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Research article

Evaluation of red blood cell count as an ancillary index to hemoglobin level in defining the severe falciparum malarial anemia among Ghanaian children in low-resource communities

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ABSTRACT

Our study evaluated red blood cell count as supporting hematological index to hematocrit level in predicting severe malarial anemia instead of the hemoglobin levels among malaria-infected children in Ghana. This case-control study was conducted at the Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana. The study recruited 139 children, of which 45 were Controls (C), 43 with severe malaria (SM), and 51 with mild malaria (MM). Validated questionnaires were administered to obtain the socio-demographic data from each respondent. Venous blood was obtained for parasitemia count and to determine the hematological profile of each participant. With point of observation, data analysis was done. The mean age of the children was 4.22 ± 2.65 years. Median levels of hemoglobin (Hb) decreased in the order; C > MM > SM ($P < 0.0001$). There was a reduction in median levels of hematocrit (HCT) ($P < 0.0001$), RBC (red blood cells count) (<0.0001), from the MM to the SM. Among patients with severe malaria, there were a positive significant spearmen's co-efficient correlations between median levels of RBC ($r = 0.652$, $P = 0.005$) and HB ($r = 0.640$, $P = 0.006$) individually against HCT. However among the mild malaria patients only RBC ($r = 0.884$, $P < 0.001$) was positively correlated against HCT. At a cut-off of $<4.0\times10^6$/uL for RBC and <8.8 g/dL for Hb, RBC (90.4%) recorded a slightly high accuracy in predicting severe falciparum malarial anemia than Hb (86.9%) among the cases. Red blood cell count may be a promising indicator to support hematocrit (<15%) in defining severe malarial anemia than hemoglobin level (<5 g/dL) among malaria-infected children from endemic areas in Ghana.

1. Introduction

Malaria is one of the most common infectious diseases in the world and one of the greatest global public health problems [1], mostly among children under 5 years [2]. About 90% of the world's severe and fatal malaria is projected to affect young children in sub-Saharan Africa [3], as major complications of severe falciparum malaria (SFM) develop rapidly and progress to death within hours or days [4]. Exposure of the signs of severe malaria and acting accordingly play significant roles in abating the increasing mortality rate. However, because these signs lack specificity, as there is no "gold standard'' methodology to identify individuals with "true'' severe malaria other than mostly severe anemia with higher microscopic parasitemia observed among patients [5]. The WHO classifies SFM as *P. falciparum* asexual parasitemia and no other confirmed cause for their symptoms or signs and with the presence of one or more of the clinical features (Cerebral malaria, pulmonary edema, acute renal failure, severe anemia, metabolic acidosis, multiple convulsions, prostration, jaundice, significant bleeding, hypoglycemia, and shock) [4].

Studies have shown that hematological changes such as low red blood cell count, hemoglobin (Hb) level, and hematocrit are amongst the most common complications of malaria and are involved in the pathology of the disease [6,7]. From the WHO (2014) report, anemia associated with SFM among children under 12 years is defined as Hb < 5 g/dl or hematocrit <15%. Confirmation of severe malarial anemia using these parameters is not substantial as the estimation of Hb levels, is accompanied by numerous setbacks including variables due to differing malaria endemicity, background hemoglobinopathy, nutritional status, demographic factors, and malaria immunity especially in the tropics [8,9].

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This is likely to be the scenario with changing epidemiology and misdiagnosis of severe falciparum malarial anemia in Africa as there are many other causes of low Hb levels such as with bacteremia, HIV-1, and hookworm infections [10,11] as well as chronic transmission of malaria in holoendemic regions.

Although parasite-driven hemolysis contributes to a reduction in Hb levels in childhood malaria, one of the primary mechanisms responsible for low Hb levels in children with severe malaria anemia is impaired or ineffective erythropoiesis [12]. It is important to note that some or all of these factors can culminate in chronically low Hb levels observed in infants and young children residing in holoendemic regions. As such, the degree of Hb is typically a poor indicator of severe malarial anemia in these settings [12], warranting the exploration of additional markers to enhance prompt detection and accurate classification.

High levels of parasitemia, particularly in non-immune individuals, can result in massive lysis and clearance of RBCs, resulting in profound anemia [13]. Acute malaria can cause severe anemia due to hemolysis of infected and uninfected RBCs, and chronic or subclinical malaria can induce anemia due to inflammation. Based on this premise, we postulate that red blood cell count could serve as a supportive indicator in predicting severe falciparum malarial anemia (<15% hematocrit) among children.

2. Materials and methods

2.1. Study design/study site

This hospital-based case-control study was carried out at the Komfo Anokye Teaching hospital (KATH), Kumasi, Ghana. Ghana is geographically placed near the equator and lies between Latitude: 7° 57′ 9.97″ N and Longitude: -1° 01′ 50.56″ W, with more than 30 million people accounting for about 2.5% of African's population (Ghana Statistical Service, 2010 Population and Housing Census). Kumasi is the regional capital of the Ashanti region with an estimated population of 4,780,380 (Ghana Statistical Service, 2010). KATH is a major Teaching Hospital and it is positioned in the middle belt of Ghana. It has over 1000 bed capacity. The relatively good road network and the cosmopolitan nature of Kumasi make the hospital accessible to all other areas. Kumasi is a malaria-endemic zone in Ghana.

2.2. Study population and sample size calculation

All children below the age of twelve years with symptoms of malaria (malaria suspects) who reported to the child health departments of KATH were screened for malaria. Children with or co-morbid with Human Immunodeficiency Virus (HIV), enteric fever, sickle cell disease and hepatitis B and C infections, and pyrexia of unknown origin were excluded from the study. Children who visited the hospital and tested negative for falciparum malaria were recruited as "Controls" using the microscopic test as diagnosis [14].

The World Health Organization (2014) defines severe falciparum malaria in children as the presence of *P. falciparum* but no other confirmed cause for signs and symptoms and vital organ dysfunction with clinical features such as impaired consciousness, prostration, multiple convulsions, acidotic breathing, acute pulmonary edema, and acute respiratory distress, shock, acute kidney injury, and or clinical jaundice plus one or more of laboratory findings such as hypoglycemia (less than 2.2 mmol/L), metabolic acidosis (plasma bicarbonate less than 5 g/dl), severe normocytic anemia (less than 5 g/dl and packed cell volume less than 15%), hemoglobinuria, hyperlactatemia (greater than 5 mmol/L), pulmonary edema (radiological) and renal impairment (serum creatinine greater than 265 μmol/L). Modifications were made where necessary to suit this study.

In this study, severe falciparum malaria was defined as the presence of *P. falciparum* asexual parasitemia with severe grade (>100,000/uL) [15] in the presence of an identified cause; a hematocrit of less than 15% in children below 12 years of age regardless of the hemoglobin levels and present with one or more symptoms of malaria [16]. Patients who present with and a positive parasitological test (microscopy) and mild grade of parasitemia (1–99999/uL) [15] but had no features of severe malaria was defined as having mild malaria.

Following the method of Charan and Biswas (2013) [17], a sample size of 140 malaria patients was proposed for the study. This research targeted 5%–10% of the population in the study area. After computing, the sample size was 140 children for 5% and 280 children for 10% of the population. At the end of the study 139 study participants were recruited in a 1:2 control-case ratio. Out of the total participants, 45 controls and 94 were cases comprising 43 and 51 been described as patients with severe malaria and mild malaria respectively.

2.3. Ethical considerations

Ethical clearance for the study was obtained from the Committee on Human Research, Publication, and Ethics (CHRPE) at the KNUST School of Medical Sciences/Komfo Anokye Teaching Hospital in Kumasi-Ghana (Reference Number: CHRPE/AP/078/17). Permission was also given by the various Medical Directors/Superintendents or Heads of Departments of the child health departments. Written informed consent was also obtained from healthcare personnel and guardians of study participants were adequately informed of the procedures, nature, risk, and the purpose of the study. Parents of qualified patients were invited to participate in the study and requested to fill or thumbprint a consent form with the help of the research team before their wards were recruited for the study according to the Helsinki declaration [18].

2.4. Questionnaire

A validated questionnaire was used to obtain socio-demographic data of the study participants from their guardians. Other clinically relevant information was obtained from the hospital archives.

2.5. Sample analysis

Three milliliters of the venous blood sample was collected from each participant and dispensed into K_2 EDTA tubes for hematological analysis and the assessment of malaria parasite density. Standard thick and thin films were prepared on clean grease-free slides. Thin films fixed with absolute methanol. Both thick and thin smears were stained, 10% Giemsa and examination for malaria parasite by light microscopy. Malaria parasite was identified by observation of the smears and the morphological appearance of the parasite in the infected red blood cells (RBCs) using a ×100 microscope objective.

Full blood count was determined using an automated hematological analyzer (Sysmex Automated Hematology Analyzer, Kobe, Japan, XP-300). From the World Health Organization (WHO, 2005) children are considered to be anemic at Hb level less than 11 g/dL [16].

Parasite load was calculated after counting asexual parasites per 200 white blood cells (WBC), assuming mean WBC count of 8000 count/μL using the following formula:

$$\text{Parasite load}/\mu\text{L} = \frac{\text{Number of observed asexual parasites} \times 8000\,\text{WBC count}/\mu\text{L}}{200\,\text{WBCs}}$$

The degree of parasitemia was graded as mild and severe when a count (x) is from 1 to 99,999/μL and >100,000/μL, parasites respectively [15].

2.6. Data analysis

Data were entered into Microsoft Excel 2013 and cleaned before being subjected to analysis. Statistical analyses were performed using GraphPad Prism 6, R statistical software, and IBM- SPSS version 20.0.

Table 1. Socio-demographic characteristics of study participants.

Variables	Control (n = 45)	Mild malaria (n = 51)	Severe malaria (n = 43)	P-value
Age (years) (mean ± SD)	3.80 ± 1.66	4.32 ± 2.81	4.27 ± 2.96	0.309
The age range of the wards (years)				
0–5	30 (32.3%)	35 (37.6%)	28 (30.1%)	0.9363
6–10	15 (34.1%)	14 (%31.8%)	15 (34.1%)	0.7109
11–12	-	2 (100.0%)	-	NA
Gender				
Male	24 (36.4%)	24 (36.4%)	18 (27.2%)	0.5581
Female	21 (28.8%)	27 (37.0%)	25 (34.2%)	0.5581
Medication				
Alaxin	-	13 (100.0%)	-	NA
Artesunate	-	3 (60.0%)	2 (40.0%)	0.7910
Artemether	-	5 (41.7%)	7 (58.3%)	0.3487
Artesunate Amodiaquine	-	24 (63.2%)	14 (36.8%)	0.1535
Lonart	-	2 (100.0%)	-	NA

P-value<0.05 = statistically significant, SD = Standard Deviation, NA = Not Applicable.

Medians were calculated for continuous variables such as the hematological parameters and Kruskal-Wallis used to compare more than two median levels of variables of continuous variables and was presented in the violin plot (R statistical software). While percentages were calculated for categorical variables data and presentation of socio-demographics and prevalence of anemia were done by using tables and bar graphs respectively. Unpaired t-test was used to compare two means of continuous variables such as the age of participants. Receiver operating characteristics (ROC) curve analyses were used for depicting the diagnostic performance of red blood cell count and hemoglobin for hematocrit as an indicator of severe malarial anemia. A p-value < 0.05 was considered statistically significant.

3. Results

The mean age (±SD) of all the children was 4.22 (±2.65) years. The mean age of those with mild and severe malaria was 4.32 (±2.81)years and 4.27 (±2.96)years respectively. A higher proportion of the study participants (67.5%) were within the age of 0–5 years. Alaxin and Lonart were the widely used anti-malarial drugs among non-severe patients, while Artemether was the predominant medication among severe malaria patients (58.3%) (Table 1).

Median levels of HCT decreased significantly in the order; control > mild malaria > severe malaria. There were statistically significant differences in the Median levels of Red blood cell (RBC), Hemoglobin (Hb),

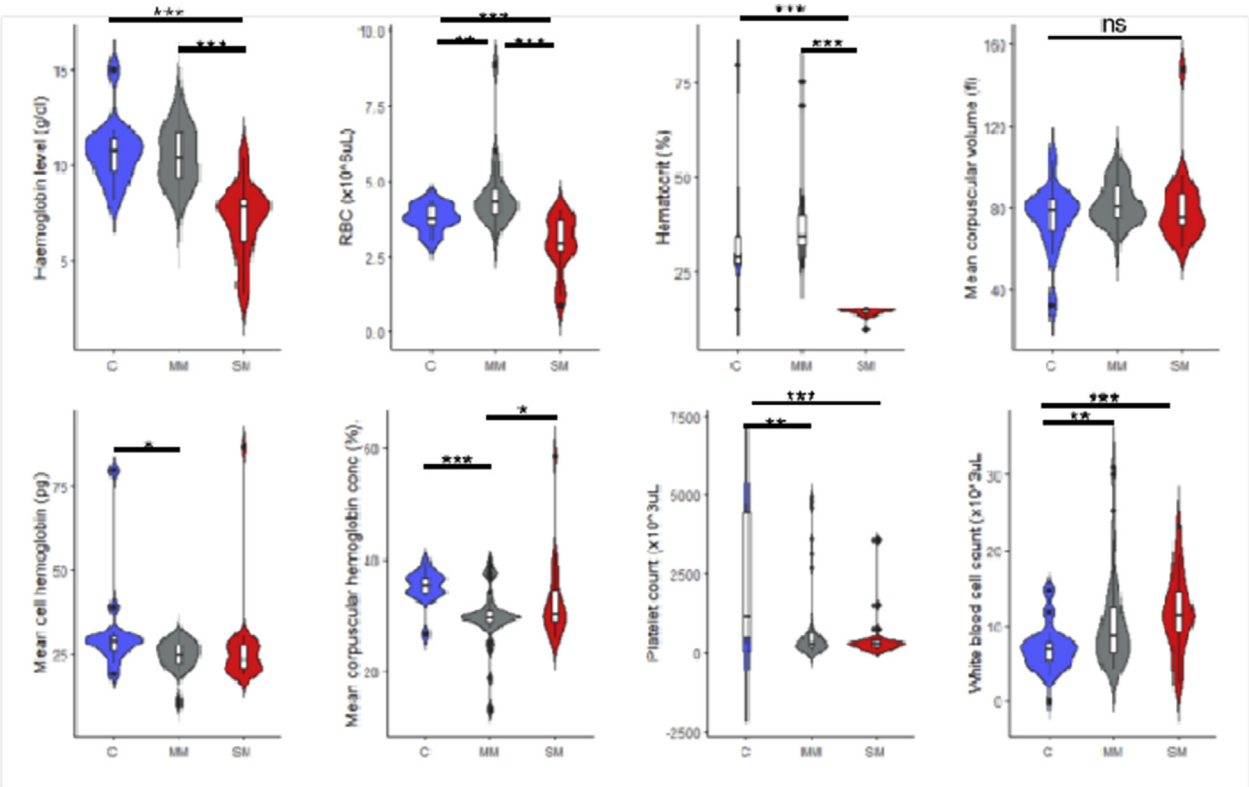

Figure 1. Comparison of hematological parameters between controls, mild and severe malaria. C=Controls, MM = Mild Malaria cases, SM = Severe Malaria cases, Significant differences in median levels; * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

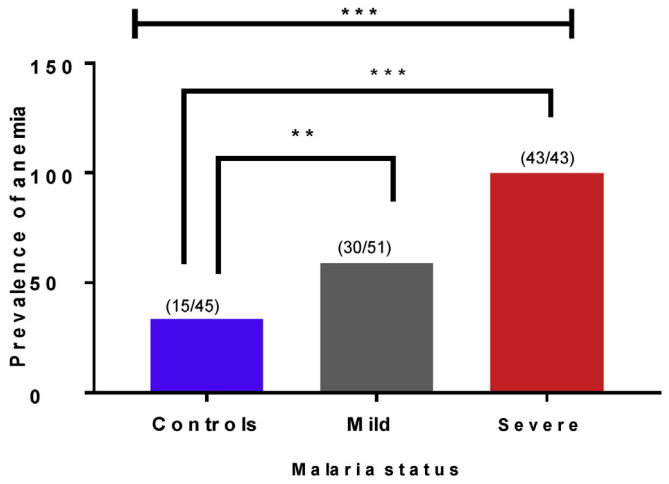

Figure 2. Prevalence of anemia among the study population. Significant differences in proportions; ** = P < 0.01, *** = P < 0.001.

Mean corpuscular hemoglobin concentration (MCHC), and Platelet count (PLT) across the various study groups. Median levels of Mean corpuscular hemoglobin (MCH) was higher among the controls than the mild and severe malaria cases. Also, median White blood cell count (WBC) increased significantly in the order; control < mild malaria < severe malaria. There was no significant difference for Mean corpuscular volume (MCV) (Figure 1).

For patients with mild malaria, 30 out of 51 were anemic, while all the patients with severe malaria were anemic. There were significant differences in the prevalence of anemia between the controls and cases (Figure 2).

Among patients with severe malaria, there were a positive significant spearmen's co-efficient correlations between median levels of RBC (r = 0.652, P = 0.005) and HB (r = 0.640, P = 0.006) individually against HCT. However among the mild malaria patients only RBC (r = 0.884, P < 0.001) was positively correlated against HCT. There was a positive correlation between age and MCV, MCH and MCHC among the severe malaria cases while age was positively correlated with MCH only in the mild malaria cases (Table 2).

Table 3. Diagnostic yield of the combined performance of hemoglobin level and parasite count, and the combined performance of red blood cell count and parasite count as possible indicators to differentiate mild and severe malaria cases.

	Hb (g/dL)	RBC($\times 10^6$/uL)	Hb*(g/dL)
Cut-off point	8.8	4	5
Sensitivity %	90.7	100	13.95
(95%CI)	(77.69–96.77)	(89.97–100.0)	(6.28–27.77)
Specificity %	90.24	83.17	100.0
(95%CI)	(76.73–96.60)	(67.87–94.35)	(89.73–100.0)
PPV%	90.70	79.63	100.0
NPV%	90.24	100.0	52.55
LR+	9.3	3.7	-
LR-	0.1	0	0.9
TP	39	43	6
TN	34	30	41
FP	4	11	0
FN	4	0	37
Accuracy %	86.9	90.4	55.95
AUC %	92.75**	93.42**	-
Kappa value	0.701**	0.766**	0.101

** = P-value<0.01 = statistically significant, PPV = Positive Predictive Value, NPV = Negative Predictive Value, CI-Confidence Interval, LR = Likelihood Ratio, TP = True Positives, TN- True Negatives, FP = False Positives. FN = False Negatives.

Using a cut-off of <4.0×10^6/uL, RBC had a sensitivity of 100.0% and specificity of 83.17%, with negative predictive value (NPV) of 100.0% and positive predictive value (PPV) 79.63%. A Hb cut-off of <8.8 g/dL, however, yielded a sensitivity, specificity, PPV and NPV of 90.70%, 90.24%, 90.70%, and 90.24% respectively differentiate mild and severe malaria with a marginally lower agreement (kappa) compared to the RBC cut-off of 4.0×10^6/uL. Conversely, using the receiver operating characteristics (ROC) curve analyses, the area under the curve (AUC) for RBC (93.42%) was higher than that for Hb (92.75%) (Table 3 and Figure 3).

Table 2. Correlation between age, hematological parameters in mild and severe malaria cases.

Parameters		Age	Hb	RBC	HCT	MCV	MCH	MCHC	PLT
Age	**r**		*0.134*	*-0.290*	*0.015*	*0.527*	*0.524*	*0.450*	*0.282*
	p-value		*0.392*	*0.059*	*0.923*	***<0.001***	***<0.001***	***0.002***	*0.106*
Hb	**r**	0.012		*0.628*	*0.640*	*-0.067*	*0.134*	*0.360*	*0.573*
	p-value	0.932		***0.007***	***0.006***	*0.797*	*0.609*	*0.156*	***0.041***
RBC	**r**	-0.228	0.107		*0.652*	*-0.717*	*-0.607*	*-0.403*	*0.194*
	p-value	0.152	0.966		***0.005***	***0.001***	***0.010***	*0.109*	*0.525*
HCT	**r**	-0.158	0.222	0.884		*-0.191*	*-0.194*	*-0.165*	*0.014*
	p-value	0.325	0.163	**<0.001**		*0.463*	*0.457*	*0.528*	*0.964*
MCV	**r**	0.172	0.404	-0.203	0.266		*0.909*	*0.639*	*-0.140*
	p-value	0.281	**0.009**	0.203	0.092		***<0.001***	***0.006***	*0.649*
MCH	**r**	0.318	0.581	-0.764	-0.493	0.525		*0.890*	*0.115*
	p-value	**0.043**	**<0.001**	**<0.001**	**<0.001**	**<0.001**		***<0.001***	*0.709*
MCHC	**r**	0.238	0.413	0.731	-0.737	-0.088	0.788		*0.436*
	p-value	0.134	**0.007**	**<0.001**	**<0.001**	0.586	**<0.001**		*0.136*
PLT	**r**	0.077	0.217	-0.043	-0.307	-0.351	0.303	0.775	
	p-value	0.651	0.197	0.807	0.077	**0.042**	0.081	**<0.001**	

P-value<0.05 = statistically significant, r = Pearson co-efficient, Italic = Severe cases, Non-italic = Mild cases, Hb = Hemoglobin, RBC = Red blood cell count, HCT = Hematocrit, MCV = Mean corpuscular volume, MCH = Mean cell hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, PLT = Platelet count, WBC = White blood cell count.

i) The italic style defines the medians of the severe cases variables. ii) The bold style of P-values of some variables depicts the rective medians been statistically significant (ie. when the p-value is less tham 0.05).

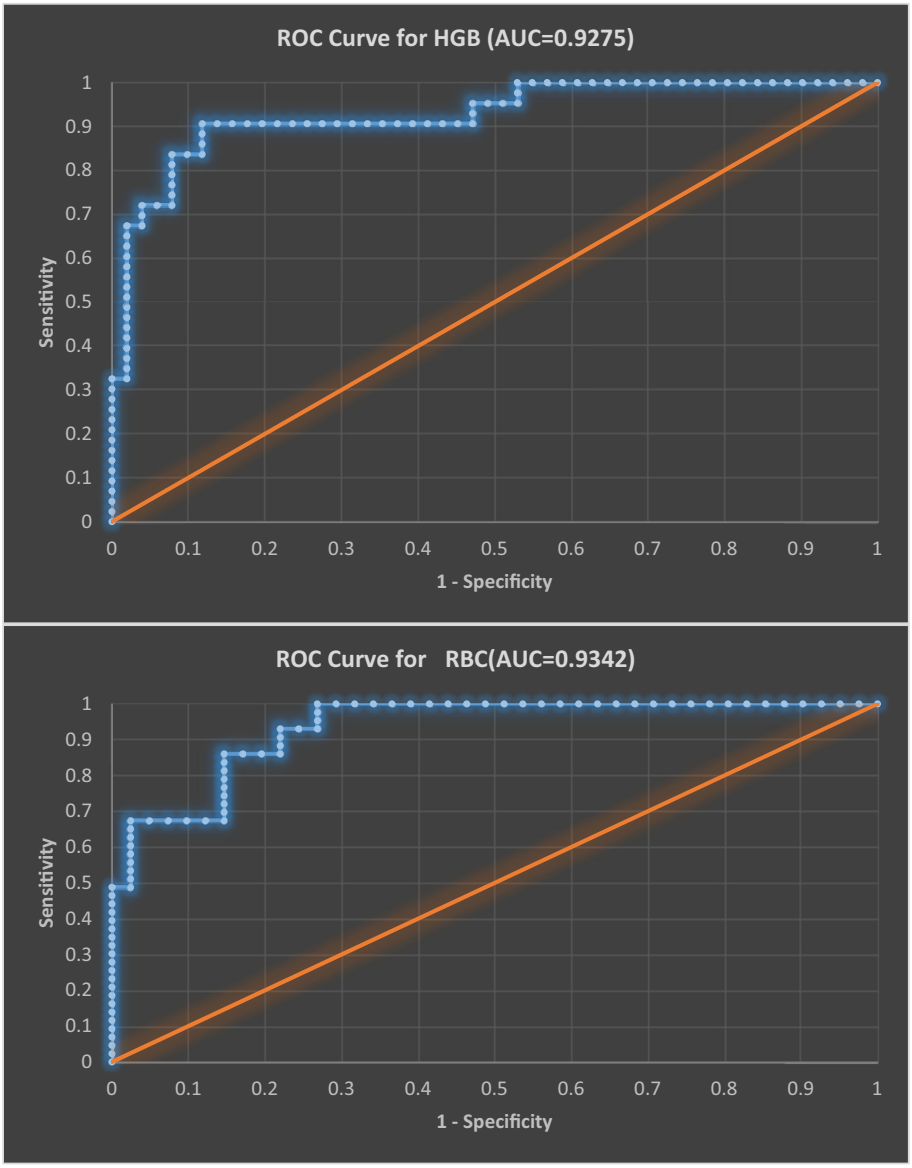

Figure 3. Receiver operating characteristics (ROC) curve analyses for depicting the overall combined diagnostic performance of hemoglobin level and parasite count, and red blood cell and parasite count in differentiating mild and severe malaria cases.

4. Discussion

This present study demonstrates a degree of variation in hematological parameters in response to malaria infection. There was a marked decrease in Hb, RBC, HCT, and PLT measurements among patients presenting with severe malaria than mild malaria [19]. This may be explained by the prompt pathophysiology of malaria following the erythrocytic cycle highlights the diagnostic value of hematological alterations in malaria-endemic areas [20]. The characteristic pattern of percentage parasitized RBC (hyper parasitemia), with consequent anemia and other hematological variations were observed in this study. A study conducted in Kenya by Maina *et al.* revealed similar variations in the hematological measures and is therefore in harmony with the findings in the current study [6].

In this study, the majority of the study participants were within 0–5 years of age (Table 1). This observation agrees with several reports that malaria usually affects children below 5 years, especially in areas of intense transmission like Kumasi where the study was conducted [21]. Underdeveloped immunity has been the major explanation for this experience [6].

This study also shows that anemia is associated with the severity of malaria infection. Median levels of hematocrit (HCT) decreased significantly in the order; control > mild malaria > severe malaria. There was a significant reduction in median levels of Hb RBC, MCHC, and PLT in the order: mild malaria > severe malaria (Figure 1). A similar trend of these results was also found in a study by Maina *et al.* in western Kenya. They concluded that infection with P. *falciparum* results in significant changes in hematological parameters of children living in malaria-endemic regions, and the most commonly affected parameters are hematocrit, hemoglobin, absolute monocyte counts and RBCs [6]. Hematocrit levels decrease as RBC, Hb, and PLT decrease [22], and the overall effects were observed in this study. Also consistent with our study findings is a review by Perkins *et al.* [23].

Although reduced RBC and PLT have been associated with malaria prevalence has been established [8], it has not been acknowledged in most health care settings as part of the significant diagnostic markers to determine the extent and severity of the condition, partly they are not routinely obtained at malaria clinics [6].

Among patients with severe malaria, there were positive significant spearmen's co-efficient correlations between RBC ($r = 0.652$, $P = 0.005$) and HB ($r = 0.640$, $P = 0.006$) individually against HCT in this study.

However among the mild malaria patients only RBC ($r = 0.884$, $P < 0.001$) was positively correlated against HCT (Table 2). This finding was dissonance with a study by Lee *et al.* [21], where there was a stronger relationship between Hb and HCT in Plasmodium falciparum malaria [24]. Other reports, however, indicate that, at higher levels of parasitemia, excessive hemolysis of parasitized RBCs may lead to anemia, thus having a direct effect on the HCT [25]. This may be the reason for the higher predictive power for RBC than Hb in this present study. In this study. Nevertheless, the density of Plasmodium parasitemia identified in this study did not influence Hb levels and this is in parallel to study Ehrhardt *et al.* on a topic "Malaria, Anemia, and Malnutrition in African Children" (2006) [26].

The WHO recommends the use of Hb (<5.0 g/dL) in the classification of mild and severe malaria cases in children under 12 years [16]. Nonetheless, a Hb cut-off of 5 g/dl presented with poor predictive power against HCT (<15%) with a severe grade of parasitemia in defining severe falciparum malarial anemia in this study. This finding buttresses the significant influence of demography and its accompanying factors on various disease profiles of severe malaria as just hemoglobin level may be less sufficient to indicate severe malarial anemia [27]. Interestingly, however, at cut-offs of $<4.0\times10^6$/uL and <8.8 g/dL for RBC and Hb with respectively, RBC and Hb* shown a high diagnostic yield accuracy differentiating mild and severe malaria. Moreover, Hb displaced a lesser measure of agreement (kappa) than RBC in predicting the severe malarial anemia (hematocrit<15%). Using the receiver operating characteristics (ROC) curve analyses, RBC recorded a slightly higher area under curve (AUC) value than Hb. Thus, RBC was able to predict true severe malarial anemia cases than Hb. This current study is in harmony with the findings of Carneiro *et al.* [24] who found inconsistency in using hemoglobin (5 g/dL) as the direct threefold reduced conversion of hematocrit in assessing anemia in malaria-endemic settings. They found out there was inconsistency in using hemoglobin (<5g/dL) as a direct three-fold reduced-conversion of hematocrit (<15%) in defining severe malarial anemia in Africa. Collectively, our study findings suggest that the use of <5 g/dL hemoglobin as an indicator for anemia with asexual falciparum parasitemia to differentiate mild and severe malaria among Ghanaian children on antimalarial regimens such as Artesunate Amodiaquine as may not be appropriate, however, RBC count of $<4.0\times10^6$/uL may be more reliable.

Nonetheless, it is worthy of note that this study is limited by the relatively small sample size. Thus, a larger sample size should be considered in further studies. Also, multi-hospital study sites should be adopted in further studies, and assessment of dietary intakes such as iron-containing foods and products should be taken into consideration to provide some initial insights.

5. Conclusion

Red blood cell count in combination with high parasitemia and HCT in children from malaria-endemic areas may be useful as a generic supportive diagnostic criterion for the classification of severe and mild malaria in children on antimalarial regimens from malaria-endemic areas. New strategic interventions may be adopted in diagnosing children with severe malarial anemia in developing countries such as Ghana.

Declarations

Author contribution statement

K. Nsiah: Conceived and designed the experiments; Wrote the paper.
B. Bahaah: Performed the experiments; Wrote the paper.
E. Acheampong: Analyzed and interpreted the data.
B. Afranie: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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