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# Erythrocyte Indices in Asymptomatic Malaria Infected Pregnant Women

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***Authors' contributions***

*Author NAM designed the study, wrote the protocol and wrote the first draft of the manuscript, performed the statistical analysis and managed the analyses of the study, managed the literature search, read and approved the final manuscript.*

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## ABSTRACT

**Background:** Red blood cell changes are one of the most common complications in malaria and they play a very crucial role in malaria pathogenesis. Malaria infections are one of the common causes of maternal anaemia especially during pregnancy. The aim of this study was to determine red cell indices of pregnant women with asymptomatic malaria.
**Place and Duration of Study:** Department of Haematology and Antenatal Unit both of Enugu State University of Science and Technology Teaching Hospital, between June and September 2022.
**Methodology:** The study population consisted of 90 pregnant women (65 pregnant women positive to malaria parasite without symptoms and 25 pregnant women negative to malaria parasite) and 26 control non-pregnant women. For the whole study population, red cell indices which include hemoglobin (HGB), packed cell volume (PCV), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width standard deviation (RDW-SD), red cell distribution width coefficient of variation (RDW-CV) were measured by automated haematology analyzer.
**Results:** In the asymptomatic malaria group (AMG), 21 (32.3%) had mild anaemia (HGB level 9.0-

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10.0 g/dl), 11 (16.9%) had moderate anaemia (HGB level 7.0-8.0 g/dl) and 2 (3.1%) had severe anaemia (HGB level <7.0 g/dl). Also in AMG group, the RDW-SD was *54.22 +/- 11.45* fl, whereas in control group it was *48.75 +/- 10.24* fl (p=0.002). Again in the AMG group the MCHC of those that had two pluses was *318.03 +/- 16.31* g/l, whereas in those that had one plus, it was 309 +/- 20.17 g/l. The comparison between the first, second and third trimester showed significant decrease in HGB (7.63 +/- 1.36 vs 11.64 +/- 0.72 g/dl) and PCV (26.98 +/- 5.14 vs 36.20 +/- 2.19 %) in third trimester compared to first trimester (p= <0.001, <0.001). whereas RDW-CV (18.96 +/- 5.04 vs 15.00 +/- 2.64 %) and RDW-SD (59.04 +/- 15.19 vs 49.16+/- 7.00 fl) (p= 0.002, 0.003) significantly increased in third trimester compared to first trimester

**Conclusion:** This study found anaemia in asymptomatic malaria infected pregnant women, significant decrease in haemoglobin and packed cell volume at third trimester, higher MCHC in those with two pluses of malaria and significant increase in red cell distribution width at third trimester.

*Keywords: Malaria; anaemia; asymptomatic; indices; trimester; plus.*

## 1. INTRODUCTION

The disease burden of malaria in Nigeria is huge; malaria is responsible for 15% anemia, 70% morbidity and 5% to 14% occurrence of low birth weight. Approximately, about 3.2 million people are at risk of malaria infection. For decades, different effort to eliminate malaria infection at international level failed to yield the needed result [1]. In Nigeria, there is about 3,000 death from malaria which lead to 40% of health expenditures, frequent visit to hospital, and up to 50% of hospital admissions yearly [2]. The problems of malaria disease vary based on the level of immunity. In Nigeria, malaria accounts for 11% of mortality among pregnant women. The problems associated with malaria include anaemia, hypoglycemia, oedema, fetal distress, spontaneous abortion, preterm delivery, low birth weight, neonatal mortality and maternal death [3]. According to WHO (2017) there is great need for malaria research among pregnant women due to the recent drug failure and parasite resistance observed among them and their neonates [4]. A malaria infection is preventable and treatable. Comprehensive studies in other places and locations are needed to provide current information on risk factors for malaria in pregnancy to heighten achievements of malaria control in Nigeria [5]. Pregnancy is known by physiologic alterations that might affect, either directly or indirectly the haematological parameters such as HGB, PCV, RBC etc. Anaemia was the second most common observed hematological abnormality in malaria infections. Study by Sharma and Shukla (2017) showed that infected red blood cells by plasmodium falciparum cause inflammation, oxidative stress, and apoptosis to the placenta [6]. The infected red blood cells pass through the placenta to avoid the host immune responses. The infected red blood cells darkens and clots the placenta base impacting maternal and neonates exchange patterns, leading to intrauterine growth retardation, low birth weight, and other detrimental pregnancy outcomes [6]. A correct evaluation of anaemia and malaria parasite infection in pregnant women are required to reduce the rate of fetal distress, spontaneous abortion, preterm delivery, low birth weight, neonatal mortality and maternal death. This study was therefore undertaken in 2022 to determine erythrocyte indices of pregnant women with asymptomatic malaria.

## 2. METHODOLOGY

The case control study was carried out in Enugu State University of Science and Technology Teaching Hospital (ESUTH) Enugu, South East Nigeria. Subjects enrolled for this study were selected by simple random sampling method, they are consisted of 65 pregnant women positive to malaria parasite without symptoms and 25 pregnant women negative to malaria parasite attending antenatal clinic in ESUTH. Also 26 non-pregnant women were used as control. Inclusion criteria include age greater than 18 years, confirmation of pregnancy by a consultant obstetrician and willingness to give a written informed consent to participate in the study. Pregnant women less than 18 years and non -consenting pregnant women were excluded from the study.

Two point five milliliters (2.5ml) of blood was collected by venepuncture using aseptic technique from each participant and introduced into Ethylene Diamine Tetra acetic Acid (EDTA) anticoagulant tube. Each sample was then mixed

gently and thoroughly to ensure anticoagulation. The samples were analyzed for malaria parasite and red cell indices parameters. Diagnosis of malaria were done by staining thick blood smear with 3% Giemsa working solution diluted in *p*H 7.2 phosphate buffer and examined for the presence of malaria parasites at 100 X oil immersion objective. Giemsa stock solution and phosphate buffer were prepared from commercially available powder. Automated haematology analyzer (Mindray/BC-5150) was used for analyzing the following red cell indices HGB, PCV, RBC, MCHC, MCH, MCV, RDW-CV and RDW-SD. The data obtained was analyzed using SPSS version 21. The results were expressed as percentage and Mean ±SD. Comparison was made using ANOVA, paired comparison was carried out using the student t-test and ($p \leq 0.05$) was considered significant.

## 3. RESULTS

Table 1 showed mean ± SD of the HGB, PCV, RBC, MCHC, MCH, MCV, RDW-CV and RDW-SD of pregnant women and non-pregnant women. The RDW-SD of pregnant women (54.22 +/- 11.45 fl) were significantly higher compared with non-pregnant women (48.75 +/- 10.24 fl), ($p = 0.002$). However, HGB, PCV, RBC, MCHC, MCH, MCV, RDW-CV were not significant. Table 2 showed no significant differences on all the parameters compared. Table 3 Compared the mean ± SD of the HGB, PCV, RBC, MCHC, MCH, MCV, RDW-CV and RDW-SD of pregnant

**Table 1. Mean ± SD of red cell indices of pregnant women positive to malaria parasite without symptom and apparently healthy non-pregnant**

| Parameters | test (n=65) | control (n=26) | p-value |
|---|---|---|---|
| RBC ($10^{12}$/l) | 3.86±0.75 | 4.27±0.36 | 0.896 |
| HGB (g/dl) | 10.19±1.72 | 11.61±0.87 | 0.563 |
| PCV (%) | 32.43±5.28 | 36.62±2.05 | 0.162 |
| MCV (fl) | 85.22±10.79 | 85.25±10.35 | 0.984 |
| MCH(pg) | 26.82±3.86 | 27.38±2.68 | 0.354 |
| MCHC (g/l) | 314.56±18.10 | 316.61±14.40 | 0.394 |
| RDW-CV (%) | 16.82±4.43 | 14.85±2.76 | 0.888 |
| RDW-SD (fl) | 54.22±11.45 | 48.75±10.24 | 0.002* |

**Table 2. Mean ± SD Red cell indices of pregnant women that tested positive for malaria and pregnant women that tested negative**

| Parameters | (+) positive (n=65) | (- ) negative (n=25) | p-value |
|---|---|---|---|
| RBC ($10^{12}$/l) | 3.86±0.75 | 3.91±0.65 | 0.832 |
| HGB (g/dl) | 10.19±1.72 | 10.211±1.30 | 0.191 |
| PCV (%) | 32.43±5.28 | 32.76±4.31 | 0.564 |
| MCV (fl) | 85.22±10.79 | 84.85±10.03 | 0.342 |
| MCH(pg) | 26.82±3.86 | 26.50±3.56 | 0.137 |
| MCHC (g/l) | 314.56±18.10 | 312.53±19.86 | 0.144 |
| RDW-CV (%) | 16.82±4.43 | 16.98±3.77 | 0.562 |
| RDW-SD (fl) | 54.22±11.45 | 54.12±11.07 | 0.953 |

**Table 3. Mean ± SD of red cell indices of pregnant women with one plus and two pluses of malaria**

| Parameters | one plus (n=19) | two pluses (n=46) | p-value |
|---|---|---|---|
| (1 to 10 parasites per 100 thick film fields) | | (11 to 100 parasites per 100 thick film fields) | |
| RBC ($10^{12}$/l) | 3.89±0.86 | 3.86±0.77 | 0.848 |
| HGB (g/dl) | 9.82±2.03 | 10.421±1.75 | 0. 137 |
| PCV (%) | 31.72±6.39 | 32.76±5.28 | 0.383 |
| MCV (fl) | 82.68±10.34 | 86.19±10.98 | 0.115 |
| MCH(pg) | 25.63±3.86 | 27.42±3.86 | 0.669 |
| MCHC (g/l) | 309.16±20.17 | 318.03±16.31 | 0.011* |
| RDW-CV (%) | 17.59±5.02 | 16.21±4.40 | 0.220 |
| RDW-SD (fl) | 55.05±14.62 | 63.34±13.61 | 0.440 |

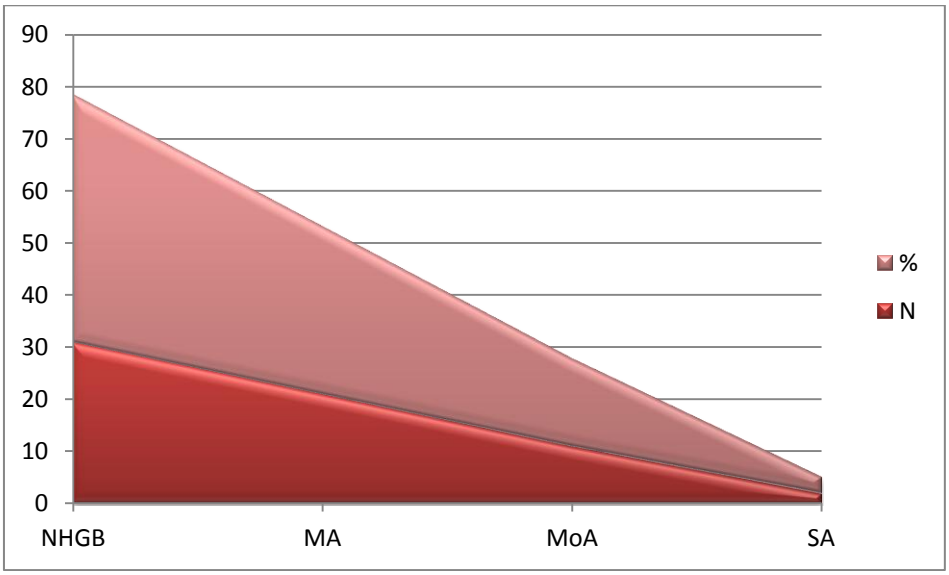


**Fig. 1. Incidence and severity of anaemia in pregnant women**

*Abbreviation: NHGB=normal haemoglobin, MA-=mild anaemia, MoA= moderate anaemia, SA= severe anaemia, N=number, % =percentage*

women that had one plus and two pluses. Mean cell haemoglobin concentration of pregnant women that had one plus (309.16 +/- 20.17 g/l) were significantly lower compared with pregnant women that had two pluses (318.03 +/- 16.31 g/l), (p = 0.011). Table 4 compared HGB, PCV, RBC, MCHC, MCH, MCV, RDW-CV and RDW-SD at first, second and third trimesters. The HGB (7.63 +/- 1.36 vs 11.64 +/- 0.72 g/dl) and PCV (26.98 +/- 5.14 vs 36.20 +/- 2.19 %) of pregnant women at third trimester were significantly lower compared with first trimester (p = <0.001, <0.001). Again, the RDW-SD (59.04 +/- 15.19 vs 49.16 +/- 7.00 fl) of pregnant women at third trimester were significantly higher compared to first trimester (p=0.03). Fig. 1 showed that overall prevalence of anaemia observed in pregnant women with asymptomatic malaria was 34 (52.3%), out of which 21 (32.3%) had mild anaemia (HGB level 9.0-10.0 g/dl), 11 (16.9%) had moderate anaemia (HGB level 7.0-8.0 g/dl) and 2 (3.1%) had severe anaemia (HGB level <7.0 g/dl).

## 4. DISCUSSION

The present study was conducted on pregnant women attending antenatal at Enugu State University of Science and Technology Teaching Hospital (ESUTH) Enugu, South East Nigeria. Studies had shown that antenatal care attendance may protect against neonatal mortality [7-9] Conditions such as anaemia, malaria, hepatitis, human immunodeficiency virus are usually screened for during antenatal visit [10-13]. Anaemia is one of the most common medical conditions in pregnancy with a prevalence of 36.5% globally according to WHO report in 2019 [14]. Recent evaluation of anaemia in pregnancy and in the low and middle income countries including Nigeria put the prevalence at 56% [15]. In this study, 52.3% prevalence of anaemia was observed which is slightly lower than 67.4% early reported from Enugu [16] and 59.6% reported from Calabar [17]. Defective hematopoeisis, bleeding and increased destruction of red blood cells are the three major causes of anaemia in pregnancy. Malaria brings about increased hemolysis of parasitized red blood cells. The extent of hemolysis depends on the strain of parasites [18]. In this study decreased HGB which is number one biomarker of anaemia [18] was observed. In this study increased RDW in pregnant women was also observed. Anisocytosis is measured by the red blood cell distribution width (RDW), demonstrated as the ratio between the standard deviation (SD) of red blood cell volumes and the mean cell volume (MCV), multiplied by 100 (red cell distribution width coefficient of variation), or as the standard deviation of erythrocyte volumes (red cell distribution width standard deviation). Anisocytosis defined as unequal variation in size of red blood cells, is identified by a high intrinsic plasticity of the external membrane and decrease

**Table 4. Mean ± SD of red cell indices of pregnant women with malaria parasite at first, second and third trimesters**

| | HGB (g/dl) | PCV (%) | RBC (x10$^{12}$/l) | MCV (fl) | MCH (pg) | MCHC (g/dl) | RDW-SD (fl) | RDW-CV (%) |
|---|---|---|---|---|---|---|---|---|
| FT (N=24) | 11.64±0.37 | 36.20±2.19 | 4.17±0.37 | 83.44±19.79 | 27.57±3.43 | 317.17±12.69 | 49.16±7.00 | 15.00±2.64 |
| ST (N=20) | 11.03±0.73 | 34.62±2.76 | 4.02±0.48 | 86.94±9.51 | 27.41±2.51 | 316.20±17.43 | 54.81±12.65 | 16.37±3.60 |
| TT (N=21) | 7.63±1.36 | 26.98±5.14 | 2.91±0.61 | 83.30±13.71 | 26.13±5.14 | 312.86±22.70 | 59.04±15.19 | 18.96±5.89 |
| F(p) value | 106.22 (<0.001) | 41.75 (<0.001) | 41.11 (<0.001) | 0.38 (0.69) | 0.90 (0.41) | 0.35 (0.71) | 3.91 (0.03) | 5.04 (0.01) |
| FT VS ST | 0.22 | 0.11 | 0.50 | 0.73 | 0.58 | 0.98 | 0.19 | 0.35 |
| FT VS TT | <0.001* | <0.001* | 0.04* | 1.00 | 0.53 | 0.72 | 0.03* | 0.02 * |
| ST VS TT | <0.001* | <0.001* | 0.04* | 0.59 | 0.57 | 0.86 | 0.60 | 0.22 |

haemoglobin content, which enable certain levels of contraction or expansion in reaction to physiological or pathological stimuli [19,20]. Red blood cell distribution width is very useful in the differential diagnosis of anaemias and other pathological conditions that can usher anisocytosis. Increased RDW values shows the presence of anisocytosis, that may be trace to the presence of small and large red blood cells or both, while values less than the lower limit of the reference interval are rare and clinically insignificant [21-23]. Studies had shown that there is sudden rise in RDW during the last 4-6 weeks of pregnancy which is showing the onset of labor and it is a sign of increased bone marrow activity. Increased RDW observed in pregnant women at third trimester in this study is in line with study done by Shehata *et al*., (1998) who observed that RDW increased significantly between 34 weeks of gestation and the onset of labor [24]. Mean corpuscular hemoglobin concentration is one of the RBC indices that can be used to diagnose and classify anaemia. The purpose of MCHC is to determine whether RBC is carrying an adequate amount of haemoglobin. Mean corpuscular hemoglobin concentration determines the concentration of hemoglobin in a RBC relative to the size of the cell itself [25-27]. In this study decrease in MCHC was observed in those with one plus of malaria, which is similar with the work done by Ahmed et al., (2021) where mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration (MCHC) were significantly lower in *P. falciparum*-infected patients [28]. Again higher MCHC which suggest folate or vitamin $B_{12}$ deficiency were seen in those with two pluses of malaria. The observation is in line with study done on pregnant women at booking by Adesina et al., (2009) [29]. The burden of anaemia and malaria in Nigeria and Enugu State requires urgent combined interventions and cost-effective measures to address the underlying causes.

## 5. CONCLUSION

This study found malaria, anaemia, significant decrease in haemoglobin, packed cell volume, higher MCHC and significant increase in red cell distribution in pregnant women. This study therefore recommends that good nutrition be encouraged among pregnant women in Enugu.

## CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

Ethical clearance was obtained from the Ethics and Research Committee of Enugu State University of Science and Technology Teaching Hospital Enugu and written informed consent was obtained from all participants in the study.

## COMPETING INTERESTS

Author has declared that no competing interests exist.

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