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Original Article

Determination of Red Cell Indices Cut-off for Identifying Iron Deficiency Anaemia among Pre-school Children with Sickle Cell Anaemia

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ABSTRACT

Nigerian pre-school children have a high risk of developing iron deficiency and there is no consistent evidence that patients with sickle cell anaemia are protected from iron deficiency anaemia. The objective is to explore red cell indices cut-off values useful as surrogate for detecting iron deficiency in children with sickle cell anaemia. Ninety-seven children with sickle cell anaemia were recruited from Children Outpatient. Reference intervals were developed using the 2.5th – 97.5th, 3.0rd – 97.0th, 5 – 95th, and 10th – 90th percentile intervals for MCV and MCH. The discriminatory performance of the proposed red cell indices criterion was assessed by use of sensitivity, specificity, accuracy, likelihood ratio and predictive values. The 2.5th, 3rd, 5th, 10th, 90th, 95th, 97th, and 97.5th percentile values were: MCV (62.7, 63.6, 66.5, 69.6, 86.3, 87.7, 89.5, and 90.1fl), and MCH (19.0, 19.5, 20.8, 21.4, 28.2, 29.1, 29.5 and 29.7pg).The various calculated cut-off points for the MCV and MCH had lower sensitivity but a higher specificity for detecting iron deficiency than the standard reference values for the general population. The calculated cut-off point for the study subjects below the 10th percentiles had the best discriminatory performance. The cut-off for iron deficiency was 69.6fl for MCV and 21.4pg for MCH either use singly or in combination. In conclusion, standard reference cut-offs of MCV and MCH based on results from western individuals without sickle cell anaemia of the same age are not in agreement with the estimated values for children with sickle cell anaemia in Nigeria.

Keywords: *Cut-off, Children, Standard reference, Pre-school*

INTRODUCTION

Sickle cell disease is one of the commonest single gene disorders in man.[1] The burden of the disease is highest in sub-Saharan Africa, especially in Nigeria, where approximately 6 million people are projected to be afflicted.[2] There is no consistent evidence that patients with sickle cell anaemia are protected from iron deficiency anaemia.[3-8] In practice iron supplementation is not usually offered in sickle cell anaemia for fear of iron overload

resulting from an added effect of multiple blood transfusions but with improving management in recent years blood transfusions are less frequent[9] and the threat of iron deficiency in these patients may be real. In addition, frequency and need for blood transfusion are not uniform for all children with sickle cell anaemia.[9]

Iron deficiency anaemia (IDA) is characterized by deficient haemoglobin synthesis, resulting in red blood cells that are abnormally small (microcytic) and contain a decreased amount of haemoglobin (hypochromic).[10.] The capacity of the blood to deliver oxygen to body cells and tissues is reduced as a consequence of inadequate haemoglobin. The diagnosis of iron deficiency is based primarily on laboratory measurements.However, conventional tests used, mean corpuscular volume (MCV), transferrin saturation and serum ferritin are limited because of varying ranges of sensitivities and specificities, as they may be modified by conditions other than iron deficiency such as SCD.[5,11,12]The identification of IDA in children with SCD is important, as IDA contributes to worsening ofanaemia[5] and may have negative long-term consequences on neurocognitive development[13,14]. and growth.[15].

The measurement of ferritin is the most accurate test indicating iron deficiency in individuals.[16 - 21] Current literatures suggests that serum ferritin less than 25ng/ml is 100% specific for iron deficiency in children with sickle cell anaemia.[22, 23] In our clinical settings there are difficulties in determining children with iron deficiency due to cost of serum ferritin estimation. As a result of these factors, we rely on red cell indices for identifying children with iron deficiency.

A study was conducted by Akodu *et al*[24] among under-five children with sickle cell anaemia recruited from Lagos Nigeria. The authors reported that a combination of low haemoglobin concentration below 11.0g/dLand low MCV below 70fl was a poor screening tool for iron deficiency anaemia in children with sickle cell anaemia. This may be due to the fact that sickle cell anaemia sometimes may be associated with relative microcytosis in the absence of iron deficiency, which is assumed to be the consequence of reduced haemoglobin production.[25] Therefore; this cut off value would include a small number of children with sickle cell anaemia without iron deficiency anaemia. Thus, the use of MCV for evaluation of iron deficiency in children with sickle cell anaemia requires careful interpretation. The aim of the present study was to explore red cell indices cut-off values useful as a surrogate for detecting iron deficiency in children with sickle cell anaemia where serum ferritin estimation is not available.

MATERIALS AND METHODS

The study was a cross-sectional study involving children with sickle cell anaemia aged11 months to 5 years who have no symptoms or sign attributable to an acute illness in the preceding four weeks attending the General Children Outpatient clinic of the Department of Paediatrics of Lagos State University Teaching Hospital, Ikeja, Lagos in south west Nigeria.

Patients were recruited consecutively until the desired sample size was attained spanning three months period. Approval for the study was obtained from the Ethics Committee of Lagos State University Teaching Hospital, Ikeja, Lagos State.

The studied sample size consisted of 97 children with sickle cell anaemia without a history of prematurity or low birth weight and who had not received a blood transfusion or haematinic within three months prior to the study. Data were summarized for two age categories: age less than two and age two to five years. The sample size calculation was based on estimated prevalence of iron deficiency anaemia (IDA) of 69%[9] among Nigerian children with 90% power and standard deviation 1.96 in a two-tailed test.

Seven milliliters of blood were drawn from a convenient peripheral vein, two millilitres and five millilitres were transferred into Na-EDTA containing tubes and plain tubes respectively. The blood in the Na-EDTA were used for complete blood count (CBC), haemoglobin concentration, mean corpuscular volume (MCV), red blood cell count, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) determination using autoanalysis. The blood inside the plain tube was centrifuged at 3000 rpm for five minutes and the clear plasma obtained was separated and stored at minus 20ºC until the serum samples were analysed for iron studies: serum iron (measured by spectrometry), total iron binding capacity, TIBC, (measured by spectrometry), transferrin saturation (calculated using serum iron and TIBC values), TS, and serum ferritin (measured by ELIZA).

A subject was considered to be iron deficient when any one of the following criteria is present: (1.) serum ferritin (SF) < 25ng/dL.[22, 23]or (2.) transferrin saturation <16%,.[22, 23]All

the data obtained were recorded and analyzed using the Statistical Package for Social Sciences (SPSS) version 17.0. Data were presented in prose, and tables. The LMS (Least Median Squares) method was used in the analysis of red cell indices percentile. Comparison of means to assess statistical significance of difference was done using student t –test. Percentiles were applied to identify cut-off points. The cut-off values for the red cell indices were determined at 2.5th, 3rd, 5th and 10thpercentiles.The discriminatory performance of the proposed red cell indices criterion was by the use of sensitivity, specificity, accuracy, likelihood ratio and predictive values. P-value less than 0.05 were regarded as statistically significant.

RESULTS

A total of 97 children with sickle cell anaemia were recruited. The age and gender distribution of the study population are given in Table I.

Overall, the age of the subjects ranged from 11 months to sixty months with a mean of 35.4 (±15.7) months. The median age was 34.5 months. The male to female ratio was 1.02: 1. Forty-two (43.3%) of the study subjects belonged to the upper socioeconomic strata (Socioeconomic indices I and II), while 41.2% and 16.0% belonged to the middle (Socioeconomic index III) and lower (Socioeconomic index IV and V) socioeconomic strata respectively.

Haematological and Serum ferritin Profile of Study Subjects

The mean haematological and serum ferritin values of study subjects are shown in Table II. Mean MCV, MCH and serum ferritin were higher among female subjects than their male counterparts but the observed difference was not significant (p >0.05). The mean haemoglobin concentration showed no significant difference irrespective of gender.

Percentiles for red cell indices distribution among study subjects

The estimation of reference intervals was performed using the percentile of the distribution. In figures 1 and 2, the percentile distributions for individual red cell indices are shown. The 2.5th, 3rd, 5th, 10th, 90th, 95th, 97th, and 97.5th percentiles were estimated. The 2.5th, 3rd, 5th, 10th, 90th, 95th, 97th, and 97.5th percentile values were: MCV (62.7, 63.6, 66.5, 69.6, 86.3, 87.7, 89.5, and 90.1), and MCH (19.0, 19.5, 20.8, 21.4, 28.2, 29.1, 29.5 and 29.7).

Table I: Age and gender distribution of the study population

Characteristics	Number	%
Gender		
Male	49	50.5
Female	48	49.5
Total	97	100.0
Age group (years)		
Male		
≤ 2	24	49.0
>2- 5	25	51.0
Total	49	100.0
Female		
≤ 2	24	50.0
>2 – 5	24	50.0
Total	48	100.0

Table II : Haematological and serum ferritin profile of study subjects

	ALL Mean (SD)	Male Mean (SD)	Female Mean (SD)	p –value
Hb concentration (g/dl)	6.7 (1.8)	6.8 (2.1)	6.6 (1.2)	0.637
MCV (fl)	77.4 (6.1)	76.5 (6.0)	78.5 (6.1)	0.159
MCH (pg)	24.8 (2.6)	24.6 (2.7)	25.1 (2.5)	0.345
Serum ferritin (ng/dl)	189.9 (104.3)	170.4 (107.5)	211.5 (97.5)	0.086

Table III – Accuracy of determined red cell indices cut-offs for identify iron deficiency

Percentile	Variables	Values	Sensitivity	Specificity	PPV	NPV	LR	Accuracy
<2.5								
	MCV	<62.7	6.3	100.0	100.0	80.0	-0.1	80.3
	MCH	<19.0	0.0	98.3	0.0	78.7	0.0	77.6
	MCV &	<62.7	0.0	98.3	0.0	78.7	0.0	77.6
	MCH	&<19.0						
<3.0								
	MCV	<63.6	6.3	98.3	50.0	79.7	-0.1	78.9
	MCH	<19.5	6.3	98.3	50.0	79.7	-0.1	78.9
	MCV &	<63.6	6.3	100.0	100.0	80.0	-0.1	80.3
	MCH	&<19.5						
<5.0								
	MCV	<66.5	6.3	96.7	33.3	79.5	-0.1	77.6
	MCH	<20.8	6.3	96.7	33.3	79.5	-0.1	77.6
	MCV &	<66.5	6.3	98.3	50.0	79.7	-0.1	78.9
	MCH	&<20.8						
<10.0								
	MCV	<69.6	12.5	91.7	28.6	79.7	-0.1	75.0
	MCH	<21.4	12.5	90.0	25.0	79.4	-0.1	73.7
	MCV &	<69.6	12.5	91.7	28.6	79.7	-0.1	75.0
	MCH	&<21.4						

PPV = Positive Predictive value NPV = Negative Predictive Value LR = Likelihood Ratio

Table IV – Comparison of validity determined using cut-off at 10th percentile with the standard reference values

Percentile	Variables	Values	Sensitivity	Specificity	PPV	NPV	LR	Accuracy
Standard reference								
	MCV	<70.0	0.0	88.3	0.0	76.8	0.0	69.7
	MCH	<27.0	12.5	13.3	3.7	36.4	-1.0	13.2
	MCV &	<70.0 &	0.0	88.3	0.0	76.8	0.0	69.7
<10.0								
	MCH	<27.0						
			12.5	91.7	28.6	79.7	-0.1	75.0
	MCV	<69.6	12.5	90.0	25.0	79.4	-0.1	73.7
	MCH	<21.4	12.5	91.7	28.6	79.7	-0.1	75.0
	MCV &	<69.6 &						
	MCH	<21.4						

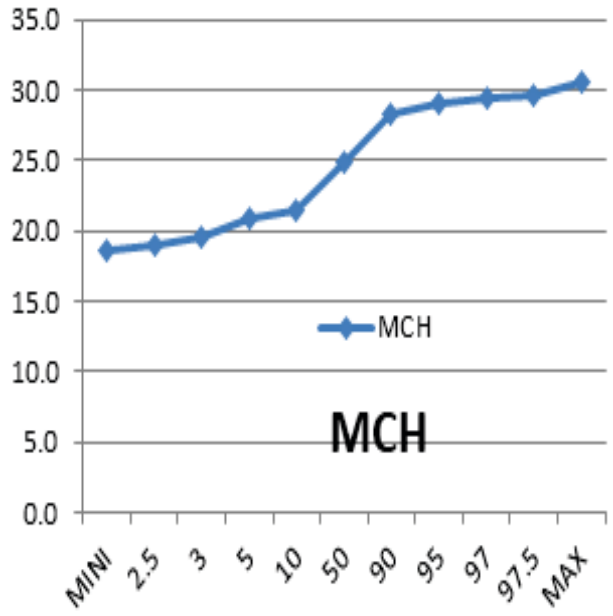

Figure 2: Percentile for the Mean Corpuscular Haemoglobin (MCH)

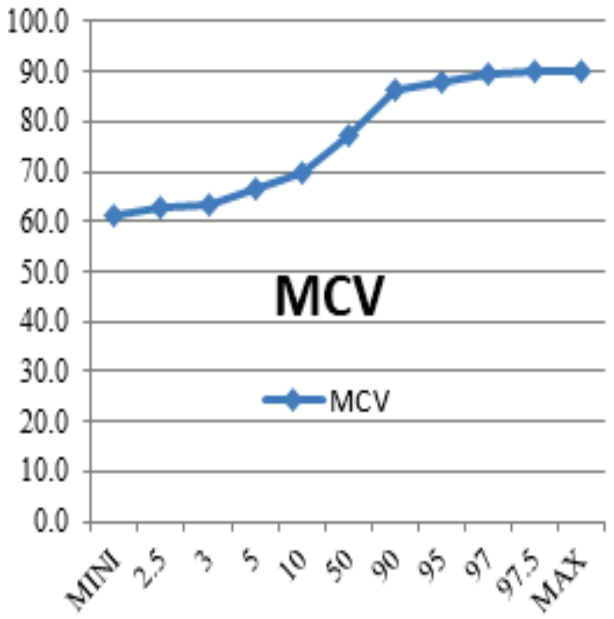

Figure 1: Percentile for the Mean Corpuscular Volume (MCV)

Accuracy of determined red cell indices cut-offsfor identification of iron deficiency

The sensitivity, specificity, accuracy, likelihood ratio and predictive values using MCV and MCH cut-off values below the 10th, 5th, 3rd, and 2.5th percentiles to identify iron deficiency among the study subjects was assessed. The various calculated cut-off points for the MCV and MCH had lower sensitivity but a slightly higher specificity for detecting iron deficiency than the standard reference values of 70fl[26] and 29pg[27] for the general population. The calculated cut-off point for the study subjects below the 10th percentiles had the best discriminatory performance. The cut-off point below which a person would be considered iron deficient was 69.6 for MCV and 21.4 for MCH either use singly or in combination.

On the basis of the standard reference values for the population microcytosis was defined as the mean corpuscular volume of <70 fl based on the lower limit for the mean cell volume reference range.[26] and mean corpuscular hemoglobin (MCH) below the lower reference cut-off values of 27 picograms was described as hypochromia[27]. Table V shows that the discriminatory performance using the determined best 10th percentile by sensitivity, specificity, accuracy, likelihood ratio and predictive values was higher compared to using the standard reference values.

DISCUSSION

Diagnostic tests are important tools in clinical practice with the use of gold-standard tests for the detection of disease in the affected individuals. In some circumstance or environment the gold-standard test either does not exist or is very difficult or expensive to perform for certain disease conditions. Therefore, we have to use alternative diagnostic tests or tools as surrogates for gold-standard tests.

The study show marked variation between the standard reference range and the reference range for children with sickle cell anaemia determined among the study subjects with the upper and lower limits of the reference range determined in the present study being generally lower than the standard reference values.

The finding of the current study showed that the lower limits for the MCV and MCH for the study subjects were lower than the universally defined lower limit of 70fl[26] and 29pg[27] for the general population. This may be due to the

fact that some of the study subjects have iron deficiency in addition to their haemoglobinopathy, both conditions been associated with microcytosis and hypochromia.

In a study of apparently healthy Ugandan children aged 1 – 5 years in 2008, Kironde *et al*[28] reported 10^{th} – 90^{th} percentile interval value of MCV of 62.0 to 83.0fl which is lower than the international 10^{th} – 90^{th} range. By these, the upper and lower limits for MCV for pre-school population studied were higher than the reported limits by Kironde*et al*.[28]. This wider range may be attributable to factors such as low dietary iron intake across the studies which would be difficult to compare. There was no remarkable difference between 5.0^{th} – 95.0^{th} percentile reference values for MCH reported by Kironde *et al*[28]among Ugandan children comparable to values from current study despite the well acknowledged undiagnosed haemoglobinopathies in the Ugandan study.

The performance (sensitivity and specificity) of different cut-offcriteria for MCV and MCH as screening tools for iron deficiency in children with sickle cell anaemia was estimated. The sensitivity of a test is defined as the proportion of subjects with iron deficiency correctly identified by a test tool.[29, 30] A sensitive test has a low false negative rate, therefore, a sensitive test can be used to rule out a disease condition.[29]The specificity of a test is defined as the proportion of subjects without iron deficiency correctly identified by a test tool.[29,30]

Diagnostic test require proper evaluation before their introduction and recommendation for use in clinical practice. To the best of the investigator's knowledge, this is the first series of report that review various cut-off values of MCV and MCH useful as a surrogate for detecting iron deficiency in children with sickle cell anaemia where serum ferritin estimation is not available. Using different MCV and MCH cut-off points for the assessment of iron deficiency showed that the estimated MCV and MCH criterion of 69.6fl and 21.4pg respectively for children with sickle cell anaemia had the highest sensitivity for detecting iron deficiency. Surprisingly when the estimated cut-off criterion of 69.6fl and 21.4pg for MCV and MCH respectively was used in combination gave sensitivity value similar to that for either of the parameter. The highest sensitivity values calculated in the current study was 12.5%. Taking into account the below 70% for the results of the sensitivity values at various MCV and MCH cut-off values, it may be concluded that MCV and MCH are not a good tests to screen for iron deficiency among subjects with sickle cell anaemia. The data from the present study demonstrated that the specificity of all the different calculated cut-off points for detecting iron deficiency was comparable and above 95% for all the cut-offs with MCV of 62.7fl and combination of the calculated cut-off points of 63.6fl and 19.5pg respectively for MCV and MCH having one hundred percent specificity values suggesting that all these calculated cut-off points of MCV and MCH are good for verification of subjects with sickle cell anaemia that have iron deficiency.

For the children with sickle cell anaemia studied the standard reference cut-off values for MCV and MCH for identifying iron-deficiency problems was compared to estimated cut-off criterion of 69.6fl and 21.4pg for MCV and MCH respectively. Using estimated cut-off criterion of 69.6fl and 21.4pg for MCV and MCH respectively for the assessment of iron deficiency showed that, compared with the standard reference cut-off points (MCV of 70fl[26] and MCH of 29pg[27]), showed that the calculated cut-off point for the MCV of 69.6fl alone or in combination with MCH cut-off point of 21.4pg had high sensitivity and the highest specificity for detecting iron deficiency than the standard reference cut-off points singly or in combination.

The positive predictive value of a test is a proportion is the probability that the patient has the disease given that the test result is positive.[29]The finding from the present study showed that the calculated cut-off point for the MCV of 62.7fl and combination of the calculated cut-off points of 63.6fl and 19.5pg respectively for MCV and MCH had one hundred percent positive predictive value. Surprisingly the estimated cut-off criterion of 69.6fl and 21.4pg respectively for MCV and MCH when assessed alone or in combination have a positive predictive value for detecting iron deficiency below thirty percent. The relatively low positive predictive value of cut-off points of 63.6fl and 19.5pg respectively for MCV and MCH for detecting iron deficiency suggests that these cut-offs with a highest sensitivity and specificity values are not the same in terms of a perfect screening tool for iron deficiency.

The negative predictive value of a test is the probability that the patient does not have the disease given that the test result is negative.[29]The performance of the different MCV and MCH cut-offs was compared in identifying subjects with iron deficiency anaemia in terms of their negative predictive values.. The negative predictive values of all the different calculated cut-off points for detecting iron

deficiency was comparable and above 75% for all the cut-offs suggesting that these cut-off points are equally good for identifying patients without iron deficiency. Other instrument of validity of all the different estimated cut-off points is the determination of their accuracy values. The accuracy test is an essential tool in the evaluation of the overall clinical utility of the test and it refers to the ability to differentiate the patient and healthy cases correctly. The accuracy values of all the different calculated cut-off points for detecting iron deficiency was comparable and above 75% for all the cut-offs.

Tests with the least error and the most accuracy are more desirable as a screening or verification tool for a disease. A better screening test should have high sensitivity and accuracy values while a test with high specificity and accuracy is better for final verification of a disease.[30] A test that have high sensitivity, specificity and accuracy values is appropriate for both screening and final verification of a disease.[30]However as a screening test, none of all the different calculated cut-off points are suitable for screening purposes due to low sensitivity values despite the high accuracy values. Hence, calculated cut-off point for the MCV of 62.7fl and combination of the calculated cut-off points of 63.6fl and 19.5pg respectively for MCV and MCH with 100% specificity and the highest accuracy are appropriate for verification of subjects with sickle cell anaemia that have iron deficiency.

CONCLUSION

In summary, these results indicate that children with sickle cell anaemia in which serum ferritin estimation for identifying iron deficiency is not possible should have either their MCV or combination of MCV and MCH for verification of iron deficiency.

Limitation of Study

The major limitation of the study is the small sample size which provided too small number of affected patients with iron deficiency to make conclusive remarks. There is a need for collaborative, multicentre study involving larger pool of children with varying degree of iron deficiency to confirm whether the calculated cut-offs are equally useful among children with sickle cell anaemia.

Conflict of Interest

None to declare

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