Reticulocyte Count Of Nigerian Children With Malaria Infection In The University Of Calabar Teaching Hospital.

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Abstracts: Background: This work was sets out to assess the reticulocyte count of Nigerian Children infected with malaria in the University of Calabar Teaching Hospital, comprising of fifty malaria infected children and forty six apparently healthy children. The malaria infected children comprised of 25 males and 25 females aged 1-10 years. Blood samples were collected and analysed for reticulocyte count using the 1% new Methylene Blue supra vital staining method and malaria parasites using, 2% Giemsa stain. The mean reticulocyte count in the malaria infected children (2. 17 ± 0.3%) was significantly higher (P<0.01) than that in the apparently healthy children (1.03 ± 0.3 %). The mean parasites density in infected children was 30,428.76 ± 11,876.85 ul. A comparism of reticulocyte level of infected male (2.204 ± 0.32%) and female children (2.133 ± 0.3 6%) showed no significant difference (p>O.O5). Equally, the reticulocyte levels of infected children between 1-5 years of age (2.1 5 ± 0.34%) was not significantly different (P> 0.05) from that of children aged between 6-10 years (2.18 ± 0.32%). The level of reticulocytosis is a reflection of parasite density in the malaria infected children. [Udomah F et al NJIRM 2015; 6(2):44-48]

Key Words: Reticulocytes, Anaemia, Malaria.

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Introduction: Malaria is a life threatening parasitic infection transmitted by the bite of a female anopheline mosquito. It was once thought that the disease came from fatid marshes, hence the name mal-aria (bad air). Scientists have since discovered the real cause of malaria to be a protozoan parasite called plasmodium[1]. Of the four species of plasmodium, the most deadly and most rampant cause of malaria in the tropics is *Plasmodium falciparum.*

Today, over 40% of the world population, especially those living in the world poorest countries are at risk of malaria[2,3]. In Africa, malaria kills a child after every 30 seconds[4]. Malaria infection causes massive destruction of red blood cells resulting in anaemia[5]. The degree of anaemia depends on the extent and frequency of the malaria infection. Repeated episodes of malaria and anaemia has severe effect and consequences on the body system including the brain, leading to impairment in children and depletion of essential metabolites including folate, enzymes and co-factors which are essential for the formation of blood cells[6]. Malaria induces haemolysis of red blood cells resulting in abnormally low levels of haemogolobin[7] and anaemia which is a major cause of prenatal mortality and low birth weight. These conditions induce erythropoietic activities on the bone marrow and are reflected on the peripheral blood as increased reticulocytes which is above the normal value.

In repeated malaria infection where blood forming metabolites especially folate has been depleted, reticulocyte response could be hampered. In the case of severe infection, reticulocyte count could become stagnated or even lower than normal instead of being increased. Reticulocyte levels in malaria infection may therefore reflect not only the bone narrow response to haemolysis but also the extent of bone marrow depletion. Since reticulocytosis is a direct function of the erythropoietic activity of the bone marrow, it is therefore one of the most valuable parameters in diagnostic haematology[8].

This study is meant to reflect the bone marrow response in malaria infection as well as the contribution of malaria to the depletion of haemopoietic metabolites in the bone marrow. It intends to use reticulocytosis to assess the rate of erythropoietic activity in the bone marrow in malaria infected children in both severe and mild malaria infection. This work will also seek to establish how the number of reticulocytes in children infected with malaria varies from those who are healthy and not infected with malaria.

In doing these, the study will not lose sight of the fact that in some disease conditions, the degree of haemolysis will not only be reflected by the number of reticulocytes in the peripheral blood but such other conditions as deficiency of certain haemopoietic factors such as iron, vitamin B12 and folate and chronic lymphocytic leukaemia in which the reticuitocyte count may be much lower than would be expected from the degree of anaemia observed and could complicate the picture[9,10]. In the bone marrow, there is usually pancytopenia, including reticulocytopenia, even in the presence of severe intravascular haemolysis with resultant anaemia[11,12]. Subjects with any of such conditions would be excluded from this study.

In summary, the study will compare the reticulocyte levels in malaria infected children to that of normal children. It will establish the relationship between parasite density and reticulocyte levels as well as asses the influence of age and sex on reticulocyte levels of malarial infected children.

Materials and Methods: Fifty (50) sick children with ages ranging from I to 10 years, admitted with the diagnosis of malaria fever to the University of Calabar Teaching Hospital (UCTH) were used for this study. All cases were confirmed by examining the patient's thick and thin blood films for malaria parasites. Two millilitres (2ml) of blood was collected from each patient. Forty six (46) age and sex matched healthy children who were free of malaria were used as controls.

The degree of malaria severity was gotten by parasite density count and reticulocyte count was performed using the supravital staining technique of new Methylene blue. All results obtained were analysed for:

1. The reticulocyte count and the parasite density in the subjects and controls
2. Reticulocyte count of male and female subjects.
3. Reticulocyte count of the subjects, age 1-5 years and 6-10 years in conjunction with the sex.

Blood Samples Collection: Antecubital vein of the subjects was selected and the skin over it was swabbed with cotton wool soaked in methylated spirit. Tourniquet was applied to occlude the vein and stop the venous returns. The vein was swabbed again. Blood was collected by vene puncture and was delivered into a bottle containing 2mg of K_2EDTA per 1 ml of blood and mixed thoroughly by inversion. All samples were properly labelled and processed as soon as possible. Where storage was inevitable, this was done at a temperature of between 4°C and 6°C and for not more than 24 hours [13].

Malaria parasite (MP) test: Thick and thin films were made on two slides using blood from the patients. The slides were labelled and then allowed to dry. Thin films were then fixed in absolute methanol and both films were stained using 2% Giemsa stain by pouring the stain on the films and allowing them to stain for 3mins. The slides bearing the thin film were rinsed in distilled water and the back wiped using cotton wool.

The parasites detection and count were done on the thick film using x 40 and x l00 oil immersion objectives respectively and reported as follows:

1- 10 parasites per 100 high power (x40) field ..... +
11-100 parasites per 100 high power (x40) field ..... ++
1-10 parasites in every high power (x40) field ..... +++
More than 10 parasites in every high power (x40) field ..... ++++

The thin film was used mostly for species identification using the oil immersion objective. The parasite density count was done by counting the number of parasites seen per number of white cells as shown below.

Malaria Parasite Counting Technique: The number of parasites seen and the number of white cells per high field were counted on the thick films until at least 200 WBC were counted.
The parasites count was calculated using the formula below:

$$\frac{\text{Number of parasites counted}}{\text{Number of white blood cells}} \times \frac{8000}{1}$$

= Number of parasites per ul of blood (WHO, 1985).

Since the average white blood cell count in our hospital community is taken as 6000 according to Alaribe [15], this replaces 8000 gotten [14]. Therefore the formula used for malaria density calculation in this work was:

Malaria Parasite Density (MD) = Number of parasites counted x 6000 / Number of white blood cells x 1 = Number of parasite per ul of blood.

Reticuloeyte Enumeration: Principle - Using the supravital staining technique such as new Methylene blue, the stain precipitates the remnant of RNA in the red cells to deep blue precipitates of reticulum which is stained against a relatively unstained background.

Technique: About 2-3 drops of 1% new Methylene blue solution were delivered into a 75 mm x 10 mm test tube by means of a Pastuer pipette. About 2-4 drops of the patients EDTA anticoagulated blood were added to the dye solution and mixed. The mixture was incubated at 37°C for 20 minutes, resuspended and a film was made and allowed to dry. It was examined, without fixing, using x 100 oil immersion objective. The reticulocytes were recognised as having reticulum or network of RNA in the cytoplasm and recorded as Y (in the table below) while the red cells were recorded as X, until a total of 1000 cells (both reticulocytes and red cells) were counted. The count was recorded and the reticulocytes calculated as shown below:

Number of RBC	Number of Reticulocytes	Total	Retic %
X	Y	X+Y= 1000	

Calculation of % reticulocyte count = $\frac{Y}{1000} \times \frac{100}{1}$

Results & Discussion: The mean reticulocytes count in the malaria infected and apparently healthy children were 2.17 ± 0.34% and 1.034 + 0.34% respectively. These values were significantly different in the two groups (P<0.0 1) as shown in table 1. The mean parasite density is also shown in table 1. For the infected children, it was 30,428.76 ± 11,876.85 ul. The mean reticulocyte value for the malaria infected male and female children were 2.204 + 0.33% and 2.133 + 0.36% respectively. There was no statistically significant difference (p>0.05) between the two as shown in table 2.

The reticulocyte count for children between one to five years was 2.17 ± 0.34% while that for children between six to ten years was 2.18 ± 0.33%. There was no significant difference between the two as shown in table 3 (p>0.05).

Table 1: Reticulocytes values and parasite density for malaria infected children and controls

	Malarial infected children	**Cont-rols**	**Cal-t**	**Crit-t**	**P - value**
Number of subjects	50	46			
Mean (SD) reticulo-cyte count (x) %	2.17 ± 0.34%	1.034 ± 0.34%	16.71	2.63	P<0.01
Mean (SD) parasite density (x) µl	30,428. 76 ± 11, 87685	-	-	-	P<0.01

Table 2: Reticulocyte count for male and female malaria infected children

	Males	**Femal es**	**Cal-t**	**Crit-t**	**P-value**
Number of subjects	25	25			
Mean (SD) reticulocyte count (x) %	2.20 ± 0.33%	2.13 ± 0.36%	0.74	2.01	P>0.01

Table 3: Age and reticulocytes values of malaria infected children

	Malarial infected Children		**Cal-t**	**Crit-t**	**P-value**
Age range of subjects	**1 - 5 years**	**6 – 10 years**			
Number of subjects	34	16			
Mean (SD) reticulocyte count (x) %	2.17 ± 0.34%	2.18 ± 0.33%	0.10	2.01	P>0.05

This study shows (table 1) that the reticulocyte value in malaria infected children was significantly

higher than that in the apparently healthy children (P<0.0l). Regarding the reticulocyte count for normal children of the age range included in this study (0.2-2%), the higher than normal count observed here might probably reflect bone marrow response to a sustained intravascular haemolysis associated with the malaria infection. It has been established that persistent intravascular haemolysis such as observed in malaria patients ultimately results in anaemia especially if the haemolysis is not adequately compensated for by the bone marrow [16,17]. Increased reticulocytes in this group of patients might be a reflection of an attempt by the bone marrow to compensate for the anaemia caused by haemolysis. This finding is in conformity with previous studies[7,18] who established that there was a resultant low level haemoglobin resulting in anaemia in malaria infection. Unlike the work carried out by White[7] which involved subjects of wider age range, the present study was focused on children and increased reticulocyte was mostly noticed in cases of increased malaria infection.

The mean reticulocyte value in infected male and female children (Table 2) did not vary significantly (P>0.05). This finding of no significant sex difference is not entirely strange because males of this age range usually produce less androgen hormones than adults. Consequently, there is no significant difference between the reticulocyte levels of the males and females at this age group. Additionally, the female children have not yet started menstrual blood loss which could have contributed to increased reticulocytosis.

Age difference also did not show significant effect on the value of reticulocyte in the subjects (Table 3). No significant difference was observed in this parameter between the age groups 1-5 and 6-10 years in the study. This is probably because children of the two age groups generally respond equally to severe malaria infection by developing anaemia. However, a similar study by Kiran and Bukhari [19] showed gender impact in reticulocyte count in 3-month and 6-month age groups, whereby the female presented higher counts in the former and lower in the latter age group. It is not known with certainty, if reticulocyte response in children under age one, is different from those of children one year and above, who actually were the subjects of the present study. However, a British study has shown that the reticulocyte count value decreases with growing age of an infant [(20)].

Our study also shows that reticulocyte count was higher when the parasite density was high and vice versa (table 1). A relationship is therefore established between reticulocyte levels and parasite density in this study. It can thus be said that the reticulocyte count is directly proortional to the density of parasites in malaria infected children. In all cases, there was reticulocytosis which was proportional to the degree of malaria infection in all the infected children.

Conclusion: There is increase in reticulocyte count when the parasite density is high. Increase reticulocyte count reflects a greater response to prevailing anaemia caused by massive destruction of red cells during severe malaria infection. Reticulocyte count in malaria infected children is directly proportional to the parasites density in most children.

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