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Original Research Article

Platelet count and platelet indices of symptomatic and asymptomatic children infected by malaria in Calabar Metropolis, Nigeria

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ABSTRACT

Background: Malaria infection has been reported to be a major problem affecting developing countries. This study aimed at the effect of malaria parasite infection on platelets count, and platelet indices; mean platelet volume (MPV), platelets distribution width (PDW) and plateletcrit (PCT) among symptomatic subjects.

Methods: A case-control study design with a total of 112 subjects (males and females) comprising of 58 symptomatic and 54 asymptomatic children infected by malaria, age range of 0-15 years from the University of Calabar Teaching Hospital were enrolled. Thick and thin film method was used to ascertain malaria parasite density.

Results: The mean values of the symptomatic malaria subjects were found to be significantly reduced in PCT, PDW and PC (0.16±0.04, 15.84±0.89 and 181.45±43.62) and significant high in MPV and MPD (9.36±1.05 and (418.80±554.40) against that of asymptomatic malaria subject ($p<0.05$). The mean MPV was significantly increased in 6-10 and 11-15 years (9.35±0.90 and 9.81±1.16) when compared with 0-5 years (8.60±0.62) age range symptomatic malaria subject groups while mean PC was significantly decrease in 6-10 and 11-15 years (172.14±35.52 and 181.55±48.49) when compared to 0-5 years (204.36±36.92) age range groups. A moderate positive significant correlation between PCT versus MPV ($r=0.327$) while PC versus PCT shows a strong ($r=0.895$) positive significant correlation ($p<0.05$).

Conclusions: There is significant increase MPD and MPV, decrease PC, PCT and PDW in the symptomatic group compared to the asymptomatic. It is important that platelet indices and platelet count should be included as a routine screening investigation to detect malaria parasite infection and treated accordingly.

Keywords: Symptomatic malaria, Platelet count, Platelet indices, Calabar

INTRODUCTION

Malaria is one of the most important parasitic infectious diseases in the world. It is a major health problem in the tropics with high morbidity and mortality.[1,2] Malaria is responsible for 781,000 deaths annually, the majority of which are in Sub-Saharan Africa. *Plasmodium falciparum* and *Plasmodium vivax* are the most common. *P. falciparum* is the most deadly.[3,4] Several clinical complications have been described in malaria infection including severe anaemia, cerebral malaria, acute pulmonary oedema and multi-organ failure. Although thrombocytopenia is often occurred, but bleeding is rare in these patients.[5,6]

Platelets are cytoplasmic fragments of bone marrow megakaryocytes, with a diameter of 3-5 µm and a volume of 4.5-11 fl.[7] A single megakaryocyte releases 1500-2000 of them to the bloodstream, where they circulate for 7-10 days. Inactivated platelets in the blood are discoid shaped and do not contain a nucleus.[8,9] Platelet distribution width (PDW) is an indicator of volume variability in platelets size and is increased in the presence of platelet anisocytosis.[10] The PDW reported varies markedly, with reference intervals ranging from 8.3 to 56.6%.[11]

Plateletcrit (PCT) is the volume occupied by platelets in the blood as a percentage and calculated according to the formula,

$$PCT=\frac{platelet\ count \times MPV}{10{,}000}.$$

The normal range for PCT is 0.22-0.24%.[5,1213] Changes in platelet counts during acute malaria were reported in the several medical literatures, such as *P. falciparum* infections; these changes are the major cause of serious and complicated disease. Peripheral destruction, excessive sequestration of platelets in spleen and excessive use of platelets associated with the disseminated intravascular coagulation phenomenon are underlying mechanisms of thrombocytopenia in malaria infection.[14,15] In spite of the preventative measures, malaria remains a major public health concern. The WHO estimated that in 2015 there were 214 million new cases of malaria resulting in 438,000 deaths.[3,16] Haematological changes, especially decrease in platelet counts associated with malaria infection are well recognized.[17] The objective of the study was therefore to compare the platelet count and platelet indices and also correlate the parameters, in symptomatic and asymptomatic malaria-infected children.

METHODS

Study area

The area chosen for this research was the children emergency room (CHER), children ward and children outpatient (CHOP) clinic of University of Calabar Teaching Hospital, Calabar, Cross River State.

Subject selection

A case control study was used with a total of one hundred and twelve (112) children were selected from CHER, CHOP of University of Calabar Teaching Hospital, Calabar, Cross River State, between the period of November 2018 to May 2019. Ethical approval was obtained from the Health Research Ethical Committee (HREC) of the University of Calabar Teaching Hospital before commencement of the study with registration number UCTH/HREC/33/606. Informed consent was also gotten from their guardian/parent and questionnaire filled.

Inclusion criteria

Children between the ages of 6 months to 15 years of both gender and children who were willing to participate in the study after parental consent were included in the study.

Exclusion criteria

Children, pregnant women, adults who did not give their consent were excluded in the study.

Collection of samples

Three milliliters (3 ml) of blood was aseptically collected from each symptomatic and asymptomatic subjects using a venepuncture and 2.5 ml of blood was dispensed into ethylene diamine tetra-acetic acid (EDTA) container in the concentration of 1mg/ml (0.04 ml) which was used for both thick and thin film and platelet analysis (platelet count and platelet indices).

Preparation of thin and thick blood film for identification of malaria

Procedure

Six and two microliters (6 µl and 2 µl) of blood was used for making thick and thin film respectively on a clean grease free slide using a spreader. The frosted end of the slide was labeled with the code number for the subject. The films were allowed to air dry with the slide placed in a horizontal position.

Fixing and staining

The thin film was fixed by dipping one third of the smear into absolute methanol for few seconds making sure that the alcohol does not touch the thick film, the smear was flooded with 10% working Giemsa solution for 10 mins and the slide was rinsed with buffer of pH 7.2. The back of the stained slide was wiped and placed in a vertical position on a rack to air dry properly. The stained blood film was examined using ×100 objective with immersion oil.

The grading of parasitaemia (%malaria) as percentage was done after counting of all malaria forms in oil immersion on thick and thin smears (global health-division of parasitic diseases and malaria, 2015). All slides were double-checked in a blinded manner.

Calculation of Plasmodium parasitaemia

$$Density = \frac{number\ of\ parasite \times 8000}{WBC\ count}.$$

Full automatic blood cell counter

Principle

The principle stated that particles pulled through an orifice concurrent with an electric current, produced a change in impedance that was proportional to the volume of the particles transversing the orifice. This pulse in impedance originated from the displacement of electrolyte caused by the particle. The coulter principle was named for its inventor Wallace H. Coulter.

Data analysis

Statistical package for social sciences (SPSS) 20.0 was used in the analysis of the sample. Generalized data was presented in tables and figure. Student t test, one way analysis of variance and Pearson correlation was used in the analysis of the data. An error probability of ≤0.05 was considered significant.

Ethical approval

The ethical approval for this study was obtained from the Health Research Ethical Committee (HREC) of the University of Calabar Teaching Hospital.

RESULTS

This study was carried out to determine the effect of malaria parasite on platelet count and platelet indices comprising of PCT, PDW and mean platelet volume (MPV).

A total of one hundred and twelve (112) subjects (males and females) comprising of both symptomatic and asymptomatic malaria-infected children who were within the ranges of 0-15 years of age recruited from University of Calabar Teaching Hospital (CHOP and (CHER) were enrolled in this study. The mean age value of the symptomatic malaria-infected children (test subjects) was 8.44±4.01 while that of the asymptomatic malaria-infected children (control subjects) was 9.21±3.92.

Table 1: Comparison between the symptomatic and the asymptomatic group.

Parameters/Groups	Symptomatic n (58)	Asymptomatic n (54)	T	P value
Age (years)	8.44±4.01	9.21±3.92	-1.029	0.306
PCT (%)	0.16±0.04	0.22±0.03	-6.997	0.000*
MPV (fl)	9.36±1.05	8.59±0.73	4.409	0.000*
PDW (fl)	15.84±0.89	16.21±0.58	-2.573	0.002*
PC (×10^9/l)	181.45±43.62	246.06±41.20	-8.047	0.000*
MPD (p/µl)	418.97±591.87	120.80±80.00	3.599	0.001*

Values are expressed as mean ± standard deviation, PC=Platelet Count, MPD=Malaria Parasite Density, *=significant at p<0.05.

Table 2: Mean±SD of PCT, MPV, PDW, PC and MPD of symptomatic group based on gender.

Parameters/groups	Male n (32)	Female n (26)	T	P value
PCT (%)	0.17±0.05	0.17±0.04	-221	0.826
MPV (fl)	9.4±1.10	9.32±1.0	167	0.868
PDW (fl)	15.94±0.93	15.73±0.85	855	0.396
PC (×109/L)	178.91±46.26	184.58±40.83	-489	0.627
MPD (p/µl)	445.69±671.60	319.23±177.81	934	0.354

Values are expressed as mean±standard deviation, PC=platelet count, MPD=malaria parasite density.

Table 3: Mean±SD values of PCT, MPV, PDW, PC and MPD of symptomatic malaria subjects based on age.

Parameters/groups	0-5 years n (14)	6-10 years n (22)	11-15 years n (22)	F	P value
PCT (%)	0.17±0.04	0.16±0.03	0.17±0.05	1.251	0.294
MPV (fl)	8.60±0.62	9.35±0.90*	9.81±1.16*	7.054	0.002*
PDW (fl)	16.10±1.22	15.90±0.77	15.90±0.77	0.235	0.792
PC (×10^9/L)	204.36±36.92	172.14±35.52*	181.55±48.49*	2.466	0.094
MPD (p/µl)	351.43±198.57	549.09±859.70	330.00±211.64	0.989	0.378

Values are expressed as mean±standard deviation, PC=platelet count, MPD=malaria parasite density, *significant at p<0.05; post hoc for age. *significant to 0-5 years; a significant to 0-5 years. b significant to 6-10 years.

Table 1 shows the comparison between symptomatic and asymptomatic malaria subject. There was a significance difference in the PCT, MPV, PDW, PC and MPD. The mean values of the symptomatic malaria subjects were found to be significantly reduced in PCT, PDW and PC (0.16±0.04, 15.84±0.89 and 181.45±43.62) and significant high in MPV and MPD (9.36±1.05 and (418.80±554.40) when compared with the asymptomatic malaria subject (p<0.05).

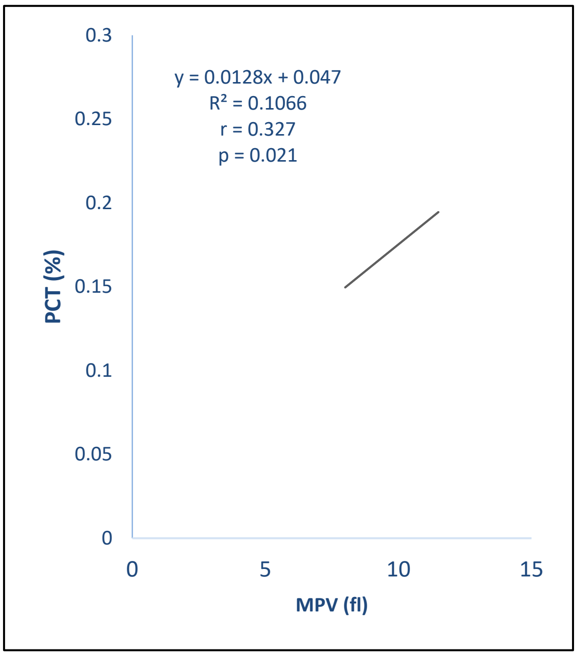

Figure 1: Correlation graph of PCT against MPV of symptomatic malaria subject.

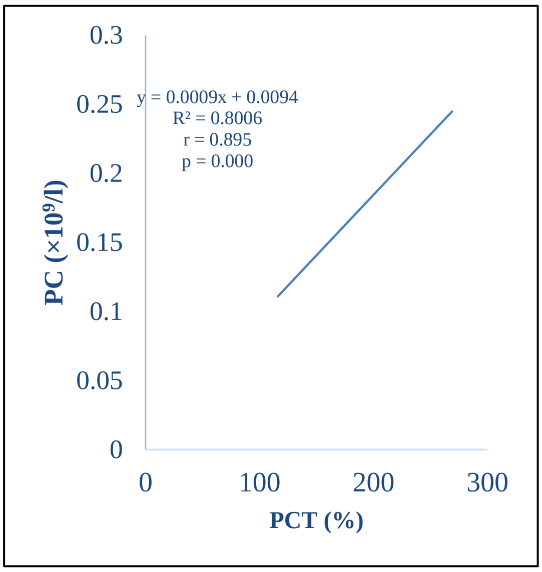

Figure 2: Correlation graph of PC against PCT of symptomatic malaria subject.

Table 2 show the mean±SD comparison within the symptomatic malaria subjects based on gender. The mean PCT, MPV, PDW, PC and MPD levels of symptomatic malaria subject were 0.17±0.05, 9.4±1.10, 15.94±0.93, 178.91±46.26, 445.69±671.60 for male and 0.17±0.04, 9.32±1.0, 15.73±0.85, 184.58±40.83, 319.23±177.81 for female respectively. It shows that there was no statistical significant difference (p>0.05) among the groups.

Table 3 shows the mean±SD comparison within the symptomatic malaria subjects based on age. There was no statistically significant (p>0.05) difference in PCT, PDW, PC and MPD, however, a significant difference (p<0.05) was observed in MPV of symptomatic. Malaria subjects when age was considered. The mean MPV was significantly increased in 6-10 and 11-15 years (9.35±0.90 and 9.81±1.16) when compared with 0-5 years (8.60±0.62) age range symptomatic malaria subject groups while mean PC was significantly decrease in 6-10 and 11-15 years (172.14±35.52 and 181.55±48.49) when compared to 0-5 years (204.36±36.92) age range groups.

Figure 1 and 2 shows the association between PCT versus MPV and PC versus PCT of symptomatic malaria subjects. There was a moderate positive significant correlation between PCT versus MPV (r=0.327) while PC versus PCT shows a strong (r=0.895) positive significant correlation (p<0.05).

DISCUSSION

Table 1 shows the result of the symptomatic subject and asymptomatic subject for PC, PCT, PDW, MPV and MPD. There was a significant difference in PCT, MPV, PDW, PC and MPD between symptomatic subject and asymptomatic subject (p<0.05). Hind et al reported similar study on 100 participants with significant difference between in symptomatic subject and asymptomatic subject.[19] In their study mean values of the symptomatic malaria subject were found to be significantly reduced in PCT, PC, MPV and MPD. It was reported that PDW was increased which was contrary to the result of the present study. This present study agreed with a study that was done Ekiti state, Nigeria with no contrary to the PDW which was decreased due to the uniformity in size of platelets as stated by Ifeanyichukwu et al.[20] There was no significant difference (p>0.05) of the symptomatic subject based on gender. Gender was not affected by PCT, MPV, PDW, PC and MPD parameters of malaria subjects in this study Table 2.

Table 3 shows the comparison within the symptomatic malaria subjects based on age. There was a significant difference (p<0.05) in PCT between 6-10 years and 11-15 years, MPV between 0-5 and 11-15 years and in PC between 0-5 and 6-10 years. This finding was in agreement with a study done in Calabar by Zaccheus et al in which there was difference in the platelet count within age 1-12 year in which it was significantly lower with a platelet count of (110±41 ×109/l) than the control group value of 116×109 /l (p<0.001).[21] There was a significant relationship between PCT versus MPV and PC versus

MPV of symptomatic malaria subject, in which there was a marked increase in MPV as platelet count decreased in symptomatic malaria subjects.

CONCLUSION

PC, MPV, PCT of symptomatic subjects were significantly decreased compared to asymptomatic subjects, there was a significant difference in platelet distribution width between symptomatic and asymptomatic subjects. Age of the symptomatic subject were not affected on platelets count and platelet indices with exception of mean platelet volume in which there was a difference between the age groups. Gender had no effects on platelets count and platelet indices. The result of this study revealed that there was a positive significant correlation between PCT versus MPV and PC versus PCT of symptomatic malaria subjects.

Recommendations

Routine investigation of platelet count and platelet indices with investigation of malaria for confirmation and monitoring of treatment in children should be carried out.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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