Original Article

ADVANCES IN BIORESEARCH
Volume 2, Issue 2, December 2011: 72- 74
ISSN 0976-4585
Journal's URL: www.soeagra.com/abr.htm
[Accepted 29 November 2011]

Effects of Cerebral Malaria on Platelet Count, Platelet Factor – 3 and Platelet Aggregate Availability in Children

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ABSTRACT
Malaria is a formidable global parasitic infection that presents a major health challenge in tropical countries especially among children. A total of 130 children (65 males and 65 females) with cerebral malaria and the same number and ratio for healthy children were considered as control; all aged 1-9 years. Standard haematological method was used to determine the Packed cell volume, platelet aggregate, platelet count and platelet factor-3. Platelet and its role in making platelet factor 3 (PF-3) available for coagulation in children with cerebral malaria were determined. Packed cell volume and platelet count were found to be significantly lower (P<0.01) in children with malaria compared to healthy ones while platelet factor–3 was significantly higher (P<0.01) in children with cerebral malaria compared to normal children. There was no significant difference of platelet aggregate between children with cerebral malaria and uninfected children at P<0.01. Cerebral malaria infection is therefore associated with thrombocytopenia and the increase of platelet factor–3 availability which in the presence of platelet aggregates may lead to hypercoagulability and various bleeding complications in children with cerebral malaria.
***Key Words**: Platelet, Platelet factor-3, Packed cell volume, Platelet aggregate, Cerebral Malaria.*

INTRODUCTION

Malaria is global parasitic infection that continues to present a major health problem in tropical countries. The disease is caused by plasmodium species transmitted by female anopheles mosquito is associated with high morbidity, high mortality especially among children thus a grave threat to life.

The primary physiological function of platelets is the production of coagulation factors necessary for intrinsic prothrombin activator formation. Active platelet extractions have been found to be phospholipids, a platelet factor 3 (PF-3) which becomes available to the coagulant enzymes and co-factor at various stages of haemostasis.

Many investigators [2-7] have observed marked alteration in haemostatic functions in malaria including thrombocytopaenia and bleeding disorders as a result of coagulation factors. Anaemia is an almost inevitable consequence of malaria infection but its pathophysiology is complex and has relatively little in common with anaemia of other infectioins [4]. Cerebral malaria occurs in approximately 2% of patients with acute *falciparum* malaria [8]. Cerebral malaria is suspected in any child with malaria whose level of consciousness is deteriorating and where no other cause is found.

And also, Vreeken,[8] reported that due to reduced humoral immune response to the sporozoite in which diagnosis is made in patients that show evidence of neurologic dysfunction e.g. seizure, convulsion, disturbance of consciousness not readily explained by metabolic abnormalities or severe febrile reactions.

Recently, it has been suggested that increased PF-3 availability may be as a result of conformational change in platelet membrane protein [9]. These together with increased platelet aggregation may contribute to a hypercoagulable state [9]. To date, there is very scanty information on the effect of cerebral malaria infection on platelet function, despite the high rate of morbidity and mortality due to *Plasmodium falciparum* infection among Nigerians. This study is aimed at determining the role of platelet count and its ability to release PF-3 for coagulation in patients with cerebral malaria.

MATERIALS AND METHODS

Sampling: A total of 130 children 65 males and 65 female of age 1-19 with acute cerebral malaria and the same numbers and sex ratio were taken for children without malaria and they were considered as control, thus totaling 260 children sampled from the out patient clinic emergency ward of Ahmadu Bello University Staff Medical Centre, Samaru, Zaria, Kaduna State, Nigeria.

Patients considered for cerebral malaria were those that showed neurologic dysfunction e.g. seizure, convulsion, disturbances of consciousness not readily explained by severe febrile reaction, etc. Patients with malaria and the control subjects who had been on any anti-malaria drugs or drugs known to inhibit platelet function such as aspirin, corticosteroids, penicillin were excluded from study. Also, verbal consent was obtained from the parents before blood was taken.

Patients with malaria were identified by thin and thick blood films for malaria parasites. Malaria positive and negative patients were recorded. Also, those that their blood film was negative were excluded from the study even if they had clinical manifestation of malaria such as fever.

Packed cell volume and platelets determination: From the children sampled, 4.5ml of blood was obtained from the anti-cubital fossa vein and was added to 0.5ml of 3.8% tri-sodium citrate solution and sample mixed gently. No haemolysed sample was used. Packed cell volume and platelet counts were done using the method of Dacie and Lewis [10].

Platelet factor 3 (PF-3) determinations: Plasma centrifuged from the blood at 500 revolutions per minute for 5 minutes in order to avoid the platelet membrane disturbance and platelet rich plasma (PRP) was decanted. PF-3 was determined according to the method of Hardisty and Hutton[11], which was done by carrying out a kaolin clotting test on the PRP. One volume of PRP was mixed with an equal volume of platelet poor plasma (PPP). The mixture was incubated with normal platelet poor plasma (PPP) and with kaolin of an equal volume of 0.02 mole calcium chloride ($CaCl_2$). The clotting time is expressed as percentage of the total PF-3 using a reverence curve. Reference curve was constructed with freezing and thawing five times the PRP obtained from healthy individuals (five males and five females). The dilution on the platelet rich plasma was made using platelet poor plasma as the diluent. The kaolin clotting time was then performed on the dilutions. The reference curve was constructed by plotting time versus percentage dilution on double logarithmic paper. Malaria parasitaemia (MP) was examined microscopically as described by Dace and lewis [11].

RESULTS

Table 1: Mean and standard deviation value of children with malaria and controls

Parameters	Children Control (n-130)	Children with malaria (n=130)	P-values
Age	8.60 ± 1.0	9.60 ± 1.0	**N/S**
PCV (%)	35.6 ± 62	26.2 ± 7.40	**P<0.001**
Platelet count (x 10%)	278.14 ± 87	166.46 ± 54.2	**P<0.001**
Platelet factor 3 (x 10%)	62.8 ± 14.0	68.5 ± 16	**P<0.001**
Platelet aggregate	**0.67 ± 0.09**	**0.72 ± 97**	**NS**

Key: *NS: not significant*

Table 2:Shows PCV, PF-3, and P.A by sex variation within the groups

Parameters	Normal children	Infected Children
PCV (%)	Male: 37.8 ± 4.6	**28.8 ± 6.4***
	Female: 33.5 ± 2.8	**25.9 ± 7.7**†
Platelet count (X10⁹/1)	Male: 260.7 ± 65	**166.3 ± 54***
	Female: 233.8 ± 67	**165.9 ± 4.3**
PF-3 (%)	Male: 63.3 ± 17.5	**66.1 ± 13.6***
	Female: 61.6 ± 7.6	**66.8 ± 18.9**
P.A.	Male: 0.69 ± 0.10	**0.72 ± 0.08**
	Female: 0.68 ± 0.09	**0.72 ± 0.08**

**shows significant difference at P≤0.01 between normal and infected and* † indicates *significant difference between sex of infected children.*

Key: PVC=parked cell volume.
PF-3 =Platelet Factor 3
P.A. =Platelet aggregate

DISCUSSION

The result revealed decreased packed cell volume, platelet counts and increased platelet factor-3 (PF-3) availability in children with malaria compared with controls at P≤0.01. Platelet aggregate was neither increased nor decreased. The result agrees with previous findings that cerebral malaria infection causes accelerated turnover of haemostatic mechanism arising from possible disorders of platelet [7]. Most of the patients with cerebral malaria infection had thrombocytopaenia, which agrees with other reports [5,6,7,10]. The mechanism for the production of thrombocytopaenia was postulated by report [10], to be due to the removal of platelets from circulation by consumption in intravascular coagulation which is evidenced in depletion of coagulation factors and presence of fibrin degradation products. Patients with malaria were observed to have low PCV. This could be due to the sequestration of parasitized red blood cells between the peripheral blood parasitaemia and the extent of haemolysis4. However, our study suggests that both iron sequestration and dyserythropoiesis may contribute to the development of anaemia but the mechanism of this marrow disturbance and gross elevation by serum feritin which often accompanies it remain unknown [9].

The increased PF-3 availability in malaria could be due to repeated malaria attacked in the presence of other infestations. Shape and functional changes of platelet, platelet-platelet interaction and centralization of platelet organelles could also be responsible for the increase in PF-35. The increased PF-3 could lead to coagulation elevation resulting from platelet hyperactivity, which may contribute to a hypercoagulability state constituting a risk factor for thrombotic complications during cerebral malaria infestation. Platelet aggregate showed no significant difference in either sex or those infected or normal as seen in other works [5,6,7] that reported increased level of PA. When male and female controls were compared with malaria children, there were significant increase in PCV, PC ($P<0.001$) respectively and lower PF-3 level in male and female control than their counterparts with malaria. Increase PF-3 in male and female children with malaria could explain possible increases in cerebral thrombotic complications during malaria infection. The increase PF-3 with presence of platelet aggregation could be associated with various bleeding disorders during malaria attack [7]. From the result it can be concluded that thrombocytopenia associated with malaria infection and the increased platelet factor-3 with presence of platelet aggregation may lead to spontaneous bleeding and thrombotic complications leading to higher morbidity and mortality in children.

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