Journal of Natural Sciences Research
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.14, No.10, 2023

Plasmodium falciparum Infection Modulates Platelet Count than Leucocyte Parameters in Carriage of Different Haemoglobin Beta Subunit (*HBB*) Genotypes

Paul Kosiyo*

Department of Medical Laboratory Science, School of Medicine, Maseno University, Private Bag, Maseno, Kenya

* E-mail of the corresponding author: paulkosiyo@gmail.com

Abstract

Background: Sickle cell disease is a prime genetic disorder due to a single nucleotide mutation resulting in haemoglobin gene (HbS) occurring in the regions where malaria is endemic. Though it primarily a disease of erythrocytes, non-erythrocytic cells are equally affected just like in malaria infection. Furthermore, leucocytes and thrombocytes have equally been hypothesized to be the driving force for sickle cell crisis. However, the modulatory trends and magnitude of *Plasmodium falciparum* infection on platelet and leucocyte parameters in sickle cell disease is not entirely explored.

Objectives: The current study therefore determined the modulatory effects of *P. falciparum* infection on platelet and leucocyte parameters in carriage of different haemoglobin beta subunit (*HBB*) genotypes.

Methodology: This cross-sectional study evaluated leucocytes and thrombocytes parameters in *P. falciparum*-infected and non-infected children (n=217, aged 1-192 months) with different *HBB* genotypes. Children with acute febrile conditions were randomly selected and enrolled at Jaramogi Oginga Odinga Teaching and Referral Hospital for a period of ten months at outpatient clinic. Haematological parameters were determined using Beckman Coulter counter ACTdiff2™ while *HBB* genotyping was done using TaqMan® SNP genotyping assay. Chi-square (χ^2) analysis was used to determine differences between proportions. Mann-Whitney U test and Kruskal Wallis test were used for comparisons of demographic and laboratory characteristics wherever applicable. Partial correlation test was used to determine relationship between leucocytes and thrombocyte parameters in carriage of different *HBB* genotypes while adjusting for age and sex as covariates.

Results: Generally, there were no differences in WBC (p=0.746), lymphocytes (p=0.103), monocyte (p=0.084) and granulocytes (p=0.354) between the infected and non-infected children. Platelet count [median (IQR); 236 (129.5), p=0.001] and PCT, [median (IQR); 0.13 (0.1), p=0.001] were markedly reduced in infected children. Specifically, monocytes had a positive correlation with platelet count (r= 0.742, p=0.002) in non-infected children with HbSS genotype. WBC revealed a positive correlation with platelet count in both infected and non-infected children (r =0.358, r^2 =0.128, p =0.047 and r = -0.638, r^2 =0.407, p= 0.047) in carriage of HbSS respectively WBC and RDW in children infected with *falciparum* in carriage of HbSS genotypes (r =0.818, r^2 =0.669, p =0.004). Monocyte count revealed a positive correlation with platelet count in non-infected children and no correlation in children infected with malaria (r= -0.742, P=0.022 and r= -0.245, P=0.525, respectively).

Conclusion: *P. falciparum* infection is responsible for a decrease the platelet count as WBC count increases in children with HbSS. Therefore, a decrease in platelet count against leukocytosis in carriage of HbSS should warrant a test for *P. falciparum* infection.

Keywords: Correlation, Haemoglobin, Haemoglobin beta sub-unit, genotype, Leucocyte, Thrombocyte.

DOI: 10.7176/JNSR/14-10-05
Publication date: August 31st 2023

1. Introduction

Even if sickle cell disease (SCD) has been robustly explored to be primarily a disease of red blood cells, studies have demonstrated that leucocytes have are equally affected (Obimba et al., 2015b, Rees et al., 2022). On the other hand, platelets have equality been shown to be elevated in SCD and could cause complications in sickle cell such as vaso-occlusion (Shome *et al.,* 2018). It has been reported that vaso-occlusion is a major vasculopathy

around a cluster of vascular injuries characterized by endothelial cell dysfunction, leukocyte activation and thrombocyte activation (Manwani and Frenette, 2013). Specifically, platelets have been shown to release many inflammatory cytokines which alter leukocyte and endothelial responses to a variety of different inflammatory stimuli of which *P. falciparum* infection could be a prime instance. Additionally, a previous study on the role of platelets on inflammation demonstrated that platelets form aggregates with leukocytes and form bonds between leukocytes and endothelium, basically mediated by platelet P-selectin (Thomas and Storey, 2015). As a result, the current study is of the opinion that there could be a modulatory effect on leukocytes and platelets parameters as a result of *P. falciparum* infection whose magnitude trend remains unclear.

Both leukocyte and platelet counts alterations have been shown to occur in malaria infection (Hoffbrand *et al.,* 2010). Furthermore, a study in Morocco revealed that both leukocytosis and thrombocytosis are the determinants of sickle cell severity through vaso-occlusion (Rees et al., 2022) however, this did not consider malaria infection. The current study is therefore based on the hypothesis *that P. falciparum* infection can modulate both leucocyte and platelet parameters to unknown extent. Even though a study in Nigeria confirmed a correlation between sickle cell and leukocytosis (mean WBC count of 10.27 $\times 10^9$/l) (Akinbami *et al.,* 2012), this did not factor in *P. falciparum* malaria infection status and did not include sickle cell traits (heterozygotes).

On the other hand, thrombocytopenia (platelet count $<150\times10^3/\mu L$) have been correlated with severe anaemia (Erhabor et al., 2014, Mboya et al., 2016). However, the overlapping effects of thrombocytosis in SCA and thrombocytopenia in *P. falciparum* infection in children with different haemoglobin beta sub-unit (*HBB*) genotypes largely remains unclear and needs further exploration. To the best of my knowledge, there is paucity of data to demonstrate relationship between leukocytes and thrombocytes in carriage of different haemoglobin beta sub-unit (*HBB*) genotypes in malaria infection. As such, this study determined the modulatory effects of *P. falciparum* infection on platelet count and leucocyte parameters in carriage of different haemoglobin beta subunit (*HBB*) genotypes in children resident in Kisumu County western Kenya.

2. Materials and Methods

Study Site: The current study was conducted at Jaramogi Oginga Odinga Teaching and Referral Hospital in western Kenya. Details on the current study site has been previously described in a previous publication (Kosiyo *et al.,* 2020).

Study Population: This cross-sectional study targeted children aged 1-192 months and resident within Kisumu County presenting with acute febrile condition (temperature>37.5ºc). A detailed description of the study population is in a previous publication (Kosiyo *et al.,* 2020).

Inclusion criteria: Children found to be infected with *P. falciparum* infection upon demonstration of asexual forms of *P. falciparum* through microscopic examination of both thick and thin smear or uninfected with *P. falciparum* but presented with acute febrile condition to form one set of control. Participants who were HIV negative, children whose parents were willing and able to provide written informed consent were included in the study.

Exclusion Criteria: Children with earlier known forms of haemoglobinopathies like α-thalassaemia disorders, history of sickle cell crises and previous blood transfusion at least in the past 3 months, evidence of acute bacterial, viral infections and parasitic infections other than *P. falciparum*, and known sickle cell patients on hydroxyurea therapy.

Ethical consideration: Approval for the current study was obtained from Jaramogi Oginga Odinga Teaching and Referral Hospital Scientific and Ethics Review Committee (JOOTRH-ERC) - Approval NO. ERC.IB/VOL.1/414.

Laboratory procedures: The details of the laboratory procedures like measurement of complete blood count parameters, malaria microscopy for demonstration of any asexual form of *P. falciparum*, DNA extraction, purification and quantification as well as *HBB* genotyping have been described in details in the previous publication (Kosiyo *et al.,* 2020). In brief, Giemsa staining technique on thick and thin smears was used for detection and speciation of *P. falciparum* respectively. Haematological parameters were determined using the Beckman Coulter ACT diff2™ (Beckman-Counter Corporation, Miami, FL, USA) in accordance to local Standard Operating Procedures (SOPs) within one hour of blood collection.

DNA extraxtion was done using PureLink® DNA Mini Kit, (Invitrogen life technologies, USA) while DNA quality and quantity was evaluated using NanoDrop ND-1000 spectrophotometer (Thermofisher Scientific, San Diego,

CO, USA) and stored at -20ºC before use. Finally, functionally tested TaqMan® SNP Genotyping Assay (Life Technologies, Grand Island, NY) was used in accordance with manufacturer's instructions. Demonstration of haemoglobin S was from biallellic discrimination (missense change [Glu6VAL]) in the single nucleotide polymorphism rs334 using the following custom primer and probe sequences: Forward-TCAAACAGACACCATGGTGCAT, Reverse-CCCCACAGGGCAGTAACG, VIC-CTGACTCCTGAGGAGAA-MGB, 6FAM-CTGACTCCTGTGGAGAA-MGB, respectively.

Statistical analyses: Statistical analyses were performed using SPSS version 26.0 software (IBM, New York, USA). Chi-square (χ^2) analysis was used to determine differences between proportions. Mann-Whitney U test and Kruskal Wallis test were used for comparisons of demographic and laboratory characteristics wherever applicable. Partial correlation test was used to determine correlation between leucocyte and thrombocyte parameters in different *HBB* genotypes while controlling for age, sex and red blood cell parameters which have been shown to cause sickle cell crisis. R^2 for correlation coefficient (r) was further used to demonstrate the degree of variability that is responsible for change towards particular trend whenever there was a correlation and statistical significance at a $p < 0.05$.

3. Results

Clinical and Laboratory characteristics of study participants: Children (n=217, aged 1-192 months) presenting with severe fever (temperature >37.5˚C) were enrolled in the study. Children were initially largely categorized on the basis of malaria infection status upon blood side microscopy to demonstration of any asexual form of *P. falciparum* malaria (trophozoite or schizont) as non-infected (n=132) and infected (n=85). The proportion of sex (p=0.240) and age (p=0.143) were comparable between the two groups.

The white blood cell count (WBC) showed no difference between the two clinical groups p=0.746. Furthermore, the lymphocytes (p=0.103), monocytes (p=0.084) and granulocytes (p=0.354) were comparable between the two groups (Table 1). Further analysis of thrombocyte parameters revealed that platelet counts were reduced in the infected group [median (IQR), 236 (129.5)] versus non-infected group [median (IQR), 278 (112.8)], p=0.001, respectively. Additionally, children with *P. falciparum* infection further had reduced plateletcrit (PCT) [median (IQR), 0.13 (0.1)] relative to non-infected [median (IQR), 0.2 (0.1)], p=0.001. Further analysis revealed that mean platelet volume (MPV) was not different between the infected and non-infected groups (p=0.119). Platelet distribution width (PDW) analysis revealed that there were no statistical differences in the two groups (p=0.629) (Table 1).

Table 1. General Demographic and Laboratory Characteristics of the Study Participants

All the study participants (Infected and non-infected n=217)				***P. falciparum* infected children based on genotype (n=85)**			
Characteristics	**Non-Infected (n=132)**	**Infected (n=85)**	**p-value**	**HbAA (n=58)**	**HBAS (n=14)**	**HbSS (n=13)**	**p-Value**
Sex, n (%)							
Male	76 (57.6)	42 (49.4)	0.240[a]	30 (51.7)	7 (50)	8 (61.5)	0.690[a]
Female	56 (42.4)	43 (50.6)		28 (48.3)	7 (50)	5 (38.5)	
Age, (months)	30 (48)	36 (55)	**0.143[b]**	36 (64.5)	36 (32.3)	16 (27)	0.660[c]
Homozygous wild type and *HBB* genotypes							
HbAA, n (%)	90 (68.2)	58 (68.2)					
HbAS, n (%)	31 (23.5)	14 (16.5)	0.743[a]				
HbSS, n (%)	11 (8.3)	13 (15.3)					
Leucocyte Parameters							
WBC ($\times10^3\mu L^{-1}$)	8.5 (6.0)	8.6 (5.2)	0.746[b]	7.8 (4.8)	9.58 (5.2)	12.68 (4.6)	0.078[c]
Lymphocytes, ($\times10^3\mu L^{-1}$)	44.1 (25.3)	38 (66.9)	0.103[b]	36.55 (27.2)	37 (36.2)	50.9 (29.1)	0.596[c]
Monocytes, ($\times10^3\mu L^{-1}$)	9.3 (4.4)	11.2 (7.2)	0.084[b]	11.2 (7.4)	12 (7.8)	8.1 (6.1)	0.282[c]
Granulocytes, ($\times10^3\mu L^{-1}$)	46.1 (26.3)	50.0 (25.8)	0.354[b]	51.4 (27.2)	48.9 (27.5)	39 (28.2)	0.494[c]
Thrombocyte parameter							
PLTC, ($\times10^3\mu L^{-1}$)	278.0 (112.8)	236 (129.5)	**0.001[b]**	220 (1127)	233 (129)	236(140)	0.399[c]
MPV, fL	5.5 (0.5)	5.40 (0.6)	0.119[b]	5.4 (0.6)	5.3 (0.9)	5.2 (0.8)	0.990[c]
PCT, %	0.2 (0.1)	0.13 (0.1)	**0.001[b]**	0.13 (0.1)	0.14 (0.1)	0.12 (0.1)	0.772[c]
PDW, %	9.4 (1.5)	9.5 (1.5)	0.629[b]	9.6 (1.4)	9.5 (2.0)	9.5 (0.40)	0.951[c]

Data are presented as the median (interquartile range; IQR) values unless stated otherwise. Study participants were initially categorized into non-infected and infected (with any density parasitaemia). *P. falciparum* were further stratified base on haemoglobin type. [a] Statistical significance was determined by the Chi-square (χ^2) analysis. [b] Statistical significance was determined using Mann Whitney test. [c] Statistical significance was determined using Kruskal Wallis test. *Abbreviations: WBC*; White blood cells, *PLCT;* Platelet count, *MPV*; mean platelet volume, *PCT;* Plateletcrit; *PDW*; Platelet distribution width.

Relationship between leucocytes and thrombocytes: Mutations in haemoglobin beta sub-unit gene (*HBB*) has been found to be associated with both leucocyte and thrombocyte abnormalities (Obimba et al., 2015a, Rees et al., 2022, Shome et al., 2018). To determine relationship between leucocyte and thrombocyte parameter in children infected with falciparum in carriage of different *HBB* genotypes, partial correlation was used while controlling for age, sex and red cell parameter and stratifying genotypes. Whenever there was a statistically significant relationship, the study further calculated r^2 to show percentage variability that is responsible for change towards particular trend.

With respect to carriage of HbSS, WBC revealed a positive correlation with platelet count in both infected and non-infected children (r =0.358, r^2 =0.128, p =0.047 and r = -0.638, r^2 =0.407, p= 0.047) respectively. However, there was only significant correlation between WBC and RDW in children infected with HbSS genotypes (r =0.818, r^2 =0.669, p =0.004). Monocyte count revealed a positive correlation with platelet count in non-infected children and no correlation in children infected with malaria (r= -0.742, P=0.022 and r= -0.245, P=0.525, respectively) (Table 2).

Additional analysis in the carriage HbAS genotype, revealed no correlation between all the leucocyte and

thrombocyte parameters in both malaria infected and non-infected children. Specifically, there was no correlation between WBC and platelet count, WBC and MPV, WBC and PCT and WBC and PDW among other parameters (Table 2).

Finally, with respect to the homozygous wild type (HbAA) genotype, WBC count showed a positive correlation with platelet count (r= 0.256, r^2 =0.066, p=0.016) and a negative correlation with MPV (r= -0.209, r^2 =0.044, p=0.050) in children without malaria infection. Furthermore, WBC count showed a positive correlation with platelet count in children without malaria infection in HbAA genotype (r= -0.209, r^2 =0.044, p=0.05). Moreover, the study revealed a positive correlation between WBC and platelet count in the infected children in this category (r= 0.350, r^2 =0.122, p=0.008). Lymphocytes showed a positive correlation with MPV (r= 0.239, r^2 =0.057, p=0.025) in children without malaria. However, there was no correlation between lymphocytes and MPV. Monocytes showed a negative correlation with PCT (r= -0.531, p=0.093) in children infected with malaria. Finally, granulocytes showed a negative correlation with MPV (r= -0.222, r^2 = 0.049, p=0.037) in children without *P. falciparum* infection (Table 2).

Table 2: Correlation of leucocyte and thrombocyte parameters with Sickle cell genotype in malaria-infected and non-infected children

			Malaria negative (132)				Malaria positive (85)			
Control Variable	**Parameter**		PLTC	MPV	PCT	PDW (%)	PLTC	MPV	PCT	PDW
Sickle cell anaemia (HbSS) (n=11)							**Sickle cell anaemia (HbSS) (n=13)**			
Age, sex and erythrocyte parameters	WBC	r	0.358	-0.087	0.359	-0.101	-0.638	-0.019	-0.551	0.818
		r^2	0.128				0.407			0.669
		p value	**0.047**	0.823	0.342	0.795	**0.047**	0.957	0.079	**0.004**
	Lymphocytes	r	-0.556	0.212	-0.350	0.226	-0.536	-0.099	-0.395	-0.160
		r^2	-	-	-	-	-	-	-	**0.026**
		p value	0.120	0.583	0.355	0.559	0.089	0.772	0.229	0.639
	Monocytes	r	0.742	-0.245	0.353	0.002	0.372	0.317	0.380	-0.531
		r^2	-	-	-	-	-	-	-	-
		p value	**0.022**	0.525	0.351	0.995	0.259	0.342	0.249	0.093
	Granulocytes	r	0.401	-0.198	0.245	-0.264	0.348	-0.017	0.221	0.314
		r^2	**0.016**	-	-	-	-	-	-	-
		p value	0.284	0.609	0.525	0.492	0.294	0.960	0.513	0.347
Sickle cell trait (HbAS) (n=31)							**Sickle cell trait (HbAS) (n=14)**			
Age, sex and erythrocyte parameters	WBC	r	0.359	-0.143	0.316	-0.027	-0.148	0.074	-0.282	-0.423
		r^2	-	-	-	-	-	-	-	-
		p value	0.061	0.469	0.101	0.891	0.645	0.819	0.375	0.170
	Lymphocytes	r	-0.074	0.147	-0.010	0.072	0.131	0.522	-0.042	0.288
		r^2	-	-	-	-	-	-	-	-
		p value	0.707	0.456	0.960	0.714	0.686	0.081	0.896	0.365
	Monocytes	r	-0.137	0.125	-0.109	-0.104	0.112	-0.396	0.187	-0.344
		r^2	-	-	-	-	-	-	-	-
		p value	0.487	0.528	0.581	0.598	0.728	0.203	0.561	0.273
	Granulocytes	r	0.089	-0.152	0.013	-0.028	-0.189	-0.509	0.007	-0.274
		r^2	-	-	-	-	-	-	-	-
		p value	0.654	0.439	0.947	.889	0.557	0.091	0.983	0.388
Homozygous wild-type (HbAA) (n=90)							**Homozygous wild-type (HbAA) (n=58)**			
Age, sex and erythrocyte parameters	WBC	r	0.256	-0.209	0.099	-0.031	0.350	0.001	-0.145	-0.016
		r^2	**0.066**	**0.044**	-	-	**0.122**	-	-	-
		p value	**0.016**	**0.050**	0.357	0.772	**0.008**	0.995	0.287	0.907
	Lymphocytes	r	-0.008	0.239	0.213	-0.031	0.052	-0.187	-0.236	-0.256
		r^2	-	-	-	-	-	-	-	-
		p value	0.938	**0.025**	0.047	0.775	0.704	0.167	0.080	0.057
	Monocytes	r	0.196	-0.067	-0.003	0.010	-0.222	0.103	-0.531	0.062

	r^2	-	-		-	-	-	-	-
	p value	0.068	0.533	0.975	0.927	0.101	0.451	0.093	0.651
Granulocytes	*r*	-0.037	-0.222	-0.218	0.030	0.034	0.147	0.090	0.231
	r^2	-	0.049	-	-	-	-	-	-
	p value	0.731	**0.037**	0.042	0.785	0.804	0.279	0.511	0.087

Data are the partial correlations (*r*). Malaria negative patients (n=132) and malaria positive patients (n=85) with acute febrile illness were categorized on the basis of haemoglobin type. All statistical significance was determined by the partial correlation test (*r*) controlling for age and sex. Values in bold are statistically significant at p≤0.05. *Abbreviations: PLTC*; Platelet count, *MPV*; Mean platelet volume, *PCT*; Plateletcrit, *PDW*; Platelet distribution width, *r*; the measure of strength of Pearson's correlation, r^2; degree of variability

4. Discussion

The mechanism behind sickle cell crisis does not only implicate erythrocytes as the primary blood cells involved but also white blood cells (WBC) and thrombocytes (Aliu *et al.,* 2020, Davila *et al.,* 2015a). The current study hypothesized that *P. falciparum* infection could modulate leukocytosis and thrombocytosis in carriage of different *HBB* genotypes. Specifically, it has been shown that these two haemtological abnormalities contribute to pathogenesis of sickle cell crisis precisely through vaso-occlusion as an underlying mechanism behind a myriad of wide-ranging complications encountered in sickle cell disease (Aliu *et al.*, 2020, Davila *et al.,* 2015a). In a recent study, it was demonstrated that leukocytosis, monocytosis and thrombocytosis are associated with HbSS genotype in *P. falciparum* malaria infection (Kosiyo *et al.,* 2021). However, in the current study specifically demonstrated how total leucocytes and specific cells of leukocyte series (monocytes, lymphocytes and granulocytes) relate with various platelet parameter in carriage of different *HBB* genotypes in the infected and non-infected children.

Positive correlation between WBC and PLCT demonstrated in non-infected children in carriage of HbSS is plausible by active haemopoiesis against background haemolysis of red cells characterized by leucopoiesis and thrombopoiesis culminating to leucocytosis and thrombocytosis which have been previously been reported in children with sickle cell disease (Fasola and Adekanmi, 2019, Purohit *et al.,* 2018). This current finding is also supported by results of previous studies which reported both leucocyte and platelet activation in children with sickle cell anaemia (HbSS) implicating leucocytosis and thrombocytosis as haematological abnormalities in sickle cell anaemia (Davila *et al.,* 2015b, Manwani and Frenette, 2013). The most interesting finding of our current study is the stronger negative correlation between WBC and PLCT in the infected children with carriage of HbSS as demonstrated by $r^2 = 0.407$ versus $r^2 = 0.128$ in non-infected children. This would mean that *P. falciparum* infection can variably modulate thrombocytosis by decreasing platelet count by 40.7% as WBC count increases in carriage of HbSS and vice versa. It further implies that a decrease in platelet count as leucocyte count increases in carriage of HbSS could be attributed to *P. falciparum* infection. It is valuable noting that that platelets in SCD not only play a haemostatic role, but also a non-haemostatic inflammatory role through cytokine secretion (Davila *et al.,* 2015a). The current study further reinforces finding by demonstrating a stronger positive correlation demonstrated between WBC and PDW (platelet distribution width). PDW can be defined as a coefficient of variation of platelet size and it is considered a marker of platelet function and activation (Brzoska et al., 2019, Hoffbrand *et al.,* 2016). Increased PDW in this case could be explained by pre-existing anisocytosis and continuous thrombocytosis which results in the release of micro- and macro-platelets into peripheral circulation as a compensatory response in sickle cell anaemia. This result corroborates that of a study in Mali which demonstrated that sickle cell patients with mean platelet count $>450\times10^3/\mu L$ could be at a greater risk of frequent occurrence Vaso-occlusion (Diallo *et al.,* 2022), however this did not consider *P. falciparum* infection.

With reference to the carriage of HbAS, absence of relationship between WBC and platelet parameters was noted in both infected and non-infected children relative those with carriage of HbAA and HbSS genotypes. The study attributes the current results to the fact that phenotypes of HbAS have been shown to be protected from the severe form of malaria (Aidoo et *al.,* 2002, Kariuki and Williams, 2020). As a result, these children have been described to enjoy what is called "selective heterozygous advantage" against malaria infection (Olatunji, 2018). My previous study from the same study site, supported these results by demonstrating that children with HbAS do not develop haematological abnormalities such as anaemia, leukocytosis, thrombocytosis and thrombocytopenia due to *P. falciparum* infection (Kosiyo *et al.,* 2021). Precisely, this protection in carriage of HbAS genotype could be ascribed to the existing postulated mechanisms, which confer protective effect of haemoglobin S against *P. falciparum* infection. These comprise (1) reduced ability of parasites to grow within the red cells (Pasvol *et al.,* 1978), (2) enhanced sickling of parasitised RBCs and eventual clearance (Luzzatto *et al.,* 1970), (3) augmented

humoral and cell-mediated immune response (Verra *et al.,* 2007) and (4) aberrant cytoskeleton within red blood cells which contains HbS results in defective trafficking and interrupted delay of parasite encoded protein-*Plasmodium falciparum* erythrocyte membrane protein 1 (P*f*EMP-1) on the surface of parasitized red blood cells (Cholera *et al.,* 2008, Cyrklaff *et al.,* 2011).

The study further disclosed that in carriage of HbAA, there is positive correlation between WBC and platelet count. This result was not surprising since is normal active haemopoiesis of all haematological end cells arise from a common pluripotent stem cell under the stimulation of a number of growth factors (Hoffbrand *et al.,* 2016). On the hand, revealed positive correlation between WBC and platelet count in the infected children with carriage of HbAA signifies leukocyte and platelet activation which has been reported as a biological conundrum in *P. falciparum* infection (Davila *et al.,* 2015b, Manwani and Frenette, 2013). Conversely this finding does not agree with that of Maina and colleagues (Maina *et al.,* 2010) which reported thrombocytopenia in malaria. This disparity could be due to the fact that our study did not involve children with severe malaria Which has been previously reported to be associated with leukocytosis and thrombocytopenia in carriage of homozygous wild-type gene. Finally, a negative correlation trend revealed between granulocytes and MPV inferred that granulocyte counts would decrease as MPV increases signifying impaired bone marrow activity due to other underlying conditions other than malaria intection. This is usually characterized by release of immature giant (macro-) platelets into the peripheral circulation in non-infected children with HbAA genotypes (Hoffbrand *et al.,* 2016).

Drawbacks in the current study included fewer number of subjects in HbAS and HbSS genotypes, likely presence of other co-infections such as bacteremia, HIV which is known to affect lymphocyte counts (Ngo Bayoï *et al.,* 2022). Furthermore, current study did not integrate differential leukocyte count to unveil more insight on benign alterations in cells of granulocytic series.

5. Conclusion

Plasmodium falciparum infection modulates both platelet count and white blood cell counts in carriage of different haemoglobin beta subunit (*HBB*) genotypes. However, *P. falciparum* infection is accountable for a pronounced decrease the platelet count as WBC count increases in children with HbSS. Therefore, a decrease in platelet count against leukocytosis in carriage of HbSS should warrant a test for *P. falciparum* infection.

The current study recommend that future studies should emphasis in the parasite density and differential leucocyte counts in children infected with *P. falciparum* malaria in carriage of *HBB* genotypes.

References

AIDOO, TERLOUW, D. J., KOLCZAK, M. S., MCELROY, P. D., TER KUILE, F. O., KARIUKI, S., NAHLEN, B. L., LAL, A. A. & UDHAYAKUMAR, V. 2002. Protective effects of the sickle cell gene against malaria morbidity and mortality. *Lancet,* 359**,** 1311-2.

AKINBAMI, A., DOSUNMU, A., ADEDIRAN, A., OSHINAIKE, O., ADEBOLA, P. & AROGUNDADE, O. 2012. Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria. *BMC Res Notes,* 5**,** 396.

ALIU, R., ILIYA, J., QUADRI, O. R., IBRAHIM, O. R. & DANIEL, E. 2020. Haematological Profile of Children With Sickle Cell Anaemia in Steady State. *Cureus,* 12**,** e11011.

BRZOSKA, T., KATO, G. J. & SUNDD, P. 2019. 31 - The Role of Platelets in Sickle Cell Disease. *In:* MICHELSON, A. D. (ed.) *Platelets (Fourth Edition)*. Academic Press.

CHOLERA, R., BRITTAIN, N. J., GILLRIE, M. R., LOPERA-MESA, T. M., DIAKITE, S. A., ARIE, T., KRAUSE, M. A., GUINDO, A., TUBMAN, A., FUJIOKA, H., DIALLO, D. A., DOUMBO, O. K., HO, M., WELLEMS, T. E. & FAIRHURST, R. M. 2008. Impaired cytoadherence of Plasmodium falciparum-infected erythrocytes containing sickle hemoglobin. *Proc Natl Acad Sci U S A,* 105**,** 991-6.

CYRKLAFF, M., SANCHEZ, C. P., KILIAN, N., BISSEYE, C., SIMPORE, J., FRISCHKNECHT, F. & LANZER, M. 2011. Hemoglobins S and C interfere with actin remodeling in Plasmodium falciparum-infected erythrocytes. *Science,* 334**,** 1283-6.

DAVILA, J., MANWANI, D., VASOVIC, L., AVANZI, M., UEHLINGER, J., IRELAND, K. & MITCHELL, W. B. 2015a. A novel inflammatory role for platelets in sickle cell disease. *Platelets,* 26**,** 726-9.

DAVILA, J., MANWANI, D., VASOVIC, L., AVANZI, M., UEHLINGER, J., IRELAND, K. & MITCHELL, W. B. 2015b. A novel inflammatory role for platelets in sickle cell disease. *Platelets,* 26**,** 726-729.

DIALLO, L., GUINDO, A., KÉITA, I., BARAÏKA, M. A., DEMBÉLÉ, A. K., TOURÉ, B. A. & DIALLO, D. A. 2022. [Platelet count in the steady state phase and clinical severity of sickle cell disease in a reference

centre for sickle cell disease in Mali]. *Pan Afr Med J,* 43**,** 52.

ERHABOR, O., MOHAMMAD, H. J., ONUIGUE, F. U., ABDULRAHAMAN, Y. & EZIMAH, A. C. 2014. Anaemia and Thrombocytopenia among Malaria Parasitized Children in Sokoto, North Western Nigeria. *Journal of Hematology & Transfusion,* 2.

FASOLA, F. A. & ADEKANMI, A. J. 2019. Haematological Profile and Blood Transfusion Pattern of Patients with Sickle Cell Anaemia Vary with Spleen Size. *Ann Ib Postgrad Med,* 17**,** 30-38.

HOFFBRAND, DOUGLAS, R. H., DAVID, M. K. & ATUL, B. M. 2016. Postgraduate Haematology 6th edition. *Willey-Blackwell*.

HOFFBRAND, A. V., CATOVSKY, D., EDWARD, G. D. & ANTHONY R.G. 2010. Postgraduate Haematology 6th edition. *Wiley-Blackwell*.

KARIUKI, S. N. & WILLIAMS, T. N. 2020. Human genetics and malaria resistance. *Hum Genet,* 139**,** 801-811.

KOSIYO, P., OTIENO, W., GITAKA, J., MUNDE, E. O. & OUMA, C. 2020. Association between haematological parameters and sickle cell genotypes in children with Plasmodium falciparum malaria resident in Kisumu County in Western Kenya. *BMC Infect Dis,* 20**,** 887.

KOSIYO, P., OTIENO, W., GITAKA, J., MUNDE, E. O. & OUMA, C. 2021. Haematological abnormalities in children with sickle cell disease and non-severe malaria infection in western Kenya. *BMC Infect Dis,* 21**,** 329.

LUZZATTO, NWACHUKU-JARRETT ES & REDDY S 1970. Increased sickling of parasitized erythrocytes as mechanism of resistance against malaria in the sickle trait. *Lancet,* 1**,** 319-21.

MAINA, R. N., WALSH, D., GADDY, C., HONGO, G., WAITUMBI, J., OTIENO, L., JONES, D. & OGUTU, B. R. 2010. Impact of Plasmodium falciparum infection on haematological parameters in children living in Western Kenya. *Malar J,* 9 Suppl 3**,** S4.

MANWANI, D. & FRENETTE, P. S. 2013. Vaso-occlusion in sickle cell disease: pathophysiology and novel targeted therapies. *Hematology Am Soc Hematol Educ Program,* 2013**,** 362-9.

MBOYA, P. K., SUMBA, P. O., OLUOCH, J. N., ABDALLAH, F. K. & MALA, A. O. 2016. Correlation between Thrombocytopenia and Anaemia in Plasmodium falciparum malaria among patients in Kisumu County-Western Kenya. . *Afr J Health Sci.,* 29**,** 76-88.

NGO BAYOÏ, C., LEHMAN, L. G., TONGA, C., KANGAM, L., WEPNJE, G. B., TCHANGA, F. C. & TOMEDI, M. E. 2022. Effects of Malaria and HIV Infection on Anemia and T-cells Levels in Children in Douala City, Cameroon. *Cureus,* 14**,** e32074.

OBIMBA, K., EZIUZOR, SAMUEL C, OZOUGWU, JEVAS, CHIBUIKE, IHEDIMBU & CHIAMAKA P 2015a. Biochemical and hematological diagnostic indices of homozygous sickle cell anemia patients in the steady state*International Journal of Medicine and Medical Sciences,* 5**,** 299-306.

OBIMBA, K., EZIUZOR, SAMUEL. C, OZOUGWU, JEVAS, CHIBUIKE, IHEDIMBU & CHIAMAKA P 2015b. Biochemical and hematological diagnostic indices of homozygous sickle cell anemia patients in the steady state. *International Journal of Medicine and Medical Sciences,* 5**,** 299-306.

OLATUNJI, P. O. 2018. Malaria and the Sickle Gene: Polymorphism Balance in favour of eradication. *Annals of Health Research,* 4**,** 88-96.

PASVOL, G., WEATHERALL, D. J. & WILSON, R. J. 1978. Cellular mechanism for the protective effect of haemoglobin S against P. falciparum malaria. *Nature,* 274**,** 701-3.

PUROHIT, P., MOHANTY, P. K., PATEL, S., DAS, P., PANIGRAHI, J. & DAS, K. 2018. Comparative study of clinical presentation and hematological indices in hospitalized sickle cell patients with severe Plasmodium falciparum malaria. *J Infect Public Health,* 11**,** 321-325.

REES, D. C., BROUSSE, V. A. M. & BREWIN, J. N. 2022. Determinants of severity in sickle cell disease. *Blood Rev,* 56**,** 100983.

SHOME, D. K., JARADAT, A., MAHOZI, A. I., SINAN, A. S., EBRAHIM, A., ALRAHIM, M., EBRAHEEM, M. S., MANSOOR, E. J., MAJED, K. S. & AZEEZ PASHA, S. A. 2018. The Platelet Count and its Implications in Sickle Cell Disease Patients Admitted for Intensive Care. *Indian J Crit Care Med,* 22**,** 585-590.

THOMAS, M. R. & STOREY, R. F. 2015. The role of platelets in inflammation. *Thromb Haemost,* 114**,** 449-58.

VERRA, F., SIMPORE, J., WARIMWE, G. M., TETTEH, K. K., HOWARD, T., OSIER, F. H., BANCONE, G., AVELLINO, P., BLOT, I., FEGAN, G., BULL, P. C., WILLIAMS, T. N., CONWAY, D. J., MARSH, K. & MODIANO, D. 2007. Haemoglobin C and S role in acquired immunity against Plasmodium falciparum malaria. *PLoS ONE,* 2**,** e978.