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Brief Original Article

Haematological parameters, haemozoin-containing leukocytes in Sudanese children with severe *Plasmodium falciparum* malaria

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Abstract

Introduction: Haemozoin –containing leucocytes (HCL) can be used to predict severe malaria.

Methodology: A case –control study was conducted in Singa, Sudan, to investigate the haematological values and HCL in children with severe *Plasmodium falciparum* malaria. The cases were children with severe *P. falciparum* malaria (67). The two groups of controls were patients with uncomplicated *P. falciparum* malaria (63) and healthy children (50).

Results: The mean (±SD) age was 5.5 (±3.8) years. In comparison with children with uncomplicated *P. falciparum* malaria, children with severe *P. falciparum* malaria had significantly lower haemoglobin and platelet counts, and significantly higher lymphocyte counts, red cell distribution width (RDW), and platelet distribution width (PDW). The rate of haemozoin –containing monocytes (percentage of children positive for this parameter in each group) was 91.0%, 84.6% and 50.0%, $P<0.001$ in children with severe *P. falciparum*, uncomplicated *P. falciparum* malaria and negative controls, respectively. Receiver Operating Characteristic (ROC) curves for blood parameters and HCL were plotted and the areas under the curve (AUC) were calculated for the prediction of severe *P. falciparum* malaria infection. The ROC curve analysis, showed a fair predictability of malaria for haemoglobin (AUC = 0.74, sensitivity = 76.0% and specificity = 60.3%, cut-off = 9.7g/dl), lymphocytes (AUC = 0.71, sensitivity = 71.3% and specificity = 62.2%, cut-off = $1.95\times10^3/mm^3$), PDW (AUC = 0.69, sensitivity = 80.1% and specificity = 66.3%, cut-off = 15.34 %) and haemozoin in the monocytes (AUC = 0.68, sensitivity = 68.2% and specificity = 65.2%, cut-off =5.5 %).

Conclusion: RDW, PDW and HCL could be used to predict severe malaria in this setting.

Key words: haematological profile; haemozoin; PDW; MPV; *Plasmodium falciparum; Sudan*.

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Introduction

In spite of the preventive measures, malaria remains a major public health problem in the tropics where malaria is responsible for 781,000 deaths per year, the majority of which are in Sub -Saharan Africa [1]. Malaria is known to cause several haematological changes such as anaemia and thrombocytopenia [2]. However, in previous years the haematological parameters were assessed manually, a method with inherent difficulty and limitations. The automated method for full blood count is now available in most of the settings and yields new parameters [e.g. red cell distribution width RDW), mean platelet volume (MPV) and platelet distribution width (PDW) that could be of diagnostic and prognostic value in different diseases including malaria [3,4]. Different blood indices have shown varied cut-off values, sensitivity and specificity in the different settings [5,6].

Haemozoin is the end product of the detoxification of haem, and is phagocytised by leucocytes mainly neutrophils and monocytes. Previous studies have shown a significant association between haemozoin –containing leucocytes (HCL) and severe malaria among African children [5–12]. There are no published data on haematological changes and HCL in Sudanese children with severe *P. falciparum* malaria. Malaria is the major health problem in Sudan [13]. The current study was conducted in central Sudan to investigate the

haematological values and HCL in children with severe *P. falciparum* malaria.

Methodology

A case–control study was conducted in the department of Paediatrics at Singa Hospital, Central Sudan, during the rainy and post rainy season (August through December 2015). The cases were patients with symptoms and signs of severe malaria, infected with the *P. falciparum*, confirmed by microscopic examination of Giemsa stained blood smears [14]. The controls were patients presented to the same clinic with uncomplicated *P. falciparum* malaria. A group of healthy children (community-based) were the negative controls. The clinical history data were gathered using a questionnaire, after an informed consent was signed by the parent/guardian.

Thin and thick blood films for malaria were prepared and stained with 10% Giemsa. If the slide was positive, the parasite density was calculated by counting the number of asexual parasites per 200 leukocytes, multiplied by the participant's own leucocyte number /μL as follows

(Number of parasites counted/WBC counted) × WBC count/μl of participant.

The blood films were considered negative if no parasites were detected in x100 (oil-immersion objective) fields of a thick blood film.

The numbers of pigmented cells (neutrophils or monocytes) were counted per 100 neutrophils in thin blood smears or200 mononuclear cells in thick blood films, and normalised to the percentage of neutrophils or monocytes obtained from a differential leukocyte count. The numbers were finally expressed as the number of pigmented cells neutrophils or monocytes /L.

Two ml of blood was taken from each participant in an ethylene diamine tetra acetic acid (EDTA) containing tube and immediately analysed for a complete haemogram using an automated haematology analyser (Sysmex XN-9000; Hyogo, Japan) as previously described and following the manufacturer's instructions [15–17]. The haemogram included haemoglobin level, leucocyte count and platelet indices, namely platelet count, mean platelet volume (MPV), and platelet distribution width (PDW).

A total sample size of 180 participants was calculated using a formula for the difference in the mean of the proposed variables (mainly haemoglobin, red cell distribution width (RDW), leucocytes, platelet counts, PDW and haemozoin –containing leucocytes) that would provide 80% power to detect a 5% difference at $\alpha = 0.05$, and assuming that 10% of the participants would have incomplete data.

Statistical analyses

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). Proportions of the studied groups were expressed in percentages (%) and comparisons between the studied groups were made applying the X^2 test. Continuous data were checked for normality using the Shapiro-Wilk test. The mean and standard deviation (SD), or median and interquartile range were used to describe the studied variables if normally or abnormally distributed, respectively. The T-test and, in case the data were not normally distributed the Mann-Whitney U (were used to evaluate the differences between the group of severe and uncomplicated malaria and between uncomplicated malaria and the negative controls. Diagnostic screening tests were used to determine the diagnostic cut-off values of various parameters (based on test sensitivity and specificity) using receiver operating characteristic (ROC) curve and the area under the curve (AUC). $P < 0.05$ was considered statistically significant.

Results

General characteristics

One hundred and eighty children were enrolled. Out of these children; 67 (37.2%) had severe *P. falciparum* malaria, 63 (35.0%) had uncomplicated *P. falciparum* malaria and 50 (27.8%) were negative controls. The age ranged from 1-16 years and the mean (±SD) was 5.5 (±3.8) years. The mean (±SD) age was 4.7 (±3.6), 5.8 (±4.1) and 5.7 (±3.0) years, ($P = 0.862$), for severe *P. falciparum* malaria, uncomplicated *P. falciparum* malaria and negative controls, respectively. Seventy-three (40.6%) were females. The females were 40.2%, 42.9% and 38.0%, ($P = 0.871$) of the children with severe *P. falciparum* malaria, uncomplicated *P. falciparum* malaria and the negative controls, respectively.

The patients with the severe form of *P. falciparum* malaria had cerebral malaria (10; 14.9%), repeated convulsions (18; 26.8%), severe anaemia (9; 13.4%), hypoglycaemia (1; 1.4%), hypotension (7; 10.4%), jaundice (6; 8.9%), hyperparasitaemia (9; 13.4%) and more than one manifestations (8; 11.9%).

Haematological parameters, HCL in children with P. falciparum malaria and negative controls

While there was no significant difference in the haemoglobin, RBCs, MPV and PDW between *P.*

falciparum -positive and *P. falciparum* -negative children, RDW, leucocytes and HCL were significantly higher in *P. falciparum* -positive children, (Table 1). While there was no significant difference in the rate of haemozoin –containing neutrophils [122/130 children (93.8%) vs. 45/50 (90.0%), $P = 0.354$], the rate of haemozoin –containing monocytes was significantly higher in *P. falciparum* positive children [110/180 (84.6%) vs. 25/50 (50.0%), $P < 0.001$].

ROC curve analysis and calculation of the AUC for haemoglobin, RDW, leucocytes, platelet counts, PDW, MPV and HCL was performed for prediction of malaria infection. The AUCs are shown in Table 2. A fair predictability of malaria for RDW (AUC = 0.73, sensitivity = 85.4% and specificity = 58.6%, cut-off = 14.45%) and HCL, and a good malaria predictability for platelet count was demonstrated (AUC = 0.82, sensitivity = 80.7% and specificity = 75.0%, cut-off = 200.0 $\times 10^3/mm^3$).

Haematological parameters, HCL in children with uncomplicated P. falciparum malaria and negative controls

While there was no significant difference in the haemoglobin, RBCs, RDW, lymphocytes and MPV between children with uncomplicated *P. falciparum* and the negative controls, neutrophils and HCL were significantly higher in children with uncomplicated *P. falciparum* (Table 3). While there was no significant difference in the rates of haemozoin –containing neutrophils [58/63 (92.1%) vs. 45/50 (90.0%), $P = 0.502$], the rates of haemozoin –containing monocytes were significantly higher in children with uncomplicated *P. falciparum* [49/63 (77.8%) vs. 25/50 (50.0%), $P < 0.001$].

Table 1. Comparison of the medians (interquartile ranges) of the blood indices and haemozoin-containing leucocytes, between *P. falciparum* malaria positive and *P. falciparum* malaria negative children.

Variables	Children *P. falciparum* malaria positive (n = 130)	Children *P. falciparum* malaria negative (n = 50)	*P*
Hemoglobin, g/dL	10.1 (8.4−11.7)	10.2 (8.7 −11.1)	0.493
Red blood cells, $\times 10^6/mm^3$	4.23 (3.58−4.63)	4.3 (3.9 −4.6)	0.337
Red cell distribution width,%	15.0 (13.7−16.7)	16.6 (15.6−19.2)	< 0.001
Leucocytes, $\times 10^3/mm^3$	7.4 (5.2−9.5)	9.1 (5.3−12.4)	0.024
Lymphocytes, $\times 10^3/mm^3$	2.5 (1.2−3.6)	3.7 (2.4−5.4)	0.001
Neutrophils, $\times 10^3/mm^3$	4.7 (2.56−6.55)	3.05 (1.9−5.8)	0.064
Platelet count, $\times 10^3/mm^3$	127.0 (74.0−195.0)	306.5 (202.5−461.0)	< 0.001
Platelets distribution width, %	15.6 (15.0−16.0)	15.6 (15.3−15.9)	0.572
Mean platelet volume, fL	8.4 (7.7−9.1)	8.1 (7.4−8.7)	0.065
Haemozoin in the neutrophils,%	8.0 (4.0−13.5)	2.5 (1.0−8.0)	0.001
Haemozoin in the monocytes, %	6.0 (2.0−10.0)	0.5 (0−4.0)	0.001

Table 2. ROC curve analysis and reliability of blood indices in predicting malaria.

Variable	Area under the curve (AUC)	*P*	Sensitivity (%)	Specificity (%)	Cut-off
Red cell distribution width, %*	0.703	< 0.001	85.4	58.6	14.450
Leucocytes, $\times 10^3/mm^3$	0.594	0.059			
Neutrophils, $\times 10^3/mm^3$	0.403	0.050			
Lymphocytes $\times 10^3/mm^3$*	0.697	< 0.001	72.3	70.0	1.250
Platelet count, $\times 10^3/mm^3$*	0.820	< 0.001	80.7	75.0	200.0
Platelets distribution width,%	0.533	0.505			
Mean platelet volume, fL	0.413	0.079			
Haemozoin in the neutrophil, %*	0.702	< 0.001	69.0	70.0	5.5
Haemozoin in the monocytes, %*	0.748	< 0.001	71.0	72.0	3.5

*Were considered for full analysis presentation because of fair and good predictability of the area under the curve.

Table 3. Comparison of the medians (interquartile ranges) of the blood indices between the children with uncomplicated *P. falciparum* malaria and the negative controls.

Variables	Children with uncomplicated *P. falciparum* malaria (n = 63)	Children *P. falciparum* malaria negative (n = 50)	*P*
Hemoglobin, g/dL	11.1 (9.8 −12.6)	10.2 (8.7 −11.1)	0.025
Red blood cells, $\times 10^6/mm^3$	4.2 (3.7 −4.6)	4.3 (3.9 −4.6)	0.181
Red cell distribution width,%	14.2 (13.5−15.7)	16.6 (15.6−19.2)	0.049
Leucocytes , $\times 10^3/mm^3$	7.2 (5.6−9.3)	9.1 (5.3−12.4)	0.042
Lymphocytes, $\times 10^3/mm^3$	1.6 (1.1−2.8)	3.7 (2.4−5.4)	0.618
Neutrophils, $\times 10^3/mm^3$	4.1 (2.2−5.5)	3.05 (1.9−5.8)	0.020
Platelet count, $\times 10^3/mm^3$	139.0 (84.0−227.0)	306.5 (202.5−461.0)	< 0.001
Platelet distribution width, %	15.0 (12.7−15.9)	15.6 (15.3−15.9)	0.013
Mean platelet volume, fL	8.5 (7.9−9.3)	8.1(7.4−8.7)	0.316
Haemozoin in the neutrophil,%	6.0 (6.0−12.0)	2.5 (1.0−8.0)	0.004
Haemozoin in the monocytes, %	4.0 (1.0−10.0)	0.5 (0−4.0)	< 0.001

Table 4. Comparison of the medians (interquartile ranges) of the blood indices between children with severe and uncomplicated *P. falciparum* malaria.

Variables	Children with severe *P. falciparum* malaria (n = 67)	Children with uncomplicated *P. falciparum* malaria (n = 63)	*P*
Hemoglobin, g/dL	8.7(7.8−10.8)	11.1 (9.8 −12.6)	<0.001
Red blood cells, $\times 10^6/mm^3$	4.1(3.4−4.6)	4.2 (3.7 −4.6)	0.188
Red cell distribution width,%	15.5 (14.2−18.6)	14.2 (13.5−15.7)	0.002
Leucocytes , $\times 10^3/mm^3$	7.5(4.8−10.4)	7.2 (5.6−9.3)	0.858
Lymphocytes, $\times 10^3/mm^3$	3.5 (1.95−5.71)	1.6 (1.1−2.8)	<0.001
Neutrophils, $\times 10^3/mm^3$	5.3 (2.9−6.6)	4.1 (2.2−5.5)	0.040
Platelet count , $\times 10^3/mm^3$	113.0 (66.2−171.5)	139.0 (84.0−227.0)	0.043
Platelet distribution width, %	15.7 (15.4−16.2)	15.0 (12.7−15.9)	< 0.001
Mean platelet volume, fL	8.4 (7.7−8.8)	8.5 (7.9−9.3)	0.210
Haemozoin in the neutrophil, %	9.0 (4.0−14.0)	6.0 (6.0−12.0)	0.101
Haemozoin in the monocytes, %	8.0 (4.0−12.0)	4.0 (1.0−10.0)	0.052

Table 5. ROC curve analysis and reliability of blood indices in predicting severe *P. falciparum* malaria.

Variable	Area under the curve (AUC)	*P*	Sensitivity (%)	Specificity (%)	Cut-off
Hemoglobin, g/dL*	0.74	< 0.001	76.0	60.3	9.7
Red cell distribution width, %*	0.65	0.003	71.5	61.2	14.65
Leucocytes, $\times 10^3/mm^3$	0.594	0.059			
Neutrophils, $\times 10^3/mm^3$	0.46	0.076			
Lymphocytes $\times 10^3/mm^3$*	0.71	< 0.001	71.3	62.2	1.95
Platelet count, $\times 10^3/mm^3$	0.57	0.144			
Platelet distribution width,% *	0.69	< 0.001	80.1	66.3	15.34
Haemozoin in the neutrophil, %	0.58	0.104			
Haemozoin in the monocytes, %*	0.68	0.015	68.2	65.2	5.5

*Were considered for full analysis presentation because of fair predictability of the area under the curve.

Haematological parameters, HCL in children with severe and uncomplicated P. falciparum malaria

In comparison with children with uncomplicated *P. falciparum* malaria, children with severe *P. falciparum* malaria had significantly lower haemoglobin and platelets, and significantly higher lymphocytes, RDW and PDW (Table 4).

While there was no significant difference in the rate of haemozoin –containing neutrophils [64/67 (95.5%) vs. 58/63 (92.1%), P = 0.483], the rate of haemozoin – containing monocytes was significantly higher in children with severe *P. falciparum* vs. children with uncomplicated *P. falciparum* malaria [61/67 (91.0%) vs.49/63 (50.0%), *P* = 0.041].

ROC curve analysis with the AUC for blood parameters and HCL was performed for the prediction of severe *P. falciparum* malaria infection. The AUCs are shown in Table 4, in which there is a fair predictability of malaria using haemoglobin (AUC = 0.74, sensitivity = 76.0% and specificity = 60.3%, cut-off = 9.7 g/dL), lymphocyte count (AUC = 0.71, sensitivity = 71.3% and specificity = 62.2%, cut-off = $1.95\times10^3/mm^3$), PDW (AUC = 0.69, sensitivity = 80.1% and specificity = 66.3%, cut-off = 15.34 %) and haemozoin in the monocytes (AUC = 0.68, sensitivity = 68.2% and specificity = 65.2%, cut-off = 5.5%) , Table 5.

Discussion

The current study has shown that children with *P. falciparum* malaria had a high RDW, leucocytes and HCL. Previous studies have reported a significant association between leucocytosis and malaria [14,18]. Leucocytosis, mainly monocytosis has a crucial role in the host defence mechanism against malaria infection. Leucocytosis reflects the production of inflammatory cytokines, which may further contribute to the pathogenesis of the disease [19]. Previous reports have shown a significant association between RDW and malaria infections [4,20]. RDW is one of the haematological indices that indicate inflammatory process in many diseases, including malaria, and is a quantitative measure of the variability in the size of erythrocytes. Increased RDW values may result from the increased cytokine production, inhibiting the maturation of erythrocytes in the bone morrow [21].

In the current study the haemozoin-containing neutrophils and monocytes have shown fair predictive values in differentiating malaria from non-malaria and severe from uncomplicated malaria (haemozoin-containing monocytes mainly). The AUC was 0.702 and 0.748, sensitivity was 69.0% and 71.05%, and specificity was 70.0% and 72.0% for haemozoin-containing neutrophil and monocytes, respectively. Previous studies have reported a significant association between HCL in children with severe malaria in different African settings [5–12]. The results of our study and these studies should be compared cautiously because of the difference in the malaria endemicity/immunity in the different settings. The haemozoin is phagocytised by leucocytes, it interacts with the immune system and results in up-regulation or down-regulation of immune mediators [22–24]. Haemozoin may also stimulate apoptosis of erythroid precursors and results in inhibition of erythropoiesis and malaria –related anaemia [25–27].

In the current study, both lymphocytes and PDW were predictors for severe malaria. These findings are in agreement with the previously reported findings by Chandra et al., that lymphocytes and PDW were predictors for malaria infections [28].

Conclusion

The presence and quantities of HCLs and other haematological parameters (RDW, PDW) could be used to predict severe malaria. Further larger longitudinal studies are needed to assess the association of HCLs and haematological parameters with severe malaria, including severe malaria due to *P. vivax.*

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Ethics approval

The study was approved by the Medical College, University of Khartoum Institutional Review Board.

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