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Glycosylated Hemoglobin Levels among Children with Sickle Cell Anemia at Muhimbili National Hospital: A Case for establishing normal values

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Abstract

Background

Improvement in the management of children with sickle cell anemia (SCA) has increased their lifespan. This has led to emergence of various endocrinopathies such as diabetes mellitus (DM). Glycosylated hemoglobin (HbA1c) levels have been shown to be low among children with sickle cell anemia (SCA) by various studies. This can lead to under diagnosis of DM in SCA children, hence delaying their treatment. There is a need to establish normal ranges of HbA1c levels among children with SCA attending Muhimbili National Hospital in Dar-e-Salaam, Tanzania.

Objectives

To determine the reference range of HbA1c levels among children with SCA attending clinics at Muhimbili National Hospital in Dar-es-salaam, Tanzania.

Methodology

This was a hospital-based cross-sectional study conducted at Pediatric clinics in Muhimbili National Hospital involving children from 9 months to 14 years. 120 children with SCA and 40 children without SCA were recruited. HbA1c levels were reported as median and IQR while hemoglobin levels were reported as mean ± standard deviation. Independent t-test and Mann Whitney test were used for analysis of continuous data.

Results

The reference range of HbA1c levels in children with SCA was from 3.4% to 5.2%. Median HbA1c level in children with SCA was 4.2% (4.1% - 4.6%) while for children without SCA it was 5.3% (4.9% - 5.5%) with p-value < 0.001. Mean hemoglobin levels were 8.26 (±1.22) g/dl in SCA children compared to 11.55 (± 1.31) g/dl in children without SCA with p-value of < 0.001

Conclusion and Recommendation

The reference range of HbA1c levels in children with SCA was from 3.4% to 5.2%. Children with SCA had significantly lower levels of HbA1c compared to children without SCA. Health personnel are advised to use HbA1c reference ranges obtained from this study when screening for diabetes mellitus among children with SCA.

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Introduction

There has been improvement in the quality of care delivered to children with sickle cell anemia (SCA) which has prolonged their lifespan (1,2). This has led to the emergence of various endocrinopathies that were not obvious in the past such as diabetes mellitus (DM) (3,4).

Glycosylated hemoglobin (HbA1c) level is among the tests used worldwide to diagnose and monitor control of DM. However, its interpretation in children with SCA has to be done with caution. This is because of the observed lower levels of HbA1c (5) in children with SCA compared to children without SCA. In a study by Lacy et al, it was observed that African Americans with the sickle cell trait (SCT) had lower levels of HbA1c than those without SCT (6). This observation in children with SCA has been linked to the various methods used to measure HbA1c levels especially the Immunoassay methods (7,8). The false lower HbA1c levels, have also been linked to decreased lifespan of red blood cells (rbc) due to continuous hemolysis (hemolytic anemia) found in SCA (9).

Considering the wide availability of immunoassay methods in our settings, using standard cut-offs of HbA1c levels to diagnose DM in children with SCA can result in a missed opportunity for intervention. Some of the patients could be missed because of the possibly false low values (10).

There are few studies focusing on HbA1c levels in children since most studies have been focusing on adult population. The objectives of this study were to determine reference range of HbA1c levels among children with SCA attending sickle cell clinic at Muhimbili National Hospital. Also, to compare HbA1c levels between children with SCA and children without SCA attending clinics at Muhimbili National Hospital.

Methodology

This was a hospital-based cross-sectional study conducted from October 2020 to April 2021 in Paediatric clinics including Sickle cell clinic at Muhimbili National Hospital in Dar-es-salaam, Tanzania. The study population included both children with SCA and without SCA.

Inclusion criteria were children from 9 months to 14 years whose parent(s) or guardians gave consent. Exclusion criteria were children with known diabetes mellitus (or with RBG ≥ 11mmol/l if diabetes status was unknown), and those who were overweight or obese because of their metabolic profiles. Children on hydroxyurea were also excluded since their elevated HbF levels would confound the HBA1c levels estimation.

A special checklist was made to recruit children without SCA due to limited availability of rapid diagnostic tests for screening sickle cell within study setting. The checklist consisted of symptoms commonly found in children with SCA. If a child had any of these symptoms, was excluded from the group of children without SCA.

A total of 160 children with SCA and 50 children without SCA were involved before the beginning of the study. Out of 160 children with SCA, 34 children were excluded because of using hydroxyurea, 4 children were excluded because of having SCT and 2 children did not give consent. Of the 50 children without SCA, 3 children were excluded because of overweight and 7 children did not give consent. Therefore, only 120 children with SCA and 40 children without SCA were enrolled in the study by using the formula of comparison of two means.

Study variables were HbA1c levels as the dependent variable and SCA status as the independent variable. Data was collected by the researcher and the assistant using structured questionnaires. The questionnaires were completed through an interview.

Blood sample was drawn from the anterior cubital fossa region of the child's arm. This region was first cleaned with 70% methylated spirit and allowed to dry. 3 milliliters of blood were drawn from that area of which 2milliliters were kept in one EDTA tube to be tested for HbA1c level and the remaining 1 milliliter was kept in another EDTA tube to be tested for hemoglobin levels. The blood samples were kept at room temperature while waiting to be taken to the laboratory. HbA1c levels were measured using COBAS INTEGRAMACHINE (which uses immunoassay method) from the Jakaya Kikwete Cardiac Institute laboratory. The HbA1c method in this study was certified by NGSP and has been enrolled in the monthly international assessment from Randox - Riqas. Hemoglobin levels were measured by using Abbott CEL-DYN RUBY hematology machine from the Muhimbili National Hospital laboratory.

Data was analyzed using SPSS version 26. First, it was tested for skewness and kurtosis to check if it followed the normal distribution curve. Then, normality was further tested with Kolmogorov-Smirnov test and Shapiro-Wilk test. Outliers were checked by using box-plot test. Data was then analyzed according to SCA status. HbA1c levels were reported as median and IQR while hemoglobin levels were reported as mean ± standard deviation. Mann Whitney test was used for analysis of HbA1c levels while independent t-test was used for analysis of hemoglobin levels. P-value of less than 0.05 was considered statistically significant.

Ethical considerations

Ethical clearance was granted from MUHAS Institutional Review Board and permission to collect data was granted from the MNH administration. A written informed consent was obtained.

Results

Out Of the 120 children with SCA, there were 68 males and 52 females. Of the 40 children without SCA, there were16 males and 24 females. The median age for children with SCA was 4 years with IOR (2-7) years while for children without SCA was 5 years with IQR (3-8) years.

Median HbA1c levels were 4.2% with IQR (4.1%- 4.6%) in children with SCA compared to 5.3% with IQR (4.9% – 5.5%) in children without SCA with a p- value < 0.001. Mean hemoglobin levels were lower (8.26 ± 1.22) g/dl in children with SCA compared to (11.55 ± 1.31) g/dl in children without SCA. This was statistically significant with a p -value < 0.001. The above information has been summarized in Table 1 below.

Table 1: Comparison of Socio-demographic and clinical characteristics between children with sickle cell anemia (HbSS) and children without sickle cell anemia (HbAA)

Variable	Hemoglobin Genotype		P – value
	HbSS n (%)	HbAA n (%)	
Age group (years)			
≤ 5	74 (76.3)	23 (23.7)	0.765
>5	46 (74.2)	16 (25.8)	
Median age (IQR) (years)	4 (2, 7)	5 (3, 8)	0.531
Sex			
Male	68 (81.0)	16 (19.0)	0.068
Female	52 (68.4)	24 (31.6)	
Residence (Districts)			
Ilala	42 (79.2)	11 (20.8)	0.886
Temeke	23 (76.7)	7 (23.3)	
Kinondoni	18 (69.2)	8 (30.8)	
Ubungo	16 (69.6)	7 (30.4)	
Kigamboni	5 (83.3)	1 (16.7)	
Outside Dar es salaam	16 (80.0)	4 (20.0)	
Median HbA1c (IQR)	4.2 (4.1 - 4.6)	5.3 (4.9 - 5.5)	<**0.001**
Mean Hb ± SD (g/dL)	8.26 (± 1.22)	11.55 (± 1.31)	**< 0.001**

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The reference range of HbA1c levels among children with sickle cell anemia was found to be from 3.4% to 5.2%.

Median HbA1c level in children with SCA was significantly lower than in children without SCA with p-value < 0.001. This can also be seen in figure 1 below.

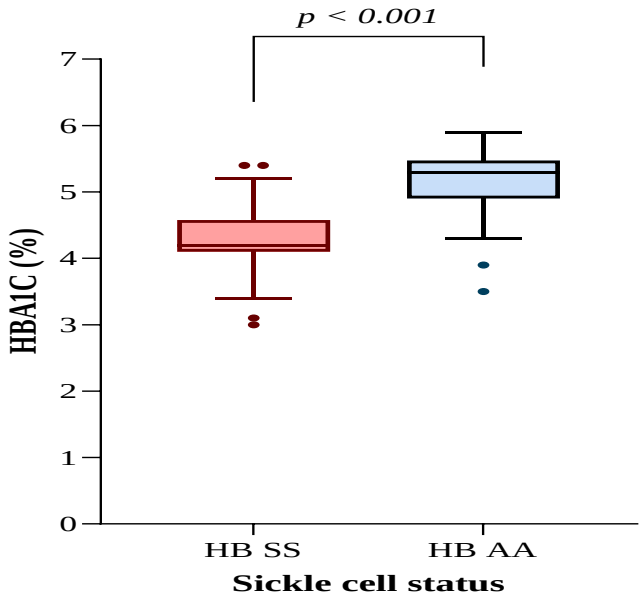

Figure 1. Comparison of median HbA1c levels between children with SCA (HbSS) and children without SCA (HbAA)

Discussion

The reference range of HbA1c levels for children with SCA was 3.4% to 5.2%. According to the American Diabetes Association (ADA), HbA1c levels of <5.7% is considered normal and above that is either pre-diabetes (5.7% – 6.4%) or diabetes (≥ 6.5%). However, in our study, the upper limit of normal HbA1c levels in children with SCA was 5.2% which was lower than 5.7% set by ADA. This means, there are some SCA children with DM that can be missed if we wait to reach HbA1c level of above 5.7%.

This study also demonstrated that children with SCA had lower levels of HbA1c compared to children without SCA. Therefore, we concur with findings from other African countries as well as outside Africa. For instance, a study done in Sudan by Atabani et al (10) revealed lower levels of HbA1c in children with SCA (4.9% ± 1.3%) compared to children without SCA (5.6% ± 0.2%) with p- value of less than 0.0025 Another study involving African-Americans

adults by Lacy et al (6) also revealed lower levels of HbA1c (5.72%) in patients with SCT compared to 6.01% in those without SCT.

There are two possibilities which have been mentioned in previous studies as to why children with SCA appear to have lower HbA1c levels compared to children without SCA. First possibility is the reduced lifespan of rbc in children with SCA. It is known that the lifespan of normal rbc is about 120 days compared to lifespan of sickled rbc which is about 10 to 20 days. This means that, with decreased lifespan of rbc, there is less time for glycosylation process of the sickled red blood cells resulting in lower levels of HbA1c among children with SCA (6).

Second possibility is the use of different methods to measure HbA1c levels. Some of these methods especially the immunoassay methods have been shown to be affected by hemoglobin variants compared to other methods (7) such as the Boronate affinity chromatography. This means using Immunoassay method in children with SCA, is more likely to give lower levels of HbA1c compared to the actual levels in a given child. The effect of different HbA1c methods has also been shown in a study by Bleyer et al (11). In their study, changes in HbA1c levels, were attributed to the methods used to measure HbA1c level rather than the decreased lifespan of sickled rbc.

In our study, we used the immunoassay method due to its wide availability. This is probably because other methods are more expensive and hence not easily affordable. Based on the findings of this study, the use of immunoassay method could be responsible for the lower reference range of HbA1c levels in children with SCA than what is known conventionally. Hence, we need reference ranges of HbA1c levels corresponding to SCA children in our community.

Tanzania is one of the countries with highest prevalence of SCA (1) globally. Improved quality of life and prolonged lifespan in children with SCA have increased their risk of developing various endocrinopathies such as DM (3). Therefore, there is a need for regular screening of these endocrinopathies.

There has been an increase in number of children with DM (12). The increase in prevalence of DM has also been shown in sickle cell patients by Zhou et al whereby it increased from 15.7% in 2009 to 16.5% in 2014 in USA (15). There is no information on the prevalence of DM among sickle cell patients in Tanzania.

It is important to consider sickle cell status, when screening and monitoring for DM with HbA1c test especially when using immunoassay methods. Improper interpretation of HbA1c

levels, could group some of the SCA children with DM into non-diabetic group. This can delay initiation of their treatment leading to poor control of their diabetic state. Poor control of DM is a common problem even in children without SCA (13).

Delay in the management of DM can cause various complications such as retinopathy and neuropathy (14). These complications are now witnessed even in some adult patients with sickle cell anemia (15). It is therefore important to have proper means of screening for DM in children with SCA so as to decrease the risk of developing these complications in their adulthood.

The strength of this study is that it gives a highlight about the lower HbA1c levels in children with SCA compared to children without SCA especially when using the Immunoassay method. It also provides a reference range of HbA1c levels which can be used to screen for DM among children with SCA when using immunoassay method.

The limitations of this study are the use of a checklist to recruit children without SCA. This might have included some of the SCA children who had not yet developed the symptoms. Also, the use of simple glucometer to exclude children with DM could have missed children with asymptomatic DM as some may have pre-diabetes or sub-clinical diabetes.

Conclusion

The reference range of HbA1c levels in children with SCA was from 3.4% to 5.2%. Children with SCA had significantly lower HbA1c levels compared to children without SCA. We recommend health personnel to use HbA1c reference ranges obtained from this study when screening for DM in children with SCA especially when using immunoassay methods.

Conflict of interest

The authors declare no conflict of interest.

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of Department of Pediatrics and Child Health at Muhimbili University and at the national Hospital.

Data Availability

The data is available in SPSS format from the author. It is also available at the data repository with written permission www.muhas.ac.tz/drp,

Authors contribution:

NMM, Conceptualization, Data curator, Field study, Collection of Data, Analysis and Writing the original manuscript. KM, Supervised the data collection process, reviewed the draft and the manuscript. KPM, Conceptualized, supervised and oversight of field activity. Initiated the primary manuscript and extensively reviewed the final manuscript. FF, Conceptualization and oversight of field activity.

Abbreviations

DM	Diabetes Mellitus
Hb	Hemoglobin
HbA	Adult Hemoglobin
HbA1c	Glycosylated Hemoglobin
IQR	Interquartile range
MNH	Muhimbili National Hospital
MUHAS	Muhimbili University of Health and Allied Sciences
RBC	Red Blood Cells
RBG	Random Blood Glucose
SCA	Sickle Cell Anemia
SCD	Sickle Cell Disease
SCT	Sickle Cell Trait

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Supplementary Material: Checklist Used to Screening Children without Sickle Cell Anemia

A. MEDICAL HISTORY OF THE CHILD

1. Age of the child ……

2. Has the child been transfused before?

a) Yes

b) No

3. Does the child have history of bone pain (arms and legs)?

a) Yes

b) No

4. Does the child have history of recurrent pain not relieved by common pain-medications like paracetamol and ibuprofen?

a) Yes

b) No

5. Does the child have history of swollen and painful hands and/or feet?

a) Yes

b) No

6. Is there history of left-sided upper abdominal swelling (splenomegaly) to the child?

a) Yes

b) No

7. Is there history of yellowish discoloration of the eyes to the child?

a) Yes

b) No

B. MEDICAL HISTORY OF CHILD'S FAMILY

8. Is there anyone in the family with Sickle Cell Anemia?

a) Yes

b) No

9. Is there any sibling with history of recurrent blood transfusion and/or recurrent painful episodes?

a) Yes

b) No

Interpretation

If any of the responses to above questions is Yes, it means increased likely hood of having sickle cell anemia and hence exclusion from the group of children without sickle cell anemia.