Original Article

Assessing Agreement of Hemoglobin and Three- Fold Conversion of Hematocrit as Methods for Detecting Anemia in Children Living in Malaria-Endemic Areas of Calabar, Nigeria

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ABSTRACT

Background: One of the major causes of anemia, defined as the reduction in the level of hemoglobin or red blood cells (RBCs) in the blood, in children in sub-Saharan Africa is malaria. Anemia is diagnosed by using either the hematocrit method or by measuring the hemoglobin concentration. **Aims:** To evaluate the relationship and agreement between hemoglobin and three-fold conversion of hematocrit results of participants in a clinical trial. **Materials and Methods:** This is a cross-sectional study that obtained data from a multi-center clinical trial that took place from 2007 to 2008 in public health facilities in Calabar, Nigeria. The hemoglobin and hematocrit results of 494 children who had ≥2000 parasite density recruited were pooled to evaluate the relationship and agreement between the two methods. The difference between the measures against the mean of the two measures was plotted according to the theory of Bland and Altman. **Results:** The mean age of the children was 34 months, with approximately equal number of boys and girls. The measured hemoglobin was lower than the calculated hemoglobin in 84.5% of the children. The result showed that lower the hemoglobin concentration, the higher the chances that the three-fold hematocrit conversion overestimates hemoglobin levels in the participants. **Conclusions:** The three-fold hematocrit conversion of hemoglobin estimation is a less reliable method than the measured hemoglobin in anemic children in the study setting.

KEYWORDS: *Anemia, hematocrit, hemoglobin, malaria, packed cell volume*

INTRODUCTION

Anemia is defined as a reduction in the level of hemoglobin or red blood cells (RBCs) in the blood.[1] This reduction may be owing to excessive blood loss from trauma, breakdown of the red blood cells due to parasitic diseases or hemoglobinopathies, and from malnutrition.

One of the major causes of anemia in sub-Saharan is malaria. It occurs as a result of lysis of uninfected and malaria-infected RBCs, increase splenic sequestration of RBCs, and transient reduction in erythropoiesis attributed to effect malaria infection on the bone marrow.[2,3] In addition, some of the other factors responsible for anemia, especially among children are malnutrition, helminthiasis, and sickle cell disease.[4]

Anemia can be diagnosed by using either the hematocrit method, also known as packed cell volume (PCV), or by measuring the haemoglobin concentration.[5-7] The

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two measurements are often used inter-changeably for determining anemia either for surveys or clinical trials.[6]

It has been argued that using these two methods interchangeably may give false hemoglobin values.[8,9] This is as a result of an overestimation of the hemoglobin concentration by the hematocrit method, which in turn leads to an underestimation of the prevalence of anemia. Haemo-concentration and increased erythrocyte rigidity during malaria infection can also alter the accuracy of the three-fold conversion method of hemoglobin determination.[6]

Owing to inadequate resources and the need to give prompt treatment to children living in malaria-endemic areas, most of which are resource-limited, the hemoglobin concentration is derived by dividing the hematocrit value by three.[10] This conversion method is refered to as the three-fold conversion.[11]

There are concerns about the three-fold conversion method as a means of determining if a person is anemic or not due to lack of confidence in the hematocrit.[8] In Nigeria, it is common for clinicians to divide hematocrit by three to derive hemoglobin value. This is largely due to a lack of point of care hemoglobinometer and the burden of the cost of these tests on the patients who are mostly poor.[12] It is important to determine the accuracy of the three-fold conversion method for the measurement of hemoglobin particularly in Nigeria, a malaria endemic area. This is because anemia, in addition to parasite density, is used to monitor patients' response to antimalarial treatment both clinically and programmatically.[13]

Aims

The aim of this study was to evaluate the agreement between hemoglobin and hematocrit results of participants in a clinical trial.

Materials and Methods

Compliance with ethical standard

Ethical approval for the original study[14] was obtained from the Ethical Review Committee of the Cross River State Ministry of Health and Ethics committee of the University of Calabar Teaching Hospital.

Informed consent

Informed consent was obtained from participants in the original study.

Study setting

The study took place from November 2007 to December 2008 (a period covering both the wet and dry seasons) in public health facilities in Calabar, South–East, Nigeria.

Study design

This was a cross-sectional study that obtained data from two sites in Cross River State that were part of a multi-center clinical trial.[14] (ClinicalTrials.gov NCT00393679; Pan African Clinical Trials Registry PACTR2009010000911750). The first health facility was the Ikot Ansa Primary Health Center, and the second was the Pamol clinic located in a rubber plantation. However, all sites were located in the semi-urban community with holoendemic *P. falciparum* malaria transmission areas, hence their suitability for the trials. The results of hemoglobin and hematocrit of 494 children under 5 years of age who had ≥2000 parasite density recruited in a multi-center clinical trial[14] were used to evaluate the accuracy of the three-fold conversion method as described by Rodríguez-Morales *et al*.[9]

Anemia

The definition of different levels of anemia in this study is according to the following cut-off points [Table 1] recommended by the WHO for children aged six to 59 months.[1]

Inclusion criteria

In the original study,[14] children with the following criteria were included 6–59 months old with suspected uncomplicated clinical malaria attending the health facility where the study was carried out with the following, Weight >5 kg; mono-infection with *P. falciparum,* and a parasitemia of 2000–200,000 asexual parasites per ul, fever >37.5 degree, or history of fever in the preceding 24 h, hemoglobin value >7.0d/l, and signed informed consent by the parents or guardian.

Exclusion criteria

Participants were excluded if they have at least one of the following criteria: Participation in any other investigational drug study (antimalarial or others) during the previous 30 days, known hypersensitivity to the study drugs, danger signs: not able to drink or breast-feed, vomiting (>twice in 24 h), recent history of convulsions (>1 in 24 h), unconscious state, and unable to sit or stand. Others were the presence of intercurrent illness or any condition (cardiac, renal, and hepatic diseases), which would place the subject at undue risk or interfere with the results of the study, including known G6PD deficiency severe malnutrition (defined as weight for height <70% of the median NCHS/WHO reference) and ongoing prophylaxis with drugs having antimalarial activity such as cotrimoxazole for the prevention of *Pneumocisticarini* pneumonia in children born to HIV+ women. Children infected with other malaria species and those with mixed malaria infection.

Sampling technique

Children 6–59 months old with suspected uncomplicated clinical malaria attending the health facility were recruited into the study and randomized to one of the study treatments if they fulfilled the inclusion criteria. The details of the trial are described elsewhere.[14]

Sample collection and laboratory procedure

Samples for hematocrit were obtained simultaneously with that of malaria microscopy. Hematocrit measurement for each participant was obtained by centrifuging sample collected in heparinized capillary tube using Hawksley micro-hematocrit tube and centrifuge (Hawksley and Sons Ltd, Sussex, UK) at 11,000 revolutions per minute (rpm) for 5 min. Venous blood samples were put into bottles containing EDTA to determine hemoglobin and other hematologicalparameters. Hemoglobin concentration was measured with hemophotometry (ERMA INC. particle counter, model PCE-210).

Statistical analysis used

We used the method described by Rodríguez-Morales[9] according to the theory of Bland and Altman,[15] which states that "two methods designed to measure the same thing will inevitably give a positive linear correlation, and the most useful comparison is gained by plotting the difference between the measures against the mean of the two measures." Thus, the hemoglobin (g/dl) measurement and hematocrit (%) divided by three was compared on the same scale (grams of hemoglobin per deciliter) using this method. The difference between actual hemoglobin measurement and hematocrit divided by three (Hb - Hct/3) and their average (Hb + Hct/3)/2) for each participant were calculated. The value obtained was used to plot the linear regression curve to determine the correlation between the two methods and accuracy of the three-fold conversion (Hct/3) for hemoglobin estimation using the Bland-Altman method. The 95% limits of agreement were calculated as +/– 1.96 standard deviations.[15]

Results

This study obtained the actual hemoglobin and hematocrit measurement of 494 children with malaria who were included in a clinical trial.

Table 2 shows the mean age of the children was 34 months, with approximately equal number of boys and girls. The mean hematocrit was 28.5%. The mean measured hemoglobin and calculated hemoglobin (Hct/3) were 9.1 g/dl and 9.5 g/dl, respectively.

Table 3 shows the measured hemoglobin was lower than the calculated hemoglobin in 84.5% of the children. There were no statistically significant differences between measured hemoglobin and calculated hemoglobin with respect to anaemia status.

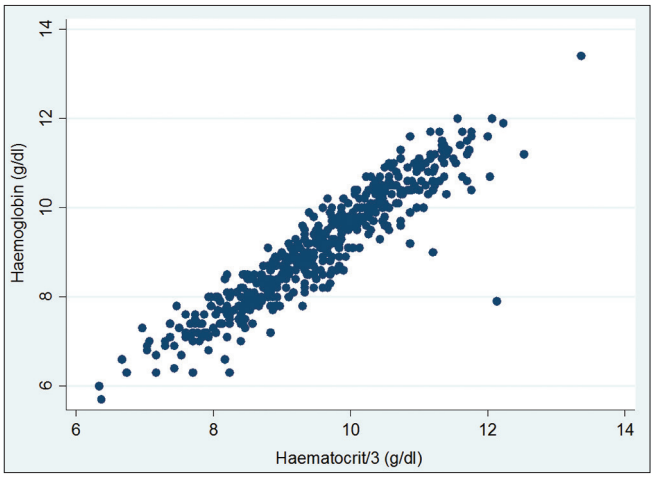

Figure 1: Scatter plot of the correlation between measured hemoglobin and calculated hemoglobin (Hct/3) in Calabar

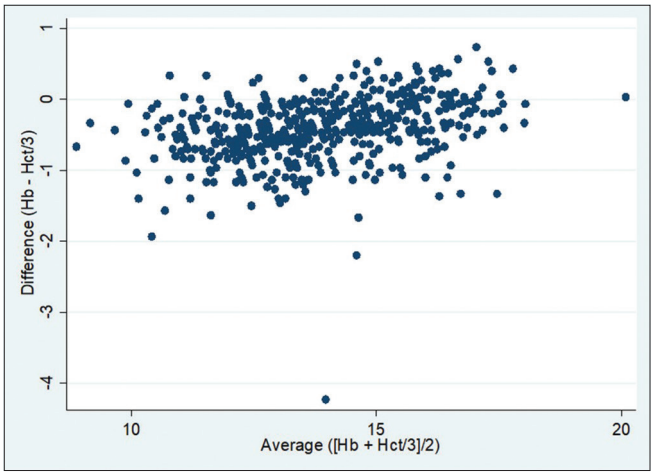

Figure 2: Scatter plot of difference against the average of measured hemoglobin and calculated hemoglobin in Calabar

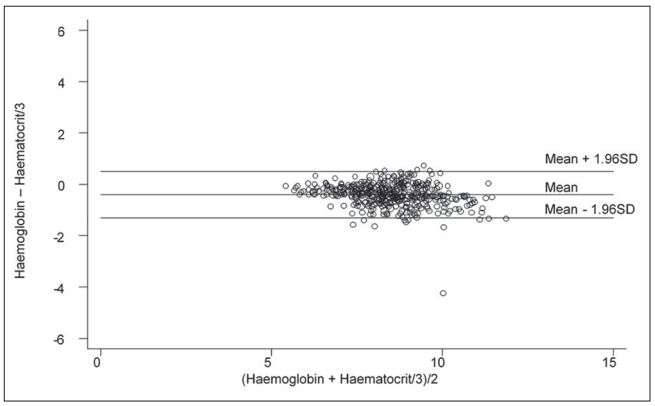

Figure 3: Bland and Altman graph of the difference in measured and calculated hemoglobin (Hct/3), by their average values in Calabar

Figure 1 shows a statistically significant direct correlation between measured hemoglobin and calculated hemoglobin (Hct/3) (r = 0.326, $P < 0.001$), both were expressed as g/dl.

The correlation matrix in Figure 2 shows a statistically significant direct correlation between the difference in measured and calculated hemoglobin (Hct/3) and their average values measured in g/dl in both axes (r = 0.326, $P < 0.001$).

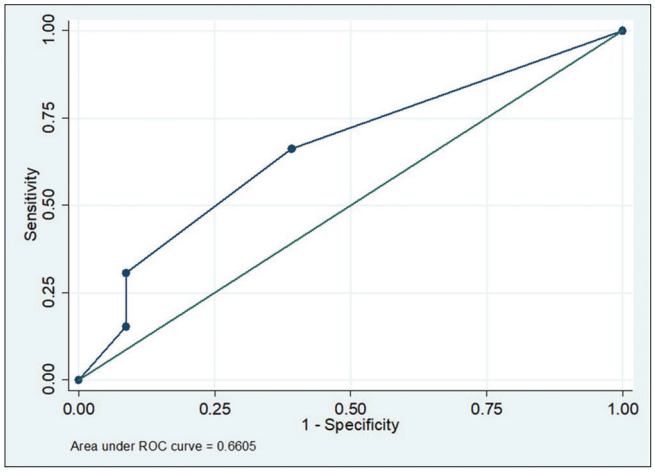

Figure 4: ROC curve of hematocrit and diagnosis of anemia in children under 5 years, adjusted for age and sex in Calabar

Table 1: Definition of Anemia by hemoglobin[1]

Anemia status	Hemoglobin cut-off range (g/dl)
Non-anemia	≥11.0 g/dl
Mild anemia	10 -10.9 g/dl
Moderate anemia	7 -9.9 g/dl
Mild-Moderate anemia*	HB 7.0 -10.9 g/dl
Severe	(HB <7.0 g/dl)

*The mild-to-moderate range of hemoglobin is described as moderate anemia in this paper for ease of reference

The Bland and Altman plot in Figure 3 shows the differences between measured and calculated hemoglobin. The mean difference differed significantly from the null value of 0 (-0.424, 95% CI: –0.465 to –0.383); hence, a systematic difference between the measured and calculated hemoglobin. There was a direct correlation between the differences and mean of measured and calculated hemoglobin (r = 0.269, $P < 0,001$), with the limits of agreement ranging from –1.346 to 0.49.

Figure 4 shows the receiver operation characteristic curve of hematocrit and diagnosis of anemia in children under 5 years, adjusted for age and sex. The area under the curve is 66%; hence, the hematocrit value below 35% correctly identifies 66% of children with hemoglobin less than 11.0 g/dl.

Discussion

The results showed a correlation between hemoglobin and hematocrit/3 and lower mean hemoglobin than mean hematocrit/3. A plot of the difference between hemoglobin and hematocrit/3 against the mean of the two measures showed a systematic difference. These results support Bland and Altman's argument that the most useful test of agreement between two methods designed to measure the same thing is to plot the difference between the measures against the mean of the measures. Hence, a test of the relationship between two methods measuring the same thing inevitably shows

Table 2: Demographic characteristics and hematologic parameters among children under 5 years in Calabar, 2010

Variable	*n*	Mean±SD	Median (Range)
Age (months)	492**	33.6±15.9	33.0 (6 -59)
Weight (kg)	492**	12.9±3.3	13.0 (6 -24)
Measured hemoglobin (g/dl)	485***	9.1±1.3	9.0 (5.7 -13.4)
Calculated hemoglobin (HCT/3)	485***	9.5±1.2	9.5 (6.3 -12.1)
Hematocrit (%)	485***	28.5±3.5	28.5 (19 -40.1)
Measured <Calculated hemoglobin	410/485 (84.5%)		
Sex (*n*=492)			
Male *n* (%)	244 (49.6)		
Female *n* (%)	248 (50.4)		

**Missing data was <0,5%. ***Missing data was <2%

Table 3: Anemia status by measured and calculated hemoglobin in the study population

Anemia status	***Hemoglobin (*n*=485)		Z test for proportion	*P*
	Measured hemoglobin *n* (%)	Calculated hemoglobin *n* (%)		
Normal (HB ≥11.0 g/dl)	41 (8.5)	56 (11.6)	Z=-0.496	*P*=0.620
Moderate (HB 7.0 -10.9 g/dl)	428 (88.2)	424 (87.4)	Z=0.357	*P*=0.721
Severe (HB <7.0 g/dl)	16 (3.3)	5 (1.0)	Z=0.274	*P*=0.784

***Missing datum was <2%

a correlation, which is not necessarily indicative of agreement.

One of the probable reasons for the systematic difference in the plot of the difference between hemoglobin and hematocrit/3 against the mean of these same measures could be due to hemoconcentration in anemia, which could result in overestimation of hematocrit measurement,[16,17] often attributable to dehydration in an underlying infection such as malaria. Hence, malaria endemicity may have accounted for Bland and Altman's phenomenon of a systematic difference between hemoglobin and hematocrit/3 measurements in our study conducted among children in Calabar, a malaria-endemic region.

However, a study in Mato Grosso, Brazil showed there was no difference in hemoglobin and hematocrit/3 methods. This may be owing to the fact that Mato Grosso is not a malaria-endemic region.[18]

This result also showed that lower the hemoglobin concentration, the higher the chances that the three-fold hematocrit conversion overestimates hemoglobin levels, this finding is similar to studies from other malaria-endemic regions.[8,9] The implication of this is that the three-fold hematocrit conversion is misleading and a less reliable method of estimating hemoglobin levels in children with malaria, especially in malaria endemic regions.

This study concludes that the three-fold hematocrit conversion of hemoglobin estimation is a less reliable method than the measured hemoglobin in anemic children in the study setting.

Study strength and limitation

The main strength of this study was the use of data in children infected with malaria to assess the correlation and agreement between hemoglobin and hematocrit/3 measurements in assessing anemia in children. The limitation of this research was that we could not assess the agreement between the two measurements after parasite clearance or on the follow-up days (Day 7 and Day 14) post-treatment because data were only available for the hemoglobin measurements at baseline.

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Conflicts of interest

There are no conflicts of interest.

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