



Thrombocytopenia in pregnant women with malaria parasite

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Abstract

Thrombocytopenia is a condition that occurs when platelet count is below 150,000 platelets per microlitre of circulating blood. The assumed structure that cause thrombocytopenia during malaria infection are: the involvement of platelet as cofactor in causing severe malaria, coagulation disruptions, bone marrow changes, splenomegaly, oxidative stress and antibody-mediated platelet destruction. The aim of the study was to determine the platelet count and platelet indices of pregnant women infected with malaria parasite. Sixty five pregnant women identified as positive for malaria by microscopy (PfMbm), twenty five pregnant women identified as negative for malaria by microscopy (NfMbm) and twenty six non-pregnant women were enrolled. Platelet count (PC), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), platelet-large cell ratio (P-LCR), platelet large cell coefficient (P-LCC) and total white blood cell count (TWBC) were measured by automated haematology analyzer. The case control study was conducted between June and September 2022. Among 65 pregnant women identified as PfMbm, 6 (9.2%) had mild thrombocytopenia ($100 < 150 \times 10^9/L$), 4 (6.2%) had moderate thrombocytopenia ($50 < 100 \times 10^9/L$) and 3 (4.6%) had severe thrombocytopenia ($< 50 \times 10^9/L$). P-LCC levels were $67.33 \pm 19.05 \times 10^3/\mu l$ in pregnant women identified as PfMbm and $57.76 \pm 15.74 \times 10^3/\mu l$ in control group $p=0.003$. Platelet counts were $295.13 \pm 95.00 \times 10^9/L$ in NfMbm group and $233.50 \pm 106.64 \times 10^9/L$ in PfMbm group $p < 0.001$, whereas P-LCC were $67.33 \pm 19.05 \times 10^3/\mu l$ in PfMbm and $74.89 \pm 23.68 \times 10^3/\mu l$ in NfMbm group $p=0.018$. Also PC were $279.24 \pm 82.16 \times 10^9/L$ in PfMbm with one plus and $234.71 \pm 84.14 \times 10^9/L$ PfMbm group with two pluses $p=0.022$. The comparison between the first, second and third trimester showed significant decrease in PC $176.19 \pm 77.51 \times 10^9/L$ in third trimester compared to first trimester $225.17 \pm 51.51 \times 10^9/L$ $p=0.049$. The study showed presence of mild, moderate and severe thrombocytopenia, significant increase in platelet large cell coefficient in pregnant women identified as positive for malaria by microscopy. The study recommends regular malaria test and platelet count in pregnant women during antenatal period.

Keywords: Malaria, platelet, pregnant, thrombocytopenia

Introduction

Globally and at sub-Saharan Africa regional levels, malaria infection still account for highest mortality compared to all other infectious diseases put together. The main burden of malaria disease is in sub-Saharan Africa, with Nigeria having a 25% burden and ranking number one in central Africa and 50% of the global burden [1]. Platelets are particles that developed from the cytoplasm fragmentation of megakaryocytes, and for many years, these structures were observed only by its action on haemostasis. The event is changing and more work has shown the involvement of platelets in inflammatory process and infectious diseases, including malaria [2-4]. Thrombocytopenia is one of the most common problems of the three major species of malaria (plasmodium falciparum, p. vivax and p. knowlesi) that affected humans. Haematological anomalies such as thrombocytopenia and anaemia have been seen in malaria patients with thrombocytopenia being the most common [5]. Although thrombocytopenia is a definitive diagnostic marker but predictive implications could change in different types of malaria. A finding of thrombocytopenia in patients should heighten the suspicion of malaria and these will lead to more diligent search for the malaria parasite and

performance of specific tests like multiple peripheral blood smears and enzyme linked immunosorbent assay for parasite-specific antigen. Thrombocytopenia is an early sign for acute malaria. But the cause of thrombocytopenia in malaria is poorly understood; however, increased platelet destruction and decreased platelet lifespan normal happen during malaria, which is often linked to palpable splenomegaly and circulating immune complexes [6, 7]. The findings of this study may give appropriate strategies for malaria interventions at Enugu. It could also help Enugu State University of Science and Technology Teaching Hospital, Enugu Nigeria to recognize the presence of malaria induced thrombocytopenia in pregnant women for an improved pregnancy outcome.

Materials and methods

This case control study was conducted at Enugu State University of Science and Technology Teaching Hospital, during the period of June and September, 2022 to detect malaria parasite, evaluate total white blood cells, thrombocyte number, platelet indices and to establish thrombocytopenia in pregnant women positive to malaria. Two point five ml of venous blood was collected from each

pregnant and non pregnant woman under sterile condition and drained into Ethylene Diamine Tetra acetic Acid (EDTA) container with total white blood cells, thrombocyte number and platelet indices estimated using automated hematological analyzer Mindray/BC-5150. Thick smears were prepared on clean glass slide using venous blood. The blood smears were stained with 3% Giemsa working solution diluted in pH 7.2 phosphate buffer and examined for the presence of malaria parasites at 100 X oil immersion objective. All Data obtained were analyzed using statistical package for social science (SPSS) version 21.0. ANOVA, paired t test and Pearson correlation coefficient were used. Significant level was set at (P value ≤ 0.05). The approval for this study was given by the Research Ethics Committee

of Enugu State University of Science and Technology Teaching Hospital (ESUTH) Enugu. Enrolled pregnant women were informed about the study objectives. Informed consent was obtained from each of them.

Result

Table1 showed mean ± SD of total white blood cell count (TWBC), platelet count and platelet indices of pregnant women and non-pregnant women. The platelet large cell coefficient of pregnant women (67.33±19.05 x 10³/μl) were significantly higher compared with non-pregnant women (57.76±15.74 x 10³/μl), (p = 0.003). However, other parameters were not significant.

Table 1: mean ± SD of total white blood cell count and platelet indices of pregnant women and control subjects

Parameters	test (N=65)	Control (N=26)	p-value
TWBC (10 ⁹ /L)	6.75±3.31	7.26±2.40	0.314
PC (10 ⁹ /L)	233.50±106.64	234.45±75.00	0.955
MPV (fl)	10.22±1.21	9.94±0.98	0.160
PDW (%)	15.72±1.87	15.98±0.43	0.271
PCT (%)	2.30±0.99	2.08±0.74	0.167
P-LCC (10 ³ /μl)	67.33±19.05	57.76±15.74	0.003*
P-LCR (%)	29.54±8.76	27.95±6.66	0.242

Table 2 compared mean ± SD of total white blood cell count and platelet indices of pregnant women that tested positive and also those that tested negative to malaria parasite. The platelet count of pregnant women positive to malaria (233.50 ± 106.64 x 10⁹/L) were significantly lower compared with pregnant women negative to malaria (295.13

± 95.00 x 10⁹/L), (p = <0.001). The platelet large cell coefficient of pregnant women positive to malaria (67.33±19.05 %) were significantly lower compared with pregnant women negative to malaria (74.89 ± 23.68 %), (p = 0.018)

Table 2: Comparison of total white blood cell count and platelet indices of pregnant women that tested positive and also those that tested negative to malaria parasite

Parameters	+ (N=65)	- (N=25)	p-value
TWBC (10 ⁹ /L)	6.75±3.31	6.41±2.28	0.508
PC (10 ⁹ /L)	233.50±106.64	295.13±95.00	<0.001*
MPV (fl)	10.22±1.21	10.29±0.75	0.685
PDW(%)	15.72±1.87	15.93±0.65	0.411
PCT (%)	2.30±0.99	2.42±1.09	0.476
P-LCC(10 ³ /μl)	67.33±19.05	74.89±23.68	0.018*
P-LCR(%)	29.54±8.76	30.69±5.91	0.388

+ =positive, - =negative

Table 3: Comparison of mean ± SD of TWBC, platelet count and platelet indices of pregnant women that had one plus and two pluses. The platelet count of pregnant women that had one plus (279.24 ± 82.16 x 10⁹/L) were significantly higher compared with pregnant women that

had two pluses (234.71 ± 84.14 x 10⁹/L), (p = 0.022). The platelet large cell ratio of pregnant women that had one plus (33.26±8.82 %) were significantly higher compared with pregnant women that had two pluses (28.44 ± 6.91 %), (p = 0.007)

Table 3: Comparison of total white blood cell count and platelet indices of pregnant women that had one plus and two pluses of malaria parasite

Parameters	+ (N=19)	++ (N=46)	p-value
TWBC (10 ⁹ /L)	6.40±2.83	6.78±3.50	0.571
PC (10 ⁹ /L)	279.24±82.16	234.71±84.14	0.022*
MPV (fl)	10.94±0.92	10.68±0.53	0.093
PDW(%)	15.27±3.02	15.96±0.58	0.131
PCT (%)	2.17±1.21	2.34±0.92	0.495
P-LCC(10 ³ /μl)	64.27±33.18	64.18±22.56	0.988
P-LCR(%)	33.26±8.82	28.44±6.91	0.007*

+ = one plus, ++ = two pluses

Table 4 compared of TWBC, platelet count and platelet indices of pregnant women between first, second and third trimesters. The platelet count of pregnant women at third trimester (176.19 ± 77.51 x 10⁹/L) were significantly lower

compared with their platelet count at first trimester (225.17± 51.51 x 10⁹/L), (p = 0.04). However, other parameters were not significant.

Table 4: Comparison of total WBC count, platelet count and platelet indices between the trimesters by ANOVA

	TWBC (10 ⁹ /L)	PC (10 ⁹ /L)	MPV (fl)	PDW (%)	PCT (%)	P-LCC (10 ³ /μl)	P-LCR (%)
1T (N=24)	6.28±2.04	225.17±51.51	10.31±0.84	15.92±0.46	2.38±0.69	57.38±13.03	29.76±6.17
2T (N=20)	6.65±3.71	207.55±45.56	10.20±1.47	15.33±3.24	2.40±0.93	66.35±17.98	30.40±8.90
3T (N=21)	7.34±4.00	176.19±77.51	10.10±1.34	15.89±0.78	2.08±1.32	49.57±12.47	29.90±9.18
F(p) value	0.59	3.83	0.19	0.66	0.67	6.80	0.04
	(0.56)	(0.03)	(0.83)	(0.52)	(0.51)	(0.00)	(0.96)
1T VS 2T	0.92	0.46	0.10	0.70	0.99	0.17	0.96
1T VS 3T	0.52	0.04 *	0.81	0.99	0.62	0.11	0.10
2T VS 3T	0.83	0.27	0.90	0.74	0.65	0.00*	0.98

Table 5 shows the correlation between TWBC, platelet count and platelet indices. There was a strong positive correlation between PCT, P-LCC and platelet count, which was significant ($P<0.001$ and $P<0.001$). There was also a strong negative correlation between MPV, P-LCR and platelet count, which was significant ($P=0.001$ and $P<0.001$).

Table 5: Pearson correlation coefficient between TWBC, platelet count and platelet indices of pregnant women positive to malaria

Platelet indices	TWBC		platelet count	
	r	p-value	p-value	R p-value
MPV	-0.053	0.676	-0.410	0.001**
PDW	-0.115	0.360	-0.139	0.270
PCT	0.159	0.205	0.970	<0.001**
P-LCC	0.139	0.270	0.789	<0.001**
P-LCR	-0.105	0.405	-0.475	<0.001**

The overall prevalence of thrombocytopenia observed in pregnant women identified as PFMbM was 13 (20%), out of which 6 (9.2%) had mild thrombocytopenia ($100<150 \times 10^9/L$), 4 (6.2%) had moderate thrombocytopenia ($50<100 \times 10^9/L$) and 3 (4.6%) had severe thrombocytopenia ($<50 \times 10^9/L$) (fig 1).

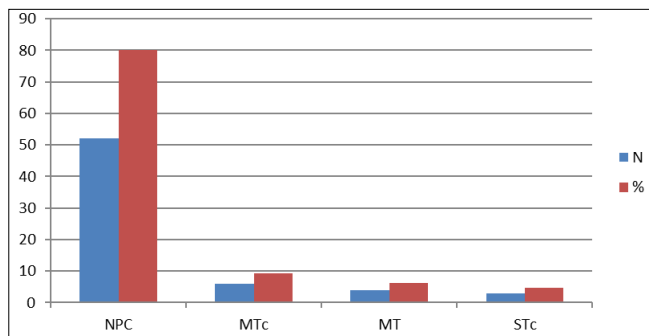


Fig 1: incidence and severity of thrombocytopenia in pregnant women that tested positive to malaria parasite

Abbreviations: NPC= normal platelet count, MTc=mild thrombocytopenia, MT= moderate thrombocytopenia, STc= severe thrombocytopenia

Discussion

This study was conducted in order to determine platelet count and platelet indices of pregnant women infected with malaria parasite. Platelet indices are biological markers of platelet activation. Platelet indices includes mean platelet volume, platelet distribution width, plateletcrit, platelet large cell ratio, platelet large cell coefficient are set of determined platelet parameters obtained as a part of the automated complete blood count. Studies demonstrated that platelet

indices may allow substantial clinical investigations concentrating on the diagnostic and prognostic values in a range of settings without leading into extra costs [8-11]. Platelet large cell coefficient is platelets larger than 12fl and smaller than 30fl. Our study showed that Platelet large cell coefficients (P-LCC) were increased in pregnant women compared to non-pregnant women. The result is in line with study done by Osama, (2021), that observed P-LCR significantly increased in pregnant women compared to the apparent healthy pregnant women [12]. Low platelet counts during malaria infection are due to platelet activation, splenic pooling, and a reduced platelet life-span to two–three days from normal seven–ten days [13, 14]. Platelet count is commonly studied in patients with malaria infection in sub-Saharan Africa countries. But those studies have continuously found lower platelet counts in patients with malaria [15-18]. Supporting the above study that observed lower platelet counts in patients with malaria, we also found significantly low levels of platelet count in pregnant women with malaria infection compared to pregnant women without malaria infection. In this study, the mean platelet count in the first trimester was $225 \times 10^9 /l$ which significantly decreased in the third trimester. The result is in agreement with study done by Jessica *et al.*, (2018), where they reported that at time of delivery, 9.9% of the women with uncomplicated pregnancies had a platelet count below 150,000 per cubic millimeter [19]. Pregnancy itself can lead to decrease platelet count for reasons that are not fully understood [20]. Thrombocytopenia in malaria was normally asymptomatic. Sudden bleeding is rare unless the platelet count falls below 10, 000 per cubic millimeter [21] when the patient would be treated with platelet transfusion to make corrections on the red. Prevalence of thrombocytopenia among pregnant women with malaria parasites was also part of this study. In this study, the prevalence of thrombocytopenia among pregnant women with malaria was 20%, which was almost comparable to the result of study conducted by Parnas *et al.*, (2006) that reported prevalence of 21.8% [22]. However, the result of this study was slightly higher than result of studies conducted by Boehlen *et al.*, (2000), Shamoan *et al.*, (2009) Nisha *et al.*, (2012) and Vyas *et al.*, (2014) [23-26].

Conclusion

The study showed presence of mild, moderate and severe thrombocytopenia in pregnant women identified as positive for malaria by microscopy. The study recommends regular malaria test and platelet count in pregnant women during antenatal period.

Conflict of interests

Authors have declared that no competing interests exist

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