenatal care in a rural community in Enugu State, Nigeria. *International Journal of Tropical Medicine; **9**(**2**)*: 40-45.

Okafor, I.M., Akpan, S.S. & Henshaw, A.E. (2022). Sero-Prevalence of Syphilis and Assessment of Haemoparasites among Prospective Blood Donors at a Tertiary Hospital

in Calabar, Southern Nigeria, *Nigerian Journal of Parasitology;* **43(1)**: 10-16.

Okafor, I.M., Asemota, E.A., Antai, A.B., Usanga, E.A. (2013). Prevalence of Iron Deficiency Anaemia among Pregnant Women in Calabar, Cross River State Nigeria. *Journal of Pharmacy and Biological Sciences; 7 : 60-64.*

Okafor, I.M., Akpan, P.A., Usanga, E.A. (2012). Prevalence and Types of Anaemia in Malaria Infected Pregnant Women Attending Antenatal Clinic in University of Calabar Teaching Hospital, Calabar, Nigeria. *Journal of Natural Science Research;* **2** : 73-78.

Okafor, I.M., Ibanga, I.A. and Asuquo, I.J. (2018). Prevalence of anaemia and Iron deficiency anaemia in a Nigerian Rural Community. *Sokoto Journal of Medical Laboratory Science;* **3(2)**: 52-58.

Okafor, I.M., Mbah, M., Usanga, E.A. (2012). The impact of anaemia and malaria parasite infection in pregnant women, Nigerian perspective. *Journal of Dental and Medical Sciences;* **1**: 34-38.

Okafor, I.M., Ogar, C.O. & Okoroiwu, H.U. (2023). Serum Ferritin and Asymptomatic Malaria, A study in Calabar, Cross River State. *African Journal of Laboratory Haematology and Blood Transfusion Science*; **2(1)** : 15-22.

Okafor, I.M., Okpokam, D.C., Antai, A.B. & Usanga, E.A. (2014). Soluble transferrin receptor as a marker in the diagnosis of iron deficiency anaemia, a study in Calabar. *International Journal of Biomedical Laboratory Science*; **3** :40-46.

Olukosi, A., and Afolabi, B. (2018). Malaria and anemia among pregnant women living in communities along the coast of Lagos Lagoon, South-west Nigeria. *International Journal of Pregnancy and Childbirth;* ***4*** **(6)**: 175–182.

Rakusan, A., Al-Bagdi, W., Gewaily, N., *et al*. (2017). Effect of hemoglobin concentration, ferritin, and leukocyte sub-populations on asymptomatic malaria in pregnant women. *Malaria Journal;* ***16*** **(1)**: 260. https://doi.org/10.1186/s12936-017-2116-8.

Rouamba, T., Samadoulougou, S., Ouédraogo, M., *et al.* (2021). Asymptomatic malaria and anaemia among pregnant women during high and low malaria transmission seasons in Burkina Faso: household-based cross-sectional surveys in Burkina Faso, 2013 and 2017. *Malaria Journal;* ***20*** **(211)**: 76-82.

Udonkang, M.I., Eluwa, M., Inyang-Etoh, P., *et al*. (2018). Efficacy of histochemical staining techniques in the detection of Plasmodium falciparum histidine-rich proteins in blood of children with malaria. *Journal of Microbiology and Infectious Disease*; **8(2)**: 55-60.

United States Agency for International Development (USAID), Asymptomatic Malaria. *Malaria Atlas Project* 2018.

White, N.J. (2018). Malaria in pregnancy: challenges and opportunities. *Malaria Journal;* ***17*** **(1)**: 1-10.

World Health Organization, Malaria in Pregnancy: World Health Organization Global Guidelines. *World Health Organization* 2018.

World Health Organization, World malaria report Geneva, *World Health Organization* 2019.

World Health Organization. Malaria. *Bulletin of World Health Organization* 2020.

Yaya, S., Uthman, O.A., Amouzou, A. & Bishwajit, G. (2018). Use of Intermittent Preventive Treatment among Pregnant Women in Sub-Saharan Africa: Evidence from Malaria Indicator Surveys. *Tropical Medicine and Infectious Disease;* ***3*** **(1)**:18.

Zakama, N., Zongo, I., Kargougou, M.R., *et al.* (2019). Prevalence and risk factors of malaria infection among pregnant women attending antenatal care in a rural area of Burkina Faso: a cross-sectional study. *BMC Pregnancy and Childbirth;* ***19*** **(1)**: 1-11.

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