Original Research Article

Erythrocyte indices in asymptomatic malaria infected pregnant women

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, hemoglobin (HGB), packed cell volume (PCV), red blood cell count (RBC), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell 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 9.0-10.0 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 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

This study was carried out in Enugu State University of Science and Technology Teaching Hospital (ESUTH) Enugu, South East Nigeria. Subjects for this study included 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. 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.

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. Automated haematology analyzer (Mindray/BC-5150) was used for analyzing the 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. RESULT

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 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). Figure 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).

Table1: 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: Red cell indices of pregnant women that showed one plus of malaria and two pluses of malaria

Parameters	one plus (n=19) (1 to 10 parasites per 100 thick film fields)	two pluses (n=46) (11 to 100 parasites per 100 thick film fields)	p-value
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

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	41.75	41.11	0.38	0.90	0.35	3.91	5.04
	(<0.001)	(<0.001)	(<0.001)	(0.69)	(0.41)	(0.71)	(0.03)	(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

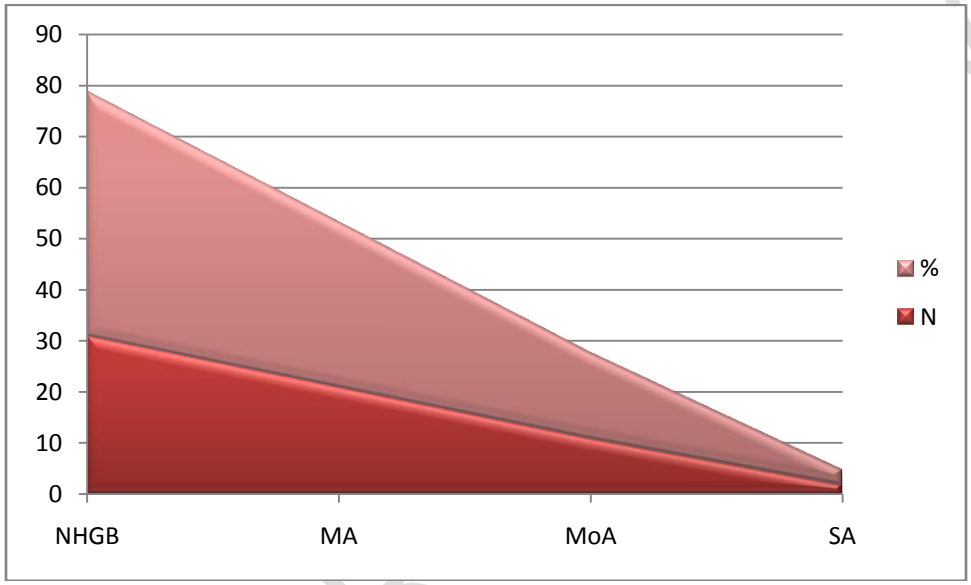

Fig 1: Incidence and severity of anaemia in pregnant women
Abbreviation: NHGB=normal haemoglobin, MA-=mild anaemia, MoA= moderate anaemia, SA= severe anaemia, N=number, % =percentage

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]. In this study increased RDW in pregnant women was 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 haemoglobin content, which enable

certain levels of contraction or expansion in reaction to physiological or pathological stimuli [14, 15]. 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 [16-18]. 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 [19]. 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 [20-22]. In this study decrease in MCHC was observed, which is similar with the work done by Ahmed *et a*l., (2021) where mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration (MCHC) were significantly lower in *P. falciparum*-infected patients [23]. Anemia is one of the commonest medical conditions in pregnancy with a prevalence of 50% worldwide [24]. The 52.3% prevalence of anaemia observed in this study is slightly lower than 67.4% early reported from Enugu [25] and 59.6% reported from Calabar [26].

CONCLUSION

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

REFERENCES

1. Segun O., Shohaimi S., Nallapan M., Lamidi-Sarumoh A., Salari N. Statistical Modelling of the Effects of Weather Factors on Malaria Occurrence in Abuja, Nigeria. International Journal of Environmental Research and Public Health.2020; 17(10):3474-3485.

2. Oluwagbemiga, A., Bamidele, A., Babatunde, A., Chimere, A., Medinat, S., & Olalekan, R. Prevalence of Malaria in Pregnant Women Attending Antenatal Clinic in Primary Health Centres in Lagos. Southwest, Nigeria. Journal of Advances in Medicine and Medical Research, 2018; 25(12), 1–9. https://doi.org/10.9734/JAMMR/2018/39620

3. Onyemaechi, N. and Malann, Y. Malaria Prevalence Investigation among Pregnant Women in Relation to their Social Well Being: A Case Study of Lugbe and Gosa, Abuja, Nigeria. International Journal of Pathogen Research. 2020; 4 (2), 7–15. https://doi.org.10.9734/ijpr/2020/v4i230107

4. World Health Organization, (2017) Malaria in Pregnant Women? http://www.org/who.int/malaria/areas/high-risk groups/pregnancy/en/.
5. Olukosi A, and Afolabi B. malaria and anemia among pregnant women living in communities along the coast of Lagos Lagoon, South-west Nigeria. International Journal of Pregnancy and Childbirth.2018; 4 (6),175–182. https://doi/10.15406/ipcb.2018.04.00122
6. Sharma L, Shukla G. placental malaria: A New Insight into the Pathophysiology. Front Med (Lausanne). 2017; 4:117. https://doi.org/10.3389/fmed.2017.00117
7. Darmstadt GL, Bhutta ZA, Cousens S, Adam T, Walker N, De Bernis L, et al. Evidence-based, cost-effective interventions: how many newborn babies can we save? The Lancet. 2005; 365(9463):977–88.
8. Raatikainen K, Heiskanen N, Heinonen S. Under-attending free antenatal care is associated with adverse pregnancy outcomes. BMC Public Health. 2007; 7(1):268.

9. 10. CHEN XK, Wen SW, Yang Q, Walker MC. Adequacy of prenatal care and neonatal mortality in infants born to mothers with and without antenatal high-risk conditions. Australian and New Zealand Journal of Obstetrics and Gynaecology. 2007; 47(2):122–7.
10. National Collaborating Centre for Women's Children's Health. Antenatal care: Routine Care for the Healthy Pregnant Woman. London, UK: RCOG Press. 2008.
11. World Health Organization. WHO statement on antenatal care. Geneva: WHO. 2011.
12. Villar J, Ba'aqeel H, Piaggio G, Lumbiganon P, Miguel Belizan J, Farnot U, et al. WHO antenatal care randomised trial for the evaluation of a new model of routine antenatal care. Lancet. 2001; 357(9268):1551–64.
13. Menendez C, D'Alessandro U, ter Kuile FO. Reducing the burden of malaria in pregnancy by preventive strategies. Lancet Infectious Diseases. 2007; 7(2):126–35.
14. Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: A simple parameter with multiple clinical applications. Crit Rev Clin Lab Sci. 2015; 52:86–105.
15. Lippi G, Salvagno GL, Guidi GC. Red blood cell distribution width is significantly associated with aging and gender. Clin Chem Lab Med. 2014; 52:e197–9.
16. Li A, Yang S, Zhang J, Qiao R. Establishment of reference intervals for complete blood count parameters during normal pregnancy in Beijing. J Clin Lab Anal. 2017;e22150.
17. Borné Y, Smith JG, Melander O, Engström G. Red cell distribution width in relation to incidence of coronary events and case fatality rates: a population-based cohort study. Heart. 2014; :1119–24.
18. Koma Y, Onishi A, Matsuoka H, Oda N, Yokota N, Matsumoto Y, et al. Increased red blood cell distribution width associates with cancer stage and prognosis in patients with lung cancer. PLoS One. 2013; 8:e80240.
19. H A Shehata [1], M M Ali, J C Evans-Jones, G J Upton, I T Manyonda Red cell distribution width (RDW) changes in pregnanc Int J Gynaecol Obstet. 1998; 62(1):43-6. doi: 10.1016/s0020-7292(98)00069-1.
20. T. Ueda, R. Kawakami, M. Horii et al., "High mean corpuscular volume is a new indicator of prognosis in acute decompensated heart failure," *Circulation Journal*, 2013; 77 (11): 2766–2771,
21. A. E. Åsberg, G. Mikkelsen, M. W. Aune, and A. Åsberg, "Empty iron stores in children and young adults-the diagnostic accuracy of MCV, MCH, and MCHC," *International Journal of Laboratory Hematology*,2014; 36(1): 98–104,.
22. M. Marković Boras, I. Brizić, and I. Mikulić, "The significance of red cell distribution width and homocysteine values in STEMI patients undergoing PCI in the population of Bosnia and Herzegovina," *European Review for Medical and Pharmacological Sciences*, 2021; 25 (10):3791–3797.
23. Ahmed M. E. Elkhalifa, Rashad Abdul-Ghani, Abdelhakam G. Tamomh, Nur Eldin Eltaher, Nada Y. Ali, Moataz M. Ali, Elsharif A. Bazie, Aboagla KhirAlla, Fatin A. DfaAlla, and Omnia A. M. Alhasan . Hematological indices and abnormalities among patients with uncomplicated falciparum malaria in Kosti city of the White Nile state, Sudan: a comparative study. BMC Infect Dis. 2021; 21: 507. . doi: 10.1186/s12879-021-06228-y
24. Omigbodun AO. Recent trends in the management of anaemia in pregnancy. Trop J Obstet Gynaecol. 2004;21(1):1–3.
25. Iloabachie GC, Meniru GI. Increasing incidence of anaemia in pregnancy in Nigeria. Orient J Med. 1990;2(4):194–198.
26. Agan TU, Ekabua JE, Udoh AE, Ekanem EI, Efiok EE, Mgbekem MA. Prevalence of anemia in women with asymptomatic malaria parasitemia at first antenatal care visit at the University of Calabar Teaching Hospital, Calabar, Nigeria Int J Womens Health. 2010; 2: 229–233. doi: 10.2147/ijwh.s11887