

## Age as a risk factor for thrombocytopenia and anaemia in children treated for acute uncomplicated falciparum malaria

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### Abstract

**Background & objectives:** Anaemia is commonly observed in children with malaria, but reports on leucocyte and platelet count abnormalities associated with malaria are inconsistent. This study examined the effect of age, gender, parasite density and temperature on haematological parameters in children with acute uncomplicated malaria.

**Methods:** Haematological parameters were determined in children with acute uncomplicated malaria, and these were correlated with age, sex, temperature and parasite density. Statistical analysis was done using SAS 9.1.

**Results:** Six hundred and ninety five children with acute uncomplicated malaria participated in the study. The mean age was 51.7 months  $\pm$  33.8. At presentation, anaemia occurred in 43.8% of the patients and children <5 yr had a significantly lower haematocrit (28.4%  $\pm$  4.8) than that of older children (32.8%  $\pm$  4.8) ( $p < 0.001$ ), but the haematocrit was not significantly different by days 14 and 28. There was no difference between both sexes. Leucocytosis was more frequently seen than leucopenia (9.5% vs 3%). Thrombocytopenia was found in 59.3% of enrolled patients. More than half of the patients with thrombocytopenia had recovered by Day 28. Baseline platelet count was related to Day 14 ( $r = 0.6$ ,  $p < 0.0001$ ) and Day 28 ( $r = 0.2$ ,  $p = 0.0015$ ) and the haematocrit on Day 28 ( $r = 0.12$ ,  $p = 0.00197$ ). Platelet count showed no correlation with temperature, parasite density and leucocyte count. Haematocrit correlated with age ( $r = 0.4$ ,  $p < 0.0001$ ); but not with parasite density or temperature. Leucocyte count showed no correlation with age or parasite density.

**Conclusion:** While thrombocytopenia was the most common haematological finding and may be of diagnostic importance, anaemia and leucocytosis were more common in the under fives.

**Key words** Anaemia – children – Nigeria – *P. falciparum* malaria – thrombocytopenia

### Introduction

Malaria remains the most important parasitic disease afflicting about 2.2 billion people globally<sup>1</sup>. In Africa, south of the Sahara, about 90 million clinical cases of malaria occur each year causing 1–2 million

deaths<sup>2</sup> of the estimated 3.5 million deaths worldwide. Most malaria deaths are due to *Plasmodium falciparum* infection<sup>3</sup>. In southern Nigeria, at least 35,000 children die annually from direct effects of malaria alone, accounting for 25–30% of all the mortalities in infants and childhood<sup>4</sup>. Pre-school children

aged <5 yr are at the greatest risk of malaria<sup>3</sup>. Haematological changes have been reported in association with malaria<sup>5-9</sup>. These include thrombocytopenia which was observed to be of diagnostic importance<sup>5,6</sup>, leucopenia and lower haemoglobin concentration<sup>7</sup> which was not affected by species of malaria infection or the ethnic group of the patients. Changes in haematological parameters may be affected by several factors including age, sex, level of parasitaemia, presence or absence of fever and the degree of fever. This study examines the haematological changes associated with acute uncomplicated malaria in children and how these are affected by the factors above.

### Material & Methods

Data used in this study were obtained from four clinical trials evaluating antimalarial drugs among children with microscopically proven acute uncomplicated falciparum malaria during years 2000 to 2003. The clinical trials included chlorproguanil-dapsone (Lapdap<sup>®</sup>) versus sulphadoxine-pyrimethamine (SP), atovaquone-proguanil (Malarone<sup>®</sup>) versus chloroquine and two studies on artemether-lumefantrine (Coartem<sup>®</sup>) and amodiaquine plus sulphadoxine-pyrimethamine (AMQ/SP). Follow-up of patients enrolled in the study evaluating chlorproguanil-dapsone vs SP was for 14 days while all other patients were followed-up for 28 days. The studies were carried out at the University College Hospital, Ibadan, Nigeria. The patients were selected from those attending the malaria clinic and the General Out Patient Department of the hospital. The study protocols received ethical approval from the Joint UCH/University of Ibadan Ethical Review Committee.

*Inclusion criteria:* Children aged 6 months to 14 yr with symptoms compatible with acute uncomplicated falciparum malaria, fever or history of fever in the 48 h before presentation, *P. falciparum* parasitaemia with parasite density >1000 asexual forms/ $\mu$ l of blood and absence of concomitant illness such as bronchopneumonia and sickle-cell anaemia.

Children who satisfied the inclusion criteria were enrolled into the study after obtaining a written informed consent from their parents/guardians. A detailed history was taken followed by a standardised physical examination. The clinical data were entered into a special Case Record Form. Thick and thin blood films from finger prick samples were obtained and Giemsa stained for identification and quantification of parasites. Parasitaemia was quantified in thick film by counting parasites relative to leucocytes. At least 200 leucocytes were counted. The count was then converted to parasite per microlitre of blood using baseline white cell count (WCC) of each patient. Venous blood was drawn into EDTA tubes and used for haematological evaluation. Cell counts were performed using a Coulter<sup>®</sup>A<sup>c</sup>.Tdiff<sup>™</sup> Analyzer (Beckman-Coulter Company, Miami, Florida). Haematological parameters (haematocrit, leucocyte count, and platelet counts) were determined and were correlated with age, sex, temperature and parasite density.

Statistical analysis was done using SAS version 9.1. To test for an association between age groups and specific haematological values, categorical variables were created for platelets, haematocrit (packed cell volume) and white cell counts (WCCs). Chi-square was used to compare proportions and categorised variables. Correlations were determined for the age group, sex, temperature, parasite density and various haematological parameters at presentation and follow-up using Pearson correlation coefficients.

### Results

A total of 695 patients were included from four clinical trials. The mean age of the patients is  $51.7 \pm 33.8$  months. There were more children <5 yr (61.4%) than >5 yr. Approximately half (53.8%) of the study population were males. The mean haematocrit on Days 0, 14 and 28 were  $30.1 \pm 5.3\%$ ,  $29.2 \pm 5.6\%$  and  $30 \pm 4.9\%$ , respectively. Anaemia (haematocrit <30%) occurred in 43.8% of the patients at presentation.

**Table 1. Haematological features (Mean  $\pm$  S.D.) of children with acute uncomplicated falciparum malaria**

| Parameters                                  | Age <60 months<br>N= 416 (61.4%) | Age $\geq$ 60 months<br>N= 262 (38.6%) | P-value |
|---|----------------------------------|--|---------|
| Haematocrit on Day 0 (%)                    | 28.4 $\pm$ 4.8                   | 32.8 $\pm$ 4.8                         | <0.0001 |
| Haematocrit on Day 14 (%)                   | 27.8 $\pm$ 5.5                   | 31.1 $\pm$ 5.1                         | <0.0001 |
| Haematocrit on Day 28 (%)                   | 30.4 $\pm$ 4.9                   | 29.7 $\pm$ 4.9                         | 0.1532  |
| White cell count ( $\times 10^9/L$ ) Day 0  | 9.2 $\pm$ 13.2                   | 7.7 $\pm$ 9.4                          | 0.1239  |
| White cell count ( $\times 10^9/L$ ) Day 28 | 7.5 $\pm$ 2.8                    | 7.3 $\pm$ 6.6                          | 0.6946  |
| Platelet count ( $\times 10^9/L$ ) Day 0    | 162.5 $\pm$ 85.3                 | 132.5 $\pm$ 55.6                       | <0.0001 |
| Platelet count ( $\times 10^9/L$ ) Day 28   | 279.8 $\pm$ 301.0                | 207.7 $\pm$ 100.7                      | 0.0066  |

Fifty-eight percent of children <5 yr old in contrast to 21% of those who are  $\geq$ 5 yr had anaemia at presentation. Children <5 yr had a significantly lower haematocrit compared with  $\geq$ 5 yr old (28.4  $\pm$  4.8% vs 32.8  $\pm$  4.8%;  $p < 0.0001$ ) but this had improved by Day 28 (Table 1). There was no difference in haematocrit between both sexes. Haematocrit correlated with age,  $r = 0.4$ ,  $p < 0.0001$ ; but not with para-

site density ( $r = -0.01$ ,  $p = 0.73$ ) or temperature ( $r = 0.05935$ ,  $p = 0.1556$ ).

The mean leucocyte counts were  $8.5 \times 10^9/L \pm 11.8 \times 10^9/L$  and  $7.5 \times 10^9/L \pm 4.7 \times 10^9/L$  on Days 0 and 28 respectively (Table 2). Leucocytosis was more frequently seen than leucopenia (9.5% vs 3%) at presentation. By Day 28, patients with leucocytosis had

**Table 2. Categorized haematological parameters of children with acute uncomplicated falciparum malaria**

| Haematological Parameters         | Day 0          |                      |         | Day 28         |                      |         |
|-----------------------------------|----------------|----------------------|---------|----------------|----------------------|---------|
|                                   | Age <60 months | Age $\geq$ 60 months | P-value | Age <60 months | Age $\geq$ 60 months | P-value |
| <i>PCV (%)</i>                    |                |                      |         |                |                      |         |
| <30%                              | 231 (35.3)     | 55 (8.4)             | <0.0001 | 100 (29.7)     | 65 (19.3)            | 0.560   |
| $\geq$ 30%                        | 168 (25.6)     | 201 (30.7)           |         | 141 (41.8)     | 31 (9.2)             |         |
| <i>WCC (/mm<sup>3</sup>)</i>      |                |                      |         |                |                      |         |
| <4,000                            | 10 (1.6)       | 9 (1.4)              |         | 8 (2.2)        | 2 (0.6)              |         |
| 4,000 – <11,000                   | 329 (51.9)     | 226 (35.6)           | 0.047   | 198 (54.5)     | 135 (37.2)           | 0.035   |
| $\geq$ 11,000                     | 45 (7.1)       | 15 (2.4)             |         | 17 (4.7)       | 3 (0.8)              |         |
| <i>Platelet (/mm<sup>3</sup>)</i> |                |                      |         |                |                      |         |
| <50,000                           | 6 (1.0)        | 6 (1)                |         | 5 (1.4)        | 5 (1.4)              |         |
| 50,000 – <100,000                 | 58 (9.2)       | 59 (9.4)             |         | 13 (3.5)       | 13 (3.5)             |         |
| 100,000 – <150,000                | 134 (21.3)     | 112 (17.8)           | <0.0001 | 30 (8.2)       | 25 (6.8)             | 0.003   |
| >150,000 – <400,000               | 170 (27)       | 74 (11.8)            |         | 140 (38.1)     | 90 (24.5)            |         |
| $\geq$ 400,000                    | 8 (1.3)        | 2 (0.3)              |         | 40 (10.9)      | 6 (1.6)              |         |

Figures in parentheses are percentages.

dropped by almost half. Almost all the patients (91.6%) had a normal white cell count by Day 28 compared to 87.5% at presentation. The WCC was not affected by age or gender. Leucocyte count showed no correlation with age, parasite density or temperature.

The mean platelet count was  $150 \times 10^9/L \pm 76 \times 10^9/L$  at presentation and  $253 \times 10^9/L \pm 247 \times 10^9/L$  at Day 28. Thrombocytopenia (platelet  $<150 \times 10^9/L$ ) was present in 59.3% of subjects at presentation; of whom 1.9% had severe thrombocytopenia (platelet count  $<50 \times 10^9/L$ ). Thrombocytopenia persisted in 22.3% of the subjects on Day 14 and 24.8% of subjects on Day 28 (Table 2). Correlation analysis demonstrated baseline platelet count was significantly positively related to Day 14 and 28 platelet counts ( $r = 0.6$ ,  $p < 0.0001$  and  $r = 0.2$ ,  $p = 0.0015$ ) respectively, and also to haematocrit on Day 28 ( $r = 0.12$ ,  $p = 0.00197$ ), and WCC on Day 28 ( $r = 0.19066$ ,  $p = 0.0003$ ). Platelet count showed no correlation with age, parasite density or temperature.

### Discussion

Anaemia has frequently been associated with malaria, with haemolysis, decreased erythropoiesis and splenic sequestration as possible causes. In the present study about 44% of the patients presented with anaemia, and this was more common in children  $<5$  yr old. This underscores the burden of anaemia in malaria, particularly in children  $<5$  yr in endemic areas. This is consistent with previous reports that anaemia due to malaria is more severe in younger children in areas of intense malaria transmission<sup>10,11</sup>. Gender had no effect on the haematocrit of children enrolled in this study. The disparity in haematocrit with gender commonly observed in adults<sup>12,13</sup> may therefore be hormonal since only children who were pre-pubertal were enrolled in the present study.

Leucocytosis was more commonly observed in this study than leucopenia, as was the findings by Sharma

*et al*<sup>14</sup>. The finding of leucocytosis has been reported in acute *Plasmodium* infection<sup>15,16</sup> and may suggest co-existing viral infection particularly with the presence of atypical lymphocytes<sup>7,17</sup> and more so in children among whom viral infection may be common. Some cases of leukamoid reaction following malaria had also been documented in the past. However, some other studies had reported leucopenia among malarial patients<sup>18,19</sup>. Age or gender had no effect on leucocyte count suggesting the cell lines are not necessarily affected by the same factors, since age was an important factor in haematocrit.

Most patients (59.3%) had thrombocytopenia at presentation but had recovered by Day 28 with only 24.8% of the study population being thrombocytopenic. Thrombocytopenia may result from a shortened life span of platelets or from pooling and destruction in the spleen<sup>20</sup>. Consumption of platelets also occurs in disseminated intravascular coagulopathy associated with severe falciparum malaria. However, only children suffering from acute uncomplicated malaria were studied and reported here. Thrombocytopenia in malaria is both non-immunologically mediated<sup>21</sup> and also immune mediated. Immune complexes are formed which activate and thus enhance platelet phagocytosis by macrophages in the spleen<sup>22,23</sup>. Contact of platelet membrane to damaged endothelial lining and with RBCs infected by parasites may trigger off this process<sup>24</sup> resulting in intravascular lysis of the activated platelets<sup>25</sup>.

The effect of malaria is more severe in children especially those  $<5$  yr of age. This is further shown by this study in which the  $<5$  yr olds have a significantly lower mean haematocrit. Thrombocytopenia occurring in this study as the most common haematological alteration may further suggest its diagnostic importance in malaria. Age has a significant influence on the platelet count judging from its effect both at presentation and on Day 28. The fact that these haematological changes were corrected by Day 28 may reflect the acute effect of *P. falciparum* on the bone

marrow. However, a co-existing viral infection should also be considered in children presenting with acute malaria and leucocytosis.

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