

## **Short Research Article**

### **The value of red blood cell indices in the diagnosis of severe malaria at a tertiary hospital in North-Eastern Nigeria.**

#### **ABSTRACT**

**Aim:** This study aimed at evaluating the red cell indices of children seen at the Federal Medical Centre, Azare, Nigeria, with a view to determining their utility in the diagnosis of severe malaria.

**Study design:** The study was a case control study.

**Place and Duration of the study:** The study was conducted at the department of Paediatrics, Federal Medical Centre, Azare, Bauchi state, Nigeria from 1<sup>st</sup> August to 31<sup>st</sup> October, 2013.

**Methodology:** One hundred and ninety-six children aged 6 months to 12 years, comprising of 98 diagnosed with severe malaria and 98 controls were recruited into the study. The following red cell indices were obtained from the subjects; haematocrit (HCT), haemoglobin concentration (HB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The data was analysed using SPSS version 16.0. Diagnostic precision was determined by calculating sensitivities, specificities, positive predictive and negative predictive values. The accuracy of these figures was assessed using 95% confidence interval.

**Results:** The HCT had a sensitivity of 79.59% and a specificity of 80.65%, while the HB had a sensitivity of 81.63% and a specificity of 71.15%. The positive predictive values for HCT and

HB were 86.67% and 84.21% respectively. MCV, MCH and MCHC had low values. MCH and MCHC however had a significant relationship with severe malaria ( $P < .001$ ).

**Conclusion:** In the presence of supporting clinical evidence, the existence of anaemia in a child in Azare may be a valuable supporting criterion in the diagnosis of severe malaria.

Keywords: Red blood cell; Indices; Severe malaria; Value; Diagnosis; Azare; Nigeria.

## INTRODUCTION

Malaria significantly impacts global mortality figures; it caused 429,000 deaths in 2015, with 70% occurring in children younger than 5 years. Ninety-two percent (92%) of all malaria mortalities occur in Africa. [1] The import of this is underscored by the fact that malaria is considered endemic in 104 countries and territories. Severe falciparum malaria is the most lethal form and preponderates in Nigeria. [1]

Malaria is preventable and treatable thus confirmation of diagnosis through microscopy or rapid diagnostic tests for every suspected case is a key element of the interventions for prevention and treatment. [1,2] However, these strategies are limited and require a certain degree of practical proficiency. These skills are not always available in semi-urban/ resource-poor settings like ours. Subsequently, it is not uncommon for clinicians to rely on clinical methods for diagnosis.[3]

Being an intra-erythrocytic parasite, plasmodium is known to trigger a wide range of haematological changes.[4] These changes may be dependent on factors such as ethnicity, level of immunity, genes, nutritional status, and socio-demographic conditions.[5] This study aimed at evaluating the red cell indices of children diagnosed with severe malaria at the Federal Medical Centre, Azare, north eastern Nigeria, with a view to determining their utility in aiding accurate diagnosis. To our knowledge no such study has ever been conducted in this part of Nigeria.

## METHODOLOGY

### Study Area:

This study was conducted at the Federal Medical Centre Azare, Bauchi state of North Eastern Nigeria. The centre serves as a tertiary health facility for the populations of Bauchi, Yobe and Jigawa states of northern Nigeria. Prior approval was obtained from the research ethics committee of the hospital.

### Study Design, Sampling and Duration

The study was a case control study. A total of 196 children aged 6 months to 12 years, comprising of 98 diagnosed with severe malaria and 98 controls were recruited into the study. The patients with severe malaria were consecutively recruited from the emergency paediatric unit (EPU) while the apparently healthy subjects (controls) were recruited from the paediatric out patients unit (POPD) from 1<sup>st</sup> August to 31<sup>st</sup> October, 2013 at the peak of the rainy season and period of highest transmission. Written Informed consent and assent (where applicable) were obtained from the caregivers and patients respectively. The ages and gender of all the subjects were also taken and documented.

Patients with evidence of other infectious diseases such as; typhoid fever, gastroenteritis, respiratory tract infections, acute bacterial meningitis or any other identified cause of anaemia other than malaria were excluded from the study. The subjects diagnosed with severe malaria were managed following standard guidelines. [6]

## **Specimen Collection**

Two milliliters (2ml) of venous blood was drawn into an EDTA anticoagulated sample bottle from all the subjects and sent to the central laboratory for analysis. Blood in the sample bottles were also used to prepare thick and thin films for demonstration of asexual forms of *Plasmodium falciparum* and to perform the rapid diagnostic test (RDT).

## **Performance of Haematological indices**

A Sysmex KX 21N haematology analyzer (serial no. 060120920) was used for analysis. The following red cell indices were obtained from the subjects; haematocrit (HCT), haemoglobin concentration (HB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). An HB level of less than 10g/dl, HCT of less than 30% and MCV of less than 80fl were regarded as the most likely predictors of severe malaria in this study.

## **Detection of Malaria Parasite**

Confirmation of severe malaria diagnosis was done with microscopy or rapid diagnostic tests (RDT) in the presence of any of the World Health Organization (WHO) case definitions for severe malaria [2,6]. The thin and thick films for malaria parasite were stained with Giemsa stain and read by medical laboratory scientists. The RDT was done using the paracheck Pf® rapid test kit for *P. falciparum* malaria (a rapid test for the detection of *Plasmodium falciparum* specific histidine rich protein-2). Results were documented as positive or negative as indicated by the coloured bands on the control windows; test ('T') and control ('C') respectively.

## Data Analysis

Collected data was entered into the statistical package for social science (SPSS) version 16.0. Categorical data were compared using the Chi-square test. Comparison between means was done using the student t-test. A *P* value less than .05 was regarded as been statistically significant. The diagnostic precision of the red blood cell indices was determined by calculating the sensitivity, specificity, and predictive values. Accuracy of these indices was assessed by using 95% confidence intervals.

## RESULTS

The biodata of the 196 study subjects is displayed in table 1. The mean age of the severe malaria group was  $4.2 \pm 2.8$  years, it differed significantly from the mean age of the control group;  $6.3 \pm 3.7$  years ( $P < .001$ ). Boys made up 51.53% (101) of the study subjects, while girls accounted for 95 subjects (48.47%). There were more girls (54) than boys (44) in the control group.

Table 1; General characteristics of study subjects.

|                  | Severe malaria<br>group n= 98(%) | Controls<br>n= 98(%) | t-value | <i>P</i> value |
|------------------|----------------------------------|----------------------|---------|----------------|
| Mean age (years) | 4.2±2.8                          | 6.3±3.7              | 3.82    | <.001          |
| Male/female      | 57(58.20)/41(41.80)              | 44(44.90)/54(55.10)  | 1.60    | .11            |

99 Table 2 shows the mean red cell indices of the severe malaria group and that of the controls. The  
 100 mean haematocrit, haemoglobin concentration, mean corpuscular haemoglobin and mean  
 101 corpuscular haemoglobin concentration of the severe malaria group differed significantly from  
 102 that of the control group ( $P < .001$  respectively). However, the mean MCV of the severe malaria  
 103 group (79.9fL) was not significantly different from that of the control group (78.6fL), ( $P = .39$ ).  
 104 The sensitivities, specificities, predictive values of all the tests are also displayed in table 2. It  
 105 shows that HB and HCT had good sensitivities and specificities, while MCV had a sensitivity of  
 106 50.00% and a specificity of 45.16%.

107 Table 2; Red blood cell indices of study population.

| Mean red cell<br>indices               | Severe<br>malaria group | Control<br>group | t-<br>value | <i>P</i><br>value | Sensitivity<br>(%) | Specificity(%) | Positive<br>predictive<br>value(%) | Negative<br>predictive<br>value(%) |
|----------------------------------------|-------------------------|------------------|-------------|-------------------|--------------------|----------------|------------------------------------|------------------------------------|
| Haematocrit<br>(%)                     | 21.60±8.60              | 35.30±4.40       | 13.20       | <.001             | 79.59              | 80.65          | 86.67                              | 71.43                              |
| Haemoglobin<br>concentration<br>(g/dl) | 7.20±2.80               | 10.80±1.60       | 9.90        | <.001             | 81.63              | 71.15          | 84.21                              | 67.27                              |
| MCV(fl)                                | 79.90±10.50             | 78.60±7.80       | -.90        | .39               | 50.00              | 45.16          | 59.04                              | 36.36                              |
| MCH(pg)                                | 26.10±3.50              | 23.80±3.10       | -4.20       | <.001             | .00                | 100.00         | .00                                | 38.75                              |
| MCHC(g/dl)                             | 32.80±2.50              | 30.60±1.80       | -6.50       | <.001             | .00                | 100.00         | .00                                | 38.75                              |

108

MCV=Mean corpuscular volume, MCH=Mean corpuscular haemoglobin,MCHC=Mean corpuscular haemoglobin concentration

Table 3 compares the gender of the severe malaria group with their mean red cell indices and shows no statistically significant association between the variables. The boys with severe malaria had a mean HCT of 22.3% while the girls had a mean value of 20.6% ( $P=.35$ ). Both genders had a mean MCHC of 32.8g/dL ( $P=.94$ ).

Table 3 Mean red cell indices by gender of subjects with severe malaria

| Mean red cell indices | Male n=57  | Female n=41 | <i>P</i> value |
|-----------------------|------------|-------------|----------------|
| HB(g/dl)              | 7.70±2.60  | 7.00±3.20   | .51            |
| HCT (%)               | 22.30±8.40 | 20.60±8.90  | .35            |
| MCV(fl)               | 79.30±9.50 | 80.80±12.00 | .54            |
| MCH(pg)               | 25.90±3.60 | 26.40±3.50  | .52            |
| MCHC(g/dl)            | 32.80±2.10 | 32.80±3.00  | .94            |

HB=Haemoglobin concentration, HCT= Haematocrit, MCV=Mean corpuscular volume, MCH=Mean corpuscular haemoglobin,MCHC=Mean corpuscular haemoglobin concentration

## DISCUSSION

The present study clearly shows that anaemia is common in severe falciparum malaria and that its presence may indicate a diagnosis of severe malaria (*P* value, high sensitivity, positive predictive and negative predictive values). This finding conforms to the widely held view. [3-4,7-10] The pathogenesis of anaemia in severe malaria is however not totally known; It is thought to stem from an interplay of several processes which include; lysis of both parasitized and unparasitized erythrocytes, mopping up of parasitized erythrocytes by the reticuloendothelial system, dyserythropoiesis, iron shunting for parasite use, bone marrow suppression and red cell sequestration in deep capillaries. [4] The observed link between severe malarial anaemia, parasitic infections, dietary deficiencies of vitamins B<sub>12</sub>, and E, folate and iron as well as drug associated anaemia is also worth noting here.[9]

Our findings do not indicate a utility for MCV, MCH, and MCHC in the diagnosis of severe malaria. Nevertheless, MCH and MCHC are relatively common enough amongst children with severe malaria to indicate that their presence should be regarded as potential warning signs (*P* values and specificities respectively). This is akin to findings from other studies. [4]. It is not known whether there is an association between red cell MCV, MCH, as well as MCHC and pathogenesis of malaria infection.[4] Yet, several studies have suggested that  $\alpha/\alpha$  thalassemia ( microcytic states ) selectively protect against severe malarial anemia, and that iron deficiency anaemia, a hypochromic microcytic state also protects against development of severe malaria in endemic areas.[11,12,13] In addition, cross-sectional studies conducted in Cameroon demonstrated a significant improvement in red cell indices with a marked reduction in the prevalence of microcytic anaemia. This was sequel to a reduction in the prevalence of malaria that ensued from institution of effective control measures.[14] These studies appear to give the

impression that there is a positive relationship between these red cell indices and prevalence of malaria. Nonetheless, the limitations of our study such as a relatively small sample size as well as the inability to match our subjects and controls for age and sex may have confounded our findings. We therefore recommend that larger, more comprehensive studies be conducted to ascertain the exact association between these red cell indices and malaria in Nigeria. Findings in the present study are in agreement with those of other studies that there is no gender preference in the effect of severe malaria on haematological parameters. [4,9]

## CONCLUSION

We have demonstrated the effects of severe malaria on some haematological indices as seen in Azare, North Eastern Nigeria. The most impacted been HB and HCT. We therefore propose that in the presence of supporting clinical evidence, the existence of anaemia in a child in Azare may be a valuable supporting criterion for the diagnosis of severe malaria. This may positively impact prognosis as institution of standard management would be effected promptly.

## ETHICAL APPROVAL

Approval was obtained from the research ethics committee of the Federal Medical Centre Azare, Nigeria before the commencement of the study.

## COMPETING INTEREST

The authors declare that there were no competing interests involved in this study.

## REFERENCES

1. World Health Organization. World malaria report. 2016: WHO Geneva; 2017(WHO/HTM/GMP/2017.4). Available: <http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/>(Accessed 16 October 2017).
2. World Health Organization. Roll Back Malaria United States Agency for International Development. New perspective: malaria diagnosis: report of a joint WHO/USAID informal consultation. 1999: WHO Geneva; 2000(WHO/CDS/RBM/2000.14). Available: <http://www.who.int/iris/handle/10665/66321> (Accessed 16 January 2018).
3. Maina RN, Walsh D, Gaddy C, Hongo G, Waitumbi J, Otieno L, et al. Impact of *Plasmodium falciparum* infection on haematological parameters in children living in Western Kenya. *Malaria Journal* 2010; (suppl 3): S4.
4. Kotepui M, Phunphuech B, Phiwklam N, Chupeerach C, Duangmano S. Effect of malarial infection on haematological parameters in a population near Thailand-Myanmar border. *Malaria Journal* 2014; 13: 218.
5. Hill AV, Allsopp CE, Kwiatkowski D, Anstey NM, Twumasi P, Rowe PA, et al. Common West African HLA antigens are associated with protection from severe malaria. *Nature* 1991; 352: 595-600.
6. World Health Organization. Guidelines for the treatment of malaria. 3rd Ed. WHO Geneva; 2015. Available: [http://apps.who.int/iris/bitstream/10665/162441/1/9789241549127\\_eng.pdf?ua=1&ua=1](http://apps.who.int/iris/bitstream/10665/162441/1/9789241549127_eng.pdf?ua=1&ua=1)(Accessed 17 October 2017).

7. George IO, Ewelike-Ezeani CS. Haematological changes in children with malaria infection in Nigeria. *J Med Med Sci* 2011; 2 (4); 768-71.
8. Imoru M, Shehu UA, Ihesiulor UG, Kwaru AH. Haematological changes in malaria-infected children in North-West Nigeria. *Turk J Med Sci* 2013; 43; 838-42.
9. Olutola A, Mokuolu O. Severe malaria anaemia in children, Anemia, Silverberg D (Ed), 2012. ISBN: 978-953-51-0138-3. Available from: <http://www.intechopen.com/books/anemia/severe-malaria-anaemia-inchildren>. (Accessed 4 April 2015).
10. Lathia TB, Joshi R. Can hematological parameters discriminate malaria from nonmalarious acute febrile illness in the tropics? *Indian J Med Sci* 2004; 58(6); 239-44.
11. May J, Evans JA, Timmann C, Ehmen C, Busch W, Thye T, et al. Hemoglobin variants and disease manifestations in severe falciparum malaria. *JAMA* 2007; 297: 2220-6.
12. Gwamaka M, Kurtis JD, Sorensen B, Holte S, Morrison R, Mutabingwa TK, et al. Iron deficiency protects against severe plasmodium falciparum malaria and death in young children. *Clin Infect Dis* 2012; 54(8): 1137-44.
13. Prentice AM, Ghattas H, Doherty C, Cox SE. Iron metabolism and malaria. *Food Nutr Bull* 2007; 28(4 suppl): S524-39.
14. Sumbele IUN, Ning TR, Bopda OSM, Nkuo-Akenji T. Variation in malariometric and red cell indices in children in the mount Cameroon area following enhanced malaria control measures: evidence from a repeated cross-sectional study. *Malaria Journal* 2014; 13: 334.