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# RESPONSES OR EFFECT OF CEREBRAL MALARIA ON PLATELET COUNT, PLATELET FACTOR – 3 AND PLATELET AGGREGATE AVAILABILITY IN CHILDREN

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## ABSTRACT

**Background and Objective:** Malaria, a formidable global parasitic infection represent a major health problem in tropical countries in terms of geographical spread, high morbidity, severe mortality especially among children. This study investigated platelet function's role and its strength to make platelet factor 3 (PF-3) available for coagulation in children with cerebral malaria.

**Subjects and Methods:** Packed cell volume, platelet aggregate, platelet count and platelet factor-3 were studied in 65 children with, cerebral malaria and 50 healthy control children (Both group were aged 1-9 years).

**Results:** Packed cell volume, platelet count were significantly lower ($P<0.01$) in children with malaria and platelet factor-3 was significantly higher ($P<0.01$) in children with cerebral malaria compared to normal children (controls). There was no significant level of platelet aggregate between children with cerebral malaria and control.

**Conclusion:** Although the pathological roles of platelet function abnormalities in cerebral malaria infection is controversial but our study conclude that cerebral malaria infection is associated with thrombocytopenia and the increase of platelet factor-3 availability, with presence of platelet aggregates, may lead to hypercoagulability and various bleeding complications in children.

**INTRODUCTION:** According to Marcfaclene [1], "If we take as our standard of importance the greatest harm, to the greatest number, then there is no question that malaria is the most important of all infectious diseases" because it is a formidable global parasitic infection which continues to represent a major health problem in tropical countries in term of geographical spread, high morbidity, severe mortality especially among children. These remain 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 infections [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.

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. Here, in this study, we have investigated the imminent role of platelet count and its strength to release PF-3 for coagulation in subjects with cerebral malaria.

**SUBJECT AND METHODS:**

**Subjects:** 65 children with acute cerebral malaria from the out patient clinic emergency ward of the Sick-bay, Ahmadu Bello University Staff Medical Centre, Samaru, Zaria, Kaduna State were compared with 50 children drawn from normal walks of life as control.

Patients with malaria were identified by thin and thick blood films for malaria parasites and packed cell volume according to the method of Dacie and Lewis [10]. Patients that showed positive in their blood films were considered to have malaria infection while those that showed negative in their blood film were excluded from the study even if they had clinical manifestation of malaria such as fever. Patients that were 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 parent before blood was taken and clearance was obtained from the appropriate authority.

**Methods:** 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 haemolyzed sample was used. Packed cell volume, platelet counts were done using the method of Dacie and Lewis [10]. Plasma was subsequently prepared by centrifugation of the blood at 500 revolutions per minute for 5 minutes in order to avoid the platelet membrane disturbance and platelet rich plasma (PRP) 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 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].

**Statistical Analysis:** All values of data was analyzed and the student t-test was used for the significance determination of the unpaired data with significance set at $P<0.05$).

**RESULT: Table 1** shows the means (SD) values of packed cell volume (PVC), platelet count (PC), and platelet aggregate (PA), age group and platelet factor-3 (PF-3) availability in children with malaria and without malaria as controls. Control children had significantly higher PCV, PC ($P<0.01$) and significantly lower PF-3 ($P<0.001$ than children with malaria. Platelet aggregate was no significant in both groups. Age group was not significant. **Table 2** shows the mean values of PCV, PC, PF-3 and PA level by sex variation within the group. There were significant sex differences in PCV, PC, and PF-3 in children with malaria. However PCV and PC were significantly higher in male in controls than female, and platelet aggregate shows no significant in both groups.

**TABLE 1:MEAN AND STANDARD DEVIATION VALUE OF CHILDREN WITH MALARIA AND CONTROLS**

| Parameters | Children Control (n-50) | Children with malaria (n=65) | 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 |
| N/S – not significant. | | | |

**TABLE 2:SHOWS PCV, AND PF-3, P.A BY SEX VARIATION WITHIN THE Groups**

| Parameters | Children Control | Children with malaria |
|---|---|---|
| PCV (%) | Male: 37.8 ± 4.6 | 28.8 ± 6.4 |
| | Female: 33.5 ± 2.8* | 25.9 ± 7.7 |
| Platelet count ($X10^9$/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 |

*P<0.001 compared with males with P<0.0025 compared with females

**DISCUSSION:** Our study revealed decreased packed cell volume, platelet counts and increased platelet factor-3 (PF-3) availability in children with malaria compared with controls. Platelet aggregate was neither increased nor decreased. Our findings agree with previous reports that cerebral malaria infection causes accelerated turnover of haemostatic mechanism arising from possible disorders of platelet. 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.

Most of the patients with cerebral malaria infection had thrombocytopaenia, which agree with other reports [5, 6, 7, 10]. The mechanism for the production of thrombocytopaenia was postulated by report [10], to be removal of platelets from the circulation by consumption in intravascular coagulation with evidence 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 haemolysis [4]. However, our study suggests that both iron sequestration and dyserythropoiesis may contribute to the development of anaemia but thee mechanism of this marrow disturbance and gross elevation by serum feritin which often accompanies it remain unknown.

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-3 [5]. 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 was not significant in our study but various studies [5, 6, 7] reported increased level of PA.

Our study was not able to detect any variation in the platelet function due to sex, between control and malaria children. 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. From our study, we conclude 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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