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Association between thrombocytes count and *Plasmodium falciparum* infection among children under five years attending Kombewa Sub-County Hospital

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Abstract

Malaria is a leading cause of morbidity and mortality especially among children, expectant women and continues to be a global health burden. Haematological changes mark some of the most common complications in malaria as they play a major role in malaria pathology. Thrombocytes in particular, have been shown to bind infected erythrocytes and kill intracellular malaria parasites thereby indicating a protective function of platelets in the early stages. However, the mechanism that leads to low thrombocytes count in malaria infected individuals is not clear. Understanding the mechanism of platelet reduction during pathogenesis of malaria infection will be fundamental in malaria severity classification, monitoring of platelet count during infection and prompt initiation of anti-malarial therapy. In trying to understand these facts, this study sort to establish the association between platelet count and *P. falciparum* infection amongst children less than five years. This was a retrospective case-control study, n=549. Children below the age of five years that attending Kombewa Sub -County Hospital were recruited. Study participants were identified using the inclusion criteria and followed horizontally to retrieve platelet count from complete blood count results. The respective malaria blood film reads were then recorded, stratified to give case and control from which random sampling was done. Chi-square test and Tukey's multiple comparison tests from Graph pad prism 5 were used in the analysis. The odds of exposure to low platelet count were then established with a confidence level of 95%. We found significant difference between the cases and controls in regard to parasite density (Chi square=157.5, p value <0.05), mean parasite density in controls =2042.1/µl compared to cases= 142880/µl. The odds of cases being exposed to malaria was 12 times more than controls (OR=12.382, 95%). We also found no variation in thrombocytes counts in relation to gender, children with thrombocytopenia were having higher parasite density, parasite density as a result of *P.falciparum* infection is not dependent on gender and children that suffered malarial infection were twelve times likely to develop thrombocytopenia. Further studies are then recommended to establish the effects of incorporation of platelet aggregation inhibitors such as aspirin in malaria treatment.

Key Words: *Plasmodium falciparum*, thrombocytopenia, infection

1. Introduction

Malaria is a leading cause in morbidity and mortality especially among children and pregnant women (1). The disease continues to be a global health burden, causing significant morbidity and mortality (2). Current estimates by the World Health Organization indicate that 40% of the world's population is at risk for malaria, while 300-500 million infections are reported per annum leading to mortality estimates that range between 0.7 and 2.7 million and high fatality rates among young children below 5 years of age (3).

In 2010, of the estimated 216 million people with malaria in the world, 81% of them were from sub-Saharan Africa. In 2010 in Africa, malaria resulted in approximately 596,000 deaths, 91% of which were in children under five years of age. Among the many approaches being pursued to control the disease, the development of safe and efficacious vaccines has been given high priority by national and international health authorities(1).

In Kenya, 28 million individuals are at the risk of contracting malaria. The cumulative human suffering and economic loss caused by malaria is immense. It is estimated that annually, 26,000 children under five years of age (72 per day) die from the direct consequence of malaria infection (3). Approximately, 170

million working days are lost each year because of malarial illness, which in turn affects the country's economy, leading to increased poverty. The distribution of malaria is not uniform, because of geographical differences in the environment such as altitude, rainfall and humidity. These factors influence transmission patterns, as they determine vector densities and intensity of biting. Kenya may be divided into four malaria ecozones; stable malaria (Nyanza, Coast, and Western regions), Seasonal malaria (Central, Eastern, and North Eastern regions) Highlands prone to malaria epidemics (mainly in Rift Valley Province and some parts of Nyanza regions, and Malaria free (Nairobi and some parts of Central regions)(4).

Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathology. These changes involve the major cell lines such as red blood cells, leucocytes and thrombocytes. In Western Kenya, severe anaemia is the predominant severe malaria syndrome.(5). Platelets in particular, have been reported to play a critical role in the pathogenesis of malarial infections by promoting the sequestration of infected red blood cells. Human platelets have been shown to bind infected erythrocytes and kill intracellular malaria parasites; thereby indicating a protective function of platelets in the early stages of erythrocytes infection distinct from their role in cerebral malaria(6).

A study by McMorran *et al*., in 2012 (7) showed that platelets are active in early infection in slowing the initial growth of malaria parasites in the bloodstream, providing greater opportunity for other defence mechanisms to control the infection and ensure survival of victims. Severe malaria has a high mortality rate (15–20%) despite treatment with effective anti-malarial drugs (8). Adjunctive therapies for severe malaria targeting the underlying disease process are therefore urgently needed. Adhesion of red cells infected with *Plasmodium falciparum* to human cells has a key role in the pathogenesis of life-threatening malaria and could be targeted with anti-adhesion therapy(9). Parasite adhesion interactions include binding to endothelial cells (cyto-adherence), rosetting with uninfected erythrocytes and platelet-mediated clumping of infected erythrocytes. There is strong indication that thrombocytes have been shown to bind infected erythrocytes and kill intracellular malaria parasites in pathogenesis of severe malaria. However, many fundamental questions regarding the role of platelets in severe malaria remain unknown. This study therefore sort to establish causes of low thrombocyte count in malaria infected children in Kombewa Sub-county a high malaria endemic site.

Methodology

Study Design

This was a retrospective case control study design that sort to establish the association between thrombocytes counts and *P. falciparum* infection. .

Study Site

The study site was Kombewa Sub County Hospital (KSCH) which is located in Kisumu West District with some of the areas a malaria holoendemic site. Over 98% of the malaria parasites circulating being P. falciparum *and the vectors* Anopheles gambie, A. fenustus *and* A. arabiensis *(10)*

The area covers approximately 369 Km^2 with a population of approximately 150, 000 people (WRP DSS, 2011).

Study Population

Children under the age of five attending Kombewa Sub-County Hospital during the study period formed the study population. *Inclusion criteria* was children who were under five years old and had Complete Blood Count (CBC) and Malaria Blood Film (MBF) as some of the test done during their visit as well as children who had data for both CBC and parasite density available. The *exclusion criteria* children under five years old whose parasite density results and/or CBC were missing

Sampling technique

Random sampling method was used as it provides equal opportunity for the subjects' participation.
Data from children under five years were obtained from the logs and recorded in the data collection work sheet, entered, organized and stratified (in terms of case and control) in a spread sheet.

Data Collection Methods

The study participants were identified using the inclusion criteria; the platelet value and gender were obtained from the CBC results and recorded in the data collection work sheet. The respective MBF reads

were also systematically retrieved and recorded on the same worksheet. The raw data was then entered in the spread sheet for cleaning, sampling and analysis using SPSS Version 16

Information that was collected about the subjects was treated with confidentiality without disclosure. Efforts were made to maximize possible benefits from the study findings to subjects and entire community. An official approval from the Independent Review and Ethical Committee, MMUST and the District Health Management Team, Kombewa Sub County Hospital was sought to allow the collection and use of the data respectively.

Results

Table 1 -2 and **Figure 1** below shows the difference in average thrombocytes counts between males and females either of the arms was not statistically significant (p>0.05, 95% CI). The average thrombocytes counts were also higher in controls (both males and females) as compared to cases.

Table 11: Average Platelet count in relation to Gender

	Gender	**N (%)**	**Average PLT Counts**
Cases	Female	99 (18.0)	96.17
	Male	84 (15.3)	108.48
Controls	Female	193 (35.2)	410.4
	Male	173 (31.5)	426.9
Total		**549 (100)**	

Table 12: Gender and Thrombocytes Counts Comparison

Tukey's Multiple Comparison Test	**Significance (P <0.05)**	**95% CI of diff**
case Female vs Case Male	No	-64.98 to 40.37
case Female vs Control Female	Yes	-358.1 to -270.3
case Female vs Control Male	Yes	-375.5 to -286.0
Case Male vs Control Female	Yes	-348.3 to -255.5
Case Male vs Control Male	Yes	-365.7 to -271.2
Control Female vs Control Male	No	-53.69 to 20.67

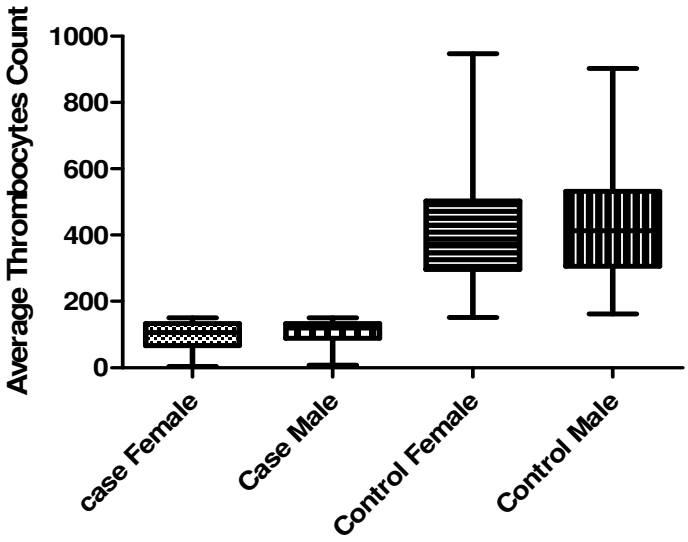

Figure 5: Average Platelet count in relation to Gender

The comparative average parasite densities were higher amongst cases (females 50 % and males 49%) and lower with an average of 1% amongst control arm, **Table 3-4** and **Figure 2**. In addition, there was statistically insignificant difference in average parasite density between male and female participants in either of the arm (p>0.05, 95% CI).

Table 13: Presence of *P. falciparum* in regard to gender

	Gender	N (%)	Average Parasitemia
Cases	Female	99 (18.0)	145,886
	Male	84 (15.3)	141,066
Controls	Female	193 (35.2)	3,537
	Male	173 (31.5)	375
Total		**549 (100)**	

Table 14: Gender and Parasite Density Comparison

Tukey's Multiple Comparison Test	Significance (P < 0.05)	95% CI of diff
Case Female vs Case Male	No	-63710 to 70400
Case Female vs Control Female	Yes	84990 to 196800
Case Female vs Control Male	Yes	87070 to 201000
Case Male vs Control Female	Yes	78440 to 196600
Case Male vs Control Male	Yes	80580 to 200800
Control Female vs Control Male	No	-44170 to 50490

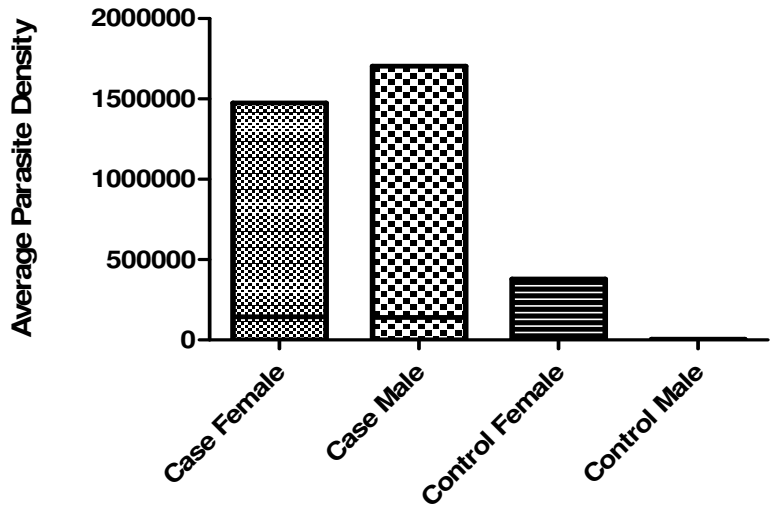

Figure 6: Parasite Density amongst Cases and Controls

As seen on the below, **Table 5** and **6**, there is statistical significance difference seen between the cases and controls in regard to parasite density (chi square=157.5, p value<0.05), mean parasite density in controls =2042.1/ul compared to cases= 142880/ul. Thrombocytopenia as an outcome seem to be influenced by the exposure (malaria)

Table 15: The relationship between Thrombocytes Counts and Parasite Density

			P. falciparum		
			NEGATIVE	POSITIVE	**Total**
Thrombocytes Counts	CONTROL	Count	286	80	366
		Expected Count	218.0	148.0	366.0
		% within Thrombocytes Counts	78.1%	21.9%	100.0%
		% within *P. falciparum*	87.5%	36.0%	66.7%
		% of Total	52.1%	14.6%	66.7%
	CASE	Count	41	142	183
		Expected Count	109.0	74.0	183.0
		% within Thrombocytes Counts	22.4%	77.6%	100.0%
		% within *P. falciparum*	12.5%	64.0%	33.3%
		% of Total	7.5%	25.9%	33.3%
Total		Count	327	222	549
		Expected Count	327.0	222.0	549.0
		% within Thrombocytes Counts	59.6%	40.4%	100.0%
		% within *P. falciparum*	100.0%	100.0%	100.0%
		% of Total	59.6%	40.4%	100.0%

Table 16 : Chi-Square Tests for thrombocytopenia versus *P.falciparum*

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.574E2[a]	1	0.000		
Continuity Correction	155.057	1	0.000		
Likelihood Ratio	161.789	1	0.000		

Table 17 : Risk Estimate

		Thrombocytes counts		
		Cases	**Control**	**Total**
***P. falciparum* Infection**	**Parasitemic**	142	80	**222**
	Aparasitemic	41	286	**327**
	Total	**183**	**366**	**500**

From **Table7**, the odds of a case being exposed to malaria is 12 times more than a control being exposed (OR=12.382, 95%-CI) i.e. those who are exposed to malaria are 12 times likely to suffer from thrombocytopenia. Odds Ratio for Thrombocytes Counts (OR) =12.382

Discussion

This study determined that the difference in average thrombocyte counts between males and females in either of the arm was not statistically significant (p>0.05, 95% CI). This concurs with a study which was done by Kotepui *et al.*, 2014 (11) to establish effect of malarial infection on haematological parameters in population near Thailand Myanmar border, that thrombocytopenia was present in 84.9% of malaria-infected patients and was independent of gender besides age and nationality (P value < 0.0001).

Normally, a person's platelet count is 150,000 to 400,000 per µl, but the normal platelet count range in children is between 150,000 and 450,000 per µl. There is a slight decrease in the platelet count in pregnancy as there is an increase in the blood produced while the number of platelets remains the same. It is normal upto100, 000 platelets per µl but if the number goes below this, and then it becomes medical emergency. Some women may experience a slight downfall in the platelet count before menstruation (12). This excerpt explain that there is no variation in platelet count in regard to gender as the level can only fall or rise due to pathological or physiological conditions.

This study found that the comparative average in parasite density was high among cases (females 50 % and males 49%) and lower with an average of 1% amongst control arm (See **Table 3-4** and **Figure 2)**. In addition, there was statistically insignificant difference in average parasite density between male and female participants irrespective of the arm (p>0.05, 95% CI). Ladhani *et al.*, in 2005 (13) also found that even though the difference in parasitemia with gender was insignificant in CM and SMA, females with uncomplicated malaria had higher than (P = 0.007) parasite loads their male counterparts.

There was statistical significant difference seen between the cases and controls in regard to parasite density (Chi square=157.5, p value<0.05), mean parasite density in controls =2042.1/µl compared to cases= 142880/µl), see **Table 5** and **6.** Thus thrombocytopenia as an outcome seems to be influenced by the exposure (malaria). These results compares with those of Utuk *et al* (14)that found in 2009 that the presence of thrombocytopenia in a patient with acute febrile illness increases the probability of malarial infection in endemic areas and may increase suspicion of malaria in settings where technical laboratory support is not available. The significantly lower thrombocytes counts in the children with malaria parasitemia than those in the control group is an observation similar to that made by Akingbola *et al.*,(15) 2006 and Essien *et al.,* 2004 (16) in Lagos.

Platelets bud from the megakaryocytes in the bone marrow and then normally circulate for about ten days in the blood. This means that they are fragments of cells and thus do not possess nuclear but have some other cellular organelles such as microtubules, which are important in holding the un inactivated platelets in crisp and discoid shape. The two key types of secretory vesicles are: dense granules which contain Adenosine Di Phosphate (ADP) and Alpha granules which contains Von Willebrand Factor, thrombin and growth factors. On a healthy endothelium platelets circulate through the vessels and their inactivated

state is supported by the absence of activating factor and release of prostacyclin (prostaglandin12). Once the RBCs are infected with *P. falciparum*, they activate platelets, which secrete platelet factor 4, which activates the immune system by turning on monocytes which contribute to the inflammation in the blood vessels through release of inflammatory mediators such as thromboxane A_2, ADP and thrombin which additionally triggers the same activation(17). This results in obstructions (Infected Erythrocytes-platelet mediated clumping) in the brain vessels causing brain damage similar to that seen in stroke. This therefore means that platelets enhance cyto-adherence by acting as bridges between endothelial cells and IEs and so target sequestration to endothelial beds not expressing adhesion receptors such as CD36 (18).
Some strains of *falciparum* malaria parasites also induce the formation of small membrane protrusions known as knobs on erythrocytes. These knobs have been identified as the site of contact with endothelial cells (ECs), and high molecular weight (MW) malarial proteins expressed on these knobs mediate this interaction. *P. falciparum* erythrocyte membrane protein-1 (PfEMP1) was subsequently identified as the candidate parasite ligand that binds to CD36, globular C1q receptor (gC1qR/ HABP1/p32) and P-selectin (19). It is a high MW protein (200–400 kDa) encoded by the *var* multi-gene family, and a member of a highly variant antigenic family that is responsible for antigen variation in *P. falciparum*. Thus, as well as mediating adhesion to ECs, it also plays a key role in immune evasion (20).
These resulting activated platelets, however, do not maintain their discoid shape and thus are not counted during analysis. Additionally, the sequestration that takes place due to cyto-adherence and clumping also reduces the pool of thrombocytes in circulation.

Conclusions

This study found that thrombocytopenia in children is most likely associated with increase in parasite density and children that suffered malarial infection were twelve times likely to develop thrombocytopenia.

References

1. WHO | World Malaria Report 2011 [Internet]. WHO. [Cited 2014 Aug 29]. Available from: http://www.who.int/malaria/world_malaria_report_2011/en/

2. Gething PW, Elyazar IRF, Moyes CL, Smith DL, Battle KE, Guerra CA, et al. (2010) ''A Long Neglected World Malaria Map: Plasmodium vivax Endemicity in'', PLoS Negl Trop Dis. 2012 Sep 6;6(9):e1814.

3. Snow RW, Guerra CA, Mutheu JJ, Hay SI. (2008) ''International Funding for Malaria Control in Relation to Populations at Risk of Stable Plasmodium falciparum Transmission'', PLoS Med. Jul 22; 5(7):e142.

4. Sabah Ahmed Omar FWM. (2007) ''Prevalence of Plasmodium

falciparum chloroquine resistant gene markers, pfcrt-76 and pfmdr1-86, eight years after cessation of chloroquine use in Mwea, Kenya'', J Infect Dev Ctries.

5. Waitumbi JN, Opollo MO, Muga RO, Misore AO, Stoute JA. (2000) ''Red cell surface changes and erythrophagocytosis in children with severe plasmodium falciparum anemia''' Blood. Feb 15; 95(4):1481–6.

6. Price RN, Uhlemann A-C, van Vugt M, Brockman A, Hutagalung R, Nair S, et al. (2006) ''Molecular and pharmacological determinants of the therapeutic response to artemether-lumefantrine in multidrug-resistant Plasmodium falciparum malaria'', Clin Infect Dis Off Publ Infect Dis Soc Am. Jun 1;42(11):1570–7.

7. McMorran BJ, Wieczorski L, Drysdale KE, Chan J-A, Huang HM, Smith C, et al. (2012) ''Platelet factor 4 and Duffy antigen required for platelet killing of Plasmodium falciparum'', Science. Dec 7; 338(6112):1348–51.

8. Moulin F. (2003)''Thrombocytopenia and Plasmodium falciparum malaria in children with different exposures'', Arch Dis Child. Jun 1; 88(6):540–1.

9. Maina RN, Walsh D, Gaddy C, Hongo G, Waitumbi J, Otieno L, et al., (2010) ''Impact of Plasmodium falciparum infection on haematological parameters in children living in Western Kenya'', Malar J.;9(Suppl 3):S4.

10. Asito AS, Moormann AM, Kiprotich C, Ng'ang'a ZW, Ploutz-Snyder R, Rochford R. (2008) ''Alterations on peripheral B cell subsets following an acute uncomplicated clinical malaria infection in children'', Malar J. Nov 18;7:238.

11. Manas Kotepui BP. (2014) ''Effect of malarial infection on haematological parameters in population near Thai/Myanmar border'', Malaria J.; 13(1):218.

12. Bain BJ. (1996) ''Ethnic and sex differences in the total and differential white cell count and platelet coun'',. J Clin Pathol. Aug 1; 49(8):664–6.

13. Ladhani S, Patel VS, El Bashir H, Shingadia D. (2005) ''Changes In Laboratory Features Of 192 Children With Imported Falciparum Malaria Treated With Quinine: Pediatr Infect'', Dis J. Nov; 24(11):1017–20.

14. Utuk E, Ikpeme E, Emodi I, Essien E. (2014) '''The Effect of Falciparum Malaria Infection on The Platelet Count of Children in a Tertiary Hospital in Uyo, Akwa Ibom State of Nigeria'', TAF Prev Med Bull.;13(3):225.

15. Akingbola TS, Shokunbi WA, Olumese PE. (2006) ''Coagulation profile in Nigerian children with cerebral malaria'', Niger Postgrad Med J. Sep; 13(3):195–9.

16. Essien EM. (1992) ''Platelets and platelet disorders in Africa'', Baillières Clin Haematol. Apr; 5(2):441–56.

17. Quintó L, Aponte JJ, Sacarlal J, Espasa M, Aide P, Mandomando I, et al. (2006) ''Haematological and biochemical indices in young African children: in search of reference intervals'', Trop Med Int Health. Nov; 11(11):1741–8.

18. Shaikh MA, Ahmed S, Diju IU, Dur-E-Yakta null. (2011) ''Platelet count in malaria patients'', J Ayub Med Coll Abbottabad JAMC. Mar; 23(1):143–5.

19. Biswas S, Mohammad MM, Patel DR, Movileanu L, van den Berg B. (2007) ''Structural insight into OprD substrate specificity'', Nat Struct Mol Biol. Nov;14(11):1108–9.

20. Zaki S. (2011) ''Malaria and dengue co-infection'', Ann Indian Acad Neurol.; 14(2):141.