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The prevalence of thrombocytopenia in *plasmodium falciparum* malaria in children at the University of Uyo Teaching Hospital, Uyo, Nigeria

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Abstract *Background:* Thrombocytopenia occurring in *falciparum* malaria infection has been documented worldwide. However, its prevalence varies from place to place, and among different population groups studied. There is paucity of data on this in Nigerian children.
Objectives: To determine the prevalence of thrombocytopenia in children presenting with *falciparum* malaria at the University of Uyo Teaching Hospital, Uyo, Akwa Ibom State, Nigeria.
Method: A prospective cross-sectional study from October 2010 to March 2011 on one hundred and eighty children with microscopically confirmed malaria aged six months to fifteen years, compared with 180 healthy children without malaria parasitaemia matched for age and gender. Their platelet counts were evaluated using the auto-analyser Sysmex KX-21N.
Results: The overall prevalence of thrombocytopenia was 5.0%, but it was higher in children with severe malaria. None of the children in the control group had thrombocytopenia.
Conclusion: The prevalence of thrombocytopenia in falciparum malaria is low in our setting, but higher in children with severe manifestations of malaria.

Keywords: Malaria, Thrombocytopenia, Prevalence, children.

Introduction

Malaria infection remains a major public health problem and cause of morbidity and mortality in all age groups.[1,2] In Nigeria, it accounts for 20 to 30% of infant and child mortality, particularly in children who are five years of age and younger. [2,3] The lethality of *P. falciparum* compared with other species of *plasmodium* probably stems from the parasite density it achieves which is typically a hundred times higher than that of other species before its proliferation is curtailed by host defence mechanisms.[2] As blood parasites for most of their complex life cycle, *plasmodia*, not surprisingly, produce haematological abnormalities that include anaemia, thrombocytopenia, splenomegaly and rarely disseminated intravascular coagulation with a bleeding diathesis.[2,3-6] Values of haematological parameters have been shown to be affected by factors such as genetics and ethnic differences, sex, age, environmental factors like dietary pattern and altitude.[3-5] Since these factors differ depending on the population and geographical area studied, it is not surprising that differences have been reported in these parameters worldwide.[3-5]

Genetic factors are shown to significantly contribute to variance in all blood cell lines. [7-9] Thus within the wide "normal reference range", there are some ethnic differences, with Caucasian values being higher than those of the African and Afro-carribeans.[7-10] It has therefore been stressed that each population must establish its own "normal reference values" for use in clinical assessments.[7,10] The normal reference range of the platelet count in the African population has been established at 100 – 300 x 10^9/L.[7-9] For the Caucasians, it is 150 – 450 x 10^9/L.[2,7,9] These differences in normal values need to be appreciated to avoid fundamental errors in the assessment and management of patients.

Thrombocytopenia, a state of reduced circulating platelet count in blood below the normal level can be caused by decreased platelet production, increased destruction, sequestration or a combination of these.[2] A major aetiologic cause for acquired thrombocytopenia of childhood is increased platelet destruction associated with several clinical conditions including protozoal infections such as malaria especially in the tropical regions.[2,3,6] This association of thrombocytopenia with malaria infection is well recognized, but prevalence varies with levels of malarial endemicity and immunity, age, malarial specie and malarial severity.[11-13] Studies from various authors in different regions of the world have also shown that considerable overlap exists in

thrombocytopenic values which occur in both uncomplicated and severe *falciparum* malarial infections.[11-13] In non-endemic countries such as United Kingdom, France, Sweden, studies done showed a varying prevalence rate of thrombocytopenia in malaria to be between 64% - 87.3%.[14-17] In a study in Dakar, Senegal where malarial transmission is hypo-endemic, that is, low and seasonal, a prevalence of 56.2% was seen.[18] In holoendemic malaria countries like Pakistan, India, Kenya, Cameroon, and Nigeria, varying prevalence rates have been documented to be as low as 13% and as high as 90%.[19-21] The prevalence of thrombocytopenia with *falciparum* parasitaemia has been mostly documented in adult populations of semi-immune and non-immune individuals, but there have been few documentation for children in the tropical region of Africa, more so in Nigeria, where malaria is endemic. This study was therefore designed to establish the prevalence of thrombocytopenia (platelet count <100 x 10^9/L)[7-9,19] in children aged six months to fifteen years with *falciparum* malaria infection as seen in a locality in Nigeria. The practice of paediatrics in the tropics does not always permit a detailed diagnostic work-up for every child with haematological manifestations therefore research on the prevalence and aetiologic patterns is important. It will serve as a guide to improve clinical treatment and outcome.

Subjects and Methods

The study was carried out in the Children Out-patient (CHOP) clinic, Children Emergency Unit (CHEU) and the Paediatric ward of the University of Uyo Teaching Hospital (UUTH), Uyo in Akwa-Ibom State. The Teaching Hospital is the only tertiary health institution in the state, and is located on the outskirts of Uyo, six kilometres from the centre of the city. Uyo, the capital city of Akwa-Ibom State is located in the South-south region of Nigeria. It lies between latitudes 4'33 and 5'33 North, longitudes 7'35 and 8'35 east, and falls within the tropical zone where the anopheline mosquito habitat exists.

Approval for the study was obtained from the hospital's Ethics committee before commencement. A written and verbal informed consent was obtained from each child if up to twelve years old and above or from the parents/ guardian(s) for younger children.

Children aged six months to fifteen years of age with fever or a history of fever of not longer than seven days duration before presentation in hospital with parasitologic evidence of malaria were included. Also included were children with fever and at least one or more features of severe malaria, as well as those who had not received any anti-malarial drug in the preceding two weeks of presentation to hospital. Any child who had received any cytotoxic drug and other drugs that interfere with platelet counts e.g non-steroidal anti-inflammatory drugs (NSAIDS) such as aspirin, Ibuprofen etc, within ten days of presentation, as well as those who had received any anti-malarial treatment within two weeks of presentation were excluded. Children with systemic diseases e.g leukaemia and other malignancies that involve the bone-marrow and those with an obvious focus of infection, with a positive blood culture examination were all excluded. Controls were afebrile apparently healthy children, matched for age and gender who showed no signs of any systemic disease and had no parasitologic evidence of malaria. They were selected from children attending child welfare clinic for growth monitoring and those presenting for immunization. Also children who presented to the out-patient clinic for school entry medical examination were recruited.

A clinical history was obtained from the care-giver and/ or the patient and included the onset and duration of fever (temperature $\geq 37.5^0$Celsius) and associated symptoms. Uncomplicated malaria was established by microscopically confirmed malaria parasitaemia with no symptoms of severity. Children with repeated convulsions, hyperpyrexia (axillary temperature $\geq 39.5^o$celsius), respiratory distress, oliguria (urinary output < 1ml/kg/ hr), cardiovascular shock, jaundice, severe prostration, haemoglobinuria, severe anaemia (Haemoglobin<5g/dl), hyperparasitaemia (involving > 5% of erythrocytes), hypoglycaemia (serum glucose < 2.2mmol/l), acidosis (bicarbonate < 15mmol/l) were classified as having severe malaria.[3,4] Children presenting with an episode or repeated episodes of convulsions had a lumbar puncture done for cerebro-spinal fluid analysis to exclude bacterial meningitis. A complete physical examination was done. Level of consciousness was assessed using the Blantyre's score for children younger than two years and Glasgow score for older children.

Thick and thin blood films for malaria parasite were prepared directly from capillary blood and the slides stained on the same day with the Giemsa stain. Each blood film was examined microscopically using the 100X objectives and the 7X eyepieces as these give a brighter and clearer image. The parasite density was determined using the method by Greenwood and Armstrong[22] who found this method to be more accurate and quicker than counting the parasites against white cells. The platelet count were determined using the fully automated blood cell analyser (Sysmex KX-21N). For any samples which contained a significant proportion of giant platelets, small platelets or did not show any platelet count, indicated by a flag signal from the machine, the manual counting method was performed for confirmation.

Thrombocytopenia was defined in this study as a platelet count < 100,000 x 10^9/L.[23-25] Severe thrombocytopenia was defined as a platelet count < 50,000 x 10^9/L.[2] Aerobic and anaerobic blood cultures were done on each child. Random blood sugar was also done using a One-Touch Ultra-2 glucometer (Life scan model Inc. Milpitas, CA.USA 95035) on every child presenting with clinical features of severe malaria. Values less than 2.2mmol/l were considered as hypoglycaemia.

Statistical analysis

Statistical analysis was performed using the SPSS (Statistical Analysis for Social Sciences) 17.0 software. Qualitative variables were expressed as number and percentage while quantitative variables were expressed as mean (X) and standard deviation (S).The arithmetic mean as a measure of central tendency, and the standard deviation (S) as a measure of dispersion where applied. The student t-test was used to compare the mean values of the quantitative variables between the subjects and the controls. For non-normally distributed quantitative variables, the Wilcoxon rank sum test was employed. The Chi-square test was used in finding a difference in the qualitative variables. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

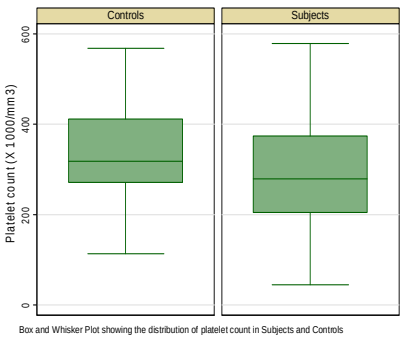
Results

A total of one hundred and eighty (180) subjects, and one hundred and eighty (180) controls were studied. The group of children aged one to five years were the most, constituting 73.3% of subjects. The mean age of the subjects, 3.75 ± 4.20 years and that of control group 3.78 ± 4.26 were comparable (p=0.96). The gender distribution and weight of subjects and controls were also comparable (p = 0.89). The mean temperature of subjects at presentation was 37.8 ± 0.84 and their mean duration of fever was 3.27 ± 1.58 days. No child in the control group had fever.

The mean malaria parasite count in subjects was 25,650.28 ± 88,312.04, with a range of 500 to 725,500 parasites/µl. *P. falciparum* was the only specie found in all the subjects. The control children had no parasitaemia. Platelet count was higher in the control group. This was statistically significant [(p = 0.0008) Fig I]. The platelet count range in subjects with uncomplicated malaria was 70 - 596 x 10^9/L significantly greater than the range, 44 – 565 x 10^9/L in subjects with severe malaria (Table 1).

Table 1: Platelet counts in subjects with uncomplicated versus severe malaria

Clinical Malaria	Platelet (x 10^9/L)		P- value
	Range	Mean ± SD	
Uncomplicated	70 – 596	313.95 ± 117.98	
Severe	44 – 565	192.54 ±141.62	P < 0.001



Box and Whisker Plot showing the distribution of platelet count in Subjects and Controls

Overall, nine (5.0%) of the 180 subjects in this study had thrombocytopenia, defined as platelet count < 100 x 10^9/L. There was no individual with thrombocytopenia among the controls. Of the 156 subjects presenting with uncomplicated malaria, three (1.9%) had thrombocytopenia, while six (25.0%) of the 24 subjects with severe malaria had thrombocytopenia. Of these, one subject (0.6%) had severe thrombocytopenia (defined by a platelet count < 50 x 10^9/L). This difference was statistically significant [(p < 0.001) Table 2a]. Comparatively, using a reference thrombocytopenic cut-off value of 150 x 10^9/L in this study, twenty-three (12.8%) of the 180 subjects would be seen to have thrombocytopenia. Of these, thirteen (54.2%) presented with severe malaria and ten (6.4%) with uncomplicated malaria. This was also statistically significant [(p < 0.001) Table 2b].

Table 2a: Prevalence of thrombocytopenia (<100,000) in clinical malaria

Platelet count (x10^9/L)	Subjects		Total	P-value
	Uncomplicated N %	Severe N %		
> 100,000	153 (98%)	18 (75%)	171 (95%)	
<100,000	3 (1.9%)	6 (25.0%)	9 (5%)	0.001

Table 2b: Prevalence of thrombocytopenia (<150,000) in clinical malaria

Platelet count (x10^9/L)	Subjects		Total	P-value
	Uncomplicated N%	Severe N %		
> 150,000	146(93.6%)	11(45.8%)	157(87.2%)	
<150,000	10 (6.4%)	13(54.2%)	23(12.8%)	0.001

Discussion

The low prevalence of 5.0% of thrombocytopenia in *falciparum* malaria found in this study is comparable to the 13.75% found by Jeremiah and Uko in Port-Harcourt.[19] This finding may be as a result of the thrombocytopenic cut-off range of 100 x 10^9/L used in both studies. Besides this, the children in that community study done in Port-harcourt were asymptomatic for malaria, which may otherwise, have had more profound effect on their platelet counts. Both studies were carried out in the south-south region of Nigeria, with similar levels of malaria transmission. The low prevalence rates obtained in both studies, may be due to the early acquisition of malarial immunity by many children in these holo-endemic study settings which confers a protective effect against severe manifestations including thrombocytopenia.[17] This immunity seems to vary in children from region to region[3] and may be a possible factor responsible for the low prevalence observed in both studies.

The higher prevalence of 23% to 59.7% documented in other studies carried out in the south-western part of Nigeria[23,24] were in contrast with that obtained from this

study. These differences in prevalence may be partly explained by the different age distributions of the children studied, the clinical severity of malaria in the groups studied, and the platelet cut-off reference ranges used by the different authors. These studies were hospital-based, had more children with severe malaria included and children with bacterial infections were not excluded. These may have contributed greatly to their higher prevalence rates of thrombocytopenia, considering the synergistic effect of these factors on depression of platelet count values. These authors[23,24] also both inexplicably used a thrombocytopenic cut-off range of 150 x 10^9/L in defining thrombocytopenia, rather than the accepted normal range of 100 x 10^9/L used in present study, being the standard reference range for the African population.[9,19]

Outside Nigeria, higher prevalence rates of 49% and 58% were also documented among children in Kenya[25] and Gabon[26] respectively, who live in similar tropical settings with stable malaria and perennial transmission. A higher cut-off of platelet reference value of 150 x 10^9/L was also used in these studies. This seems to have contributed significantly to the higher prevalence documented. As compared to this study, a higher prevalence was also obtained from children in Dakar[18] (56.2%) and France[16] (45.6%), being areas of hypo-endemic and non-endemic transmission of malaria respectively. This may be because children in these hypo and non-endemic areas of transmission are mostly non-immune to malaria.[2,16,18] They are therefore at a higher risk of severe clinical manifestations of malaria, with its attendant lowering effects on platelet count values. Besides, the prospective study in Dakar was done over a prolonged period of seventeen months, and children with severe malaria comprised a greater percentage (74.7%) of the study population. This probably explains the low platelet count values. This re-inforces the importance of a close monitoring of the platelet counts in children visiting from non-endemic settings, since they are at greater risk of thrombocytopenia.

The significantly lower platelet count in the children with malaria parasitaemia than those in the control group is an observation similar to that made by Akingbola et al[23] and Iwalokun et al[24] in Lagos, Nigeria. Likewise, the normal platelet count values seen in the control children in this study as also observed by Akingbola et al[23] all serve to affirm the adverse effect of malaria parasitaemia on platelet count values. The reduced platelet lifespan and platelet destruction in acute malaria, which partly results from the binding of malaria antigen unto platelets is perhaps responsible for the above findings. This observation was found to be irrespective of the malaria transmission level or specie in the areas studied.[21]

Children with severe manifestations of malaria had significantly lower platelet counts than those with uncomplicated malaria in this study similar to previous reports in other Nigerian children.[23,24] Reduction in platelet count values, which is a more frequent finding in severe forms of malaria, would have been responsible for the lower platelet counts seen in the children in this study.

Conclusion

In conclusion, although the overall prevalence of thrombocytopenia in children with *falciparum* malaria in our locality is low, it is significantly higher in children with severe manifestations of malaria than in the uncomplicated type. It is recommended where possible, to evaluate and monitor the platelet counts in children presenting with clinical features of severe malaria. It would also be beneficial to use standardized normal reference values representing children living in similar geographical settings.

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