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Erythrocyte indices in Pre-school Nigerian Children with Sickle Cell Anaemia in Steady State

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ABSTRACT

Background: Sickle cell disease is a genetic haemoglobinopathy with consequent haemolysis and anaemia. It is of interest to study its effect on red cell indices beside haemoglobin concentration.

Objectives: The objective of the study is to determine the values of red cell indices in preschool-age children with sickle cell anaemia.

Methods: we conducted a cross-sectional study including 97children with sickle cell anaemia aged six months to five years and 97 age-and sex-matched healthy controls with haemoglobin genotype AA (Hb AA). The red cell indices such as packed cell volume, haemoglobin concentration, mean corpuscular volume, red blood cell count, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were investigated, using an auto analyzer.

Results: The mean PCV, haemoglobin concentration and red blood cell count were significantly higher in HbAA controls ($p = 0.000$ in each case). The mean MCV was higher among HbSS subjects but it was only among females and when the result was analyzed irrespective of gender that the difference was statistically significant ($p < 0.05$).

Conclusion: Children with sickle cell anaemia in steady state have lower values of all red cell parameters and higher values of MCV, compared to haemoglobin phenotype AA controls.

Keywords: Sickle cell anaemia, Steady state, Red cell indices, Red cell count, Peripheral smear

INTRODUCTION

Sickle cell anaemia is one of the commonest single-gene disorders in man with variable distribution in different parts of the world and variable clinical manifestations[1]. In sickle cell disorders haemoglobin S (Hb S) is present in the red blood cells (RBC) instead of haemoglobin A (Hb A). It is a common cause of chronic anaemia among children of African descent [1]. The condition is characterised by chronic haemolysis, high bone marrow activity and the theoretical likelihood of derangements in red cell indices.

The major pathophysiological consequence of the molecular aberration in the HbS molecule has decreased solubility especially, in low oxygen medium. This insolubility also increases the viscosity of the blood. In high concentrations of deoxy-HbS in the erythrocytes, a solid gel is formed by polymerization of the HbS molecule (tactoids). It is this gelation that alters the shape of the RBC and is responsible for rigidity of its membrane as well as its shortened life span [2]. Healthy red blood cells typically live 90 to 120 days, but sickle cells only survive 10 to 20 days [3].

In the presence of inherent haemoglobindefects, certain factors promote sickling in vitro. These include low oxygen tension, decreased pH, increased temperature, advanced cell age and increased intracellular Hb S concentration [2]. Observations have shown that, in vivo, RBC in SS disease are capable of repeated cycles of sickling and unsickling, depending on whether they are in

the venous or arterial circulation [2].However, after several cycles, some RBC loses the capacity to return to the normal shape even after exposure to oxygen. These are called irreversibly sickled cells (ISC)[2]. Once irreversibly sickled, the cells are destroyed by the reticulo-endothelial system. Thus, the shortened life-span of red cells is partly due to the result ofhaemolytic anaemia [2].

The red blood cell indices measure the size, shape and physical characteristics of the red blood cells. They comprises three components: (i) the average red blood size estimated by the mean corpuscular volume (MCV); (ii) the amount of hemoglobin per red blood cell or the mean corpuscular hemoglobin (MCH) and (iii) the amount of hemoglobin relative to the size of the cell or hemoglobin concentration per red blood cell - the mean corpuscular hemoglobin concentration (MCHC). The clinical implications of determining the red cell indices along with the red blood cell count (RBC) are that any co-existing anaemia such as iron deficiency anaemia which may lead to worsening anaemia may be identified.

Erythrocyte indices constitute important parameters useful in the clinical care of children with and without sickle cell anaemia as a screening tool for anaemia. It seems that the red cell indices of children with sickle cell anaemia will differ from those of children without sickle cell anaemia due to chronic haemolysis and increased bone marrow activity in sickle cell anaemia.However, there is a dearth of studies on erythrocyte indices in African children with sickle cell anaemia. It is therefore expected that the information derived from the study will provide guide to the clinicians in the management of children with sickle cell anaemia most especially in the steady state.

SUBJECTS AND METHODS

A cross-sectional study was conducted among children with sickle cell anaemia attending the Sickle Cell Disorder Clinic and other Consultant Outpatient Clinics of the Department of Paediatrics, Lagos State University Teaching Hospital, Ikeja in Southwest Nigeria. Diagnoses were confirmed by alkaline haemoglobin electrophoresis between December 2009 and February 2010. The Lagos State University Teaching Hospital, an urban tertiary health centre, is a major referral centre serving the whole of Lagos State.

Study approval was obtained from the Ethics Committee of the Lagos State University Teaching Hospital. The study enrolled 197 consecutive sickle cell anaemia patients who came for a routine follow-up visit in the clinic and met the inclusion criteria. Healthy controls included children with haemoglobin (AA) genotype from the General Outpatient and follow-up clinics and healthy children attending other specialist clinics like the Paediatric Dermatology Clinic. Controls and primary subjects were matched on the basis of age and sex. One hundred and ninety-four children (97Hb SS and 97 Hb AA) were studied. In order to avoid uneven distribution of subjects in terms of age or sex, the calculated sample size was stratified.

Inclusion criteria included:

a.Age of at least 6 months to 5 years

b.Confirmed Hb SS by electrophoresis

c.Steady-state condition i.e. absence of any crisis in the preceding four weeks and absence of any symptom or sign attributable to acute illness [4].

The study exclusion criteria were:

a. Denial of consent
b. Children on long-term transfusion therapy
c. Children who had received a blood transfusion within three months prior to the study
d. Children with a history of prematurity or low birth weight
e. Any patient with disorders that may affect the haematological values such as leukemia or renal disease.

The inclusion and exclusion criteria for the controls were the same as for the subjects except that the haemoglobin genotype was AA.

Two ml of blood were drawn from a convenient peripheral vein and transferred into Na-EDTA containing tubes. The vacuum tubes were labeled and placed in a cool box containing ice-packs. The samples were protected from light at all times using sheets of black plastic. They were transported to the Research Laboratory of the Department of Paediatrics, Lagos State University College of Medicine. The fresh blood samples collected in EDTA containing tubes were used for determination

of packed cell volume (PCV), haemoglobin (Hb) concentration, mean corpuscular volume (MCV), red blood cell (RBC) count, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) determination on the same day of collection using an auto-analyzer (Coulter LH 750).

Social classification was done using the scheme proposed by Oyedeji [5] in which subjects were grouped into five classes (I – V). Socio-economic index scores (1 to 5) were awarded to each subject based on the occupational and educational levels of parents. The data was analyzed using Statistical Package for Social Science (SPSS) software. Comparison of mean values was done using Student's t-Test and level of significance was set at $p < 0.05$.

RESULTS

A total of 194 children (97 Hb SS and 97 Hb AA) were recruited. Overall, the age of the subjects ranged from 7 months to 60 months with a mean of 30.61 (±15.97) months: 32.05 ± 16.12 months and 29.18 ± 15.77 months for SS subjects and AA controls, respectively (Mann –Whitney U = 4143.50, p = 0.151). The median ages were 25.00 and 26.00 months in SS subjects and AA controls, respectively. Ninety-six (49.5%) of the study subjects belonged to the upper socioeconomic strata (Socioeconomic indices I and II), while 34.5% and 16.0% belonged to the middle (Socioeconomic index III) and lower (Socioeconomic index IV and V) socioeconomic strata, respectively.

Haematological Profile of Study Subjects

The comparisons of the mean values of red blood cell indices between Hb SS subjects and Hb AA controls are shown in Tables 1. The mean PCV, haemoglobin concentration and red blood cell count were significantly higher in Hb AA controls (p = 0.000 in each case). The mean MCV and MCH were higher among Hb SS subjects, but the difference was only statistically significant among females and when the result was analyzed irrespective of gender. In both male and female subjects, the mean MCHC was comparable between Hb SS subjects and Hb AA controls.

Table 1 – Haematological profile of study subjects

	SS Mean (SD)	AA Mean (SD)	t-value	p-value
PCV (%)				
Males	20.9(3.91)	30.2 (3.34)	12.700	0.000
Females	21.0 (3.59)	29.9 (5.10)	9.474	0.000
Males and Females	20.5 (3.57)	30.1 (4.37)	15.202	0.000
Hb concentration(g/dl)				
Males	6.9 (1.96)	9.7 (1.26)	8.472	0.000
Females	6.7 (1.21)	9.6 (1.86)	8.721	0.000
Males and Females	6.8 (1.74)	9.7 (1.62)	11.177	0.000
MCV(fl)				
Males	75.3 (7.08)	72.9 (6.32)	1.758	0.082
Females	76.9 (7.60)	73.2 (5.27)	2.696	0.008
Males and Females	77.5 (6.10)	73.0 (5.73)	-4.830	0.000
RBC count				
Males	2.9 (0.69)	4.2 (0.47)	10.999	0.000
Females	2.8 (0.58)	4.1 (0.55)	11.013	0.000
Males and Females	2.7 (0.52)	4.1 (0.52)	17.740	0.000
MCH(µg)				
Males	24.0 (2.97)	23.4 (2.90)	1.029	0.306
Females	24.7 (2.78)	23.6 (2.46)	2.035	0.045
Males and Females	24.9 (2.55)	23.5 (2.65)	-3.368	0.001
MCHC(g/dl)				
Males	31.9 (1.71)	32.0 (1.65)	0.372	0.710
Females	31.9 (1.32)	32.2 (1.63)	0.728	0.468
Males and Females	32.1 (1.52)	32.1 (1.62)	0.083	0.934

Comparison of study subjects according to ranges for red cell indices

Table 2 shows the comparison of study subjects according to ranges for red cell indices. Low PCV and Hb values are more common among subjects with sickle cell anaemia compared with Hb AA controls. The observed difference was significant (p = <0.05). On the contrary, low MCV and MCH are more common among Hb AA controls compared with Hb SS subjects but the observed difference was significant. Almost three-fifth of the subjects with low MCHC belonged to Hb SS group. However, the observed difference was not significant.

DISCUSSION

In comparison with haemoglobin AA controls, children with sickle cell anaemia were expected to have significantly lower mean haemoglobin concentration and packed cell volume. This is the obvious consequence of shortened lifespan of sickle red blood cells attendant upon chronic haemolysis. Another direct consequence of chronic haemolysis in children with sickle cell anaemia is reduced red cell mass.

Table 2 – Distribution of study subjects according to red cell indices range

	SS	AA	p-value
PCV (%)			
<30	94(68.1)	44(31.9)	0.000
≥30	3(5.4)	53(94.6)	
Hb concentration(g/dl)			
<11	96(53.6)	83(46.4)	0.001
≥11	1(6.7)	14(93.3)	
MCV(fl)			
<75	36(37.9)	59(62.1)	0.003
75 - 100	61(61.6)	38(38.4)	
>100	0(0.0)	0(0.0)	
MCH(µg)			
<27	76(46.1)	89(53.9)	0.015
27 – 32	21(72.4)	8(27.6)	
>32	0(0.0)	0(0.0)	
MCHC(g/dl)			
<32	52(54.7)	43(45.3)	0.203
32 - 36	44(44.9)	54(55.1)	
>36	1(100.0)	0(0.0)	

NB: Values in parenthesis are % of column total

In the present study, the mean cell haemoglobin concentration in children with sickle cell anaemia in a steady state (6.9g/dl, 6.7g/dl in boys and girls, respectively) was reported within the range of 6.5 – 8.3g/dl in other parts of Africa[6-9]. It is; however, lower than 8.0 – 9.0g/dl which have been reported from studies conducted in Jamaica and in the USA. [10-13]one possible explanation may be the intense malaria transmission found in Nigeria. It is well known that in areas with intense malaria transmission, malaria is a predominant cause of anaemia in young children [14]. This finding of relatively low haemoglobin concentration even among subjects with normal haemoglobin genotype living in the same environment supports this explanation. Fetal haemoglobin concentration was not assayed in the current study. Thus, it is not possible to comment on the role of that factor in explaining the lower haemoglobin concentration observed in our subjects.

In the present study, the mean packed cell volume (PCV) in children with sickle cell anaemia was lower than 25.3% reported by Jeya kumar et al[15] among twenty-four children with sickle cell disease in Ibadan, Nigeria, 23 years ago. The observed difference is possibly due to the sample size. Small sample size is known to produce exaggerated high mean values. It might have been expected that improved understanding of the disease and improved care and follow-up of affected patients would have translated into higher PCV in steady state. However, it is possible that worsening economic situation of individuals and families would counteract the gains of improved care available in hospitals.

The present study shows that the mean packed cell volume (PCV) in children with sickle cell anaemia is similar in both females and males. This is contrary to observation reported by Khan et al[16]among under-five children in an equally hospital-based study at Bilaspur in which India reported that mean PCV was higherin males than in females. The disparity in mean PCV values between current study and Bilaspur study may support the concept that there may be an ethnic difference in the effect of gender on haemoglobin concentration.

In the present study, the mean corpuscular volume (MCV) was higher in SS subjects than AA controls, especially among girls where the difference was statistically significant. The trend of higher MCV in children with sickle cell anaemia is consistent with previous studies [10,17,18]. This is most likely a consequence of chronic haemolysis on-going in children with sickle cell anaemia stimulating haemopoiesis and haemopoietic activity thus providing more rapid supply of young red blood cells. It is known that fresh red blood cells have higher MCV [19].

The present study demonstrated that microcytosis and hypochromia were frequently seen in subjects with haemoglobin genotype AA. Iron deficiency anaemia is characterized by deficient haemoglobin synthesis, resulting in microcytosis and hypochromia[20]. Iron deficiency anaemia is said to be uncommon in individuals with sickle cell disease because of availability of an adequate iron source potentially from increased red cell turnover and from blood transfusions[21].

From the result of the present study macrocytosis is uncommon in children with sickle cell anaemia. Macrocytosis is a common feature seen in children with vitamin B12 or folic acid deficiency[22]. Vitamin B12 or folic acid deficiency state and thus macrocytosis would commonly be expected to be seen in haemoglobin SS subjects as a result of increased demand of erythropoiesis. The apparently

paradoxical finding of less macrocytosis among HbSS subjects might be the result of routine supplementation with folic acid and B complex vitamins in our clinic. This is in agreement with other studies[23] using the same supplementation practice.

Overall, female children with sickle cell anaemia had significantly higher mean corpuscular volume than their Hb AA counterparts. The mean red cell count was significantly higher among HbSS subjects than Hb AA controls. Both microcytosis and hypochromia were significantly more often found in subjects with HbAA than children with sickle cell anemia.

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