GLYCOSYLATED HEMOGLOBIN LEVELS AMONG NON-DIABETIC CHILDREN WITH SICKLE CELL ANEMIA AT MUHIMBILI NATIONAL HOSPITAL. A CASE FOR ESTABLISHING NORMAL VALUES.

Nancy Modest Mugyabuso, MD

A Dissertation Submitted in (Partial) Fulfillment of the Requirement for the Degree of Master of Medicine in Pediatric and Child Health of the Muhimbili University of Health and Allied Sciences.

October 2021

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences dissertation entitled **Glycosylated Hemoglobin Levels among Non-Diabetic Children with Sickle Cell Anemia at Muhimbili National Hospital. A Case for Establishing Normal Values**, as partial fulfillment of the requirement for degree of Master of Medicine in Paediatrics and Child Health of the Muhimbili University of Health and Allied Sciences.

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Prof Karim P Manji

(Supervisor)

_____

Date

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Dr Kandi Muze

(Co-Supervisor)

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Date

DECLARATION AND COPYRIGHT

I, **Nancy Modest Mugyabuso,** declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature…… Date…….

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DEDICATION

This work is dedicated to my beloved parents Mr. Modest Mugyabuso and Mrs. Winifrida Mugyabuso for their love, kindness and great support.

To my brothers, Denis Mugyabuso and Eric Mugyabuso.

And to GOD the almighty, who is our creator and source of all lives.

ABSTRACT

Background

There has been great improvement worldwide in the management of children with sickle cell anemia accompanied with improvement in their life quality. However, with the increase in their lifespan, as expected there is emergence of various endocrinopathies such as Diabetes mellitus

As of January 2010, the American Diabetes Association, began advocating the use of Glycosylated Hemoglobin (HbA1c) in diagnosing and monitoring Diabetes mellitus. It has been shown in some of the studies that HbA1c levels in children with sickle cell anemia are lower compared to children without sickle cell anemia. Therefore, there is a need to establish normal range of HbA1c levels corresponding to children with sickle cell anemia.

It is important to consider this decrease of HbA1c levels in children with sickle cell anemia when screening them for Diabetes mellitus so as not to miss children with false lower levels of HbA1c. Delay in diagnosis can delay treatment leading to poor diabetic control.

Objective

To determine normal range of glycosylated hemoglobin levels among non-diabetic children with sickle cell anemia attending clinics at Muhimbili National Hospital in Dar-es-salaam

Methodology

This was a hospital-based cross sectional study conducted at Paediatric clinics in Muhimbili National Hospital involving children from 9 months to 14 years. 120 children with sickle cell anemia and 40 children without sickle cell anemia were recruited consecutively from these clinics. Data was reported as median and interquartile range or mean ± standard deviation. Chi-square was used for categorical data while for continuous data independent t-test was used for normally distributed data and Mann Whitney test for non-parametric data.

Results

Median age for children with sickle cell anemia was 4 years with interquartile range (IQR) of (2 - 7) years while median age for children without sickle cell anemia was 5 years with IQR of (3 - 8) years. The reference range of HbA1c levels in children with sickle cell anemia was from 3.4% to 5.2%. Median HbA1c level in children with sickle cell anemia was 4.2% with IQR of (4.1% - 4.6%) while for children without sickle cell anemia median HbA1c levels was 5.3% with IQR of (4.9% - 5.5%). The median HbA1c in children with sickle cell anemia was significantly lower than median in children without sickle cell anemia

Conclusion and Recommendation

The reference range of HbA1c levels in children with sickle cell anemia was from 3.4% to 5.2%. Children with sickle cell anemia had lower levels of HbA1c compared to children without sickle cell anemia.

Health personnel are advised to use HbA1c reference ranges obtained from this study when screening for Diabetes mellitus in children with sickle cell anemia.

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ABBREVIATION

DM- Diabetes Mellitus

ENT- Ear, Nose and Throat

Hb- Hemoglobin

HbA- Adult Hemoglobin

HbA1c- Glycosylated Hemoglobin

IFCC- International Federation of Clinical Chemistry

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

DEFINITION OF KEY TERMS

1. Glycosylated Hemoglobin- It is a blood test which reflects blood glucose levels in the previous 2 to 3 months.
2. Sickle Cell Anemia- It is the homozygous form (HbSS) of Sickle Cell hemoglobinopathy.
3. Overweight- children whose weight-for-height/length is above +2 standard deviation (for under-fives) or whose body mass index (BMI) by age and sex is from 85^{th} to 95^{th} percentile from growth charts (if above five years)
4. Obesity- children whose weight-for-height/length is above +3 standard deviation (for under-fives) or whose body mass index (BMI) by age and sex is above 95^{th} percentile from growth charts (if above five years)
5. Diabetes mellitus was defined as random blood glucose $\geq$ 11.1mmol/l

CHAPTER ONE

1.1 INTRODUCTION AND LITERATURE REVIEW

1.1.1 GLYCOSYLATED HEMOGLOBIN

Hemoglobin is the protein carrying oxygen within the red blood cells. There are different types of hemoglobin which include adult hemoglobin (HbA), sickled hemoglobin (HbS) and other minor forms like glycosylated hemoglobin (HbA1c).

HbA1c is formed by attachment of glucose in the blood to hemoglobin through irreversible and non-enzymatic process (1,2). This is a slow process which occurs throughout the lifespan of a red blood cell. HbA1c levels give similar information to what might be given by frequent glucose monitoring throughout the day for 3 consecutive months (3,4). Hence, with prolonged levels of hyperglycemia there is expected increase in HbA1c levels. This allows HbA1c levels to become excellent marker of the overall glycemic control in the lifetime of a normal red blood cell which is about 120 days (5). Intense glucose control in diabetic patients has been associated with delayed onset and slow progression of diabetic complications such as retinopathy, nephropathy and neuropathy (6). This allows HbA1c levels to be used in both diagnosis and monitoring of Diabetes Mellitus.

Since January 2010, the American Diabetes Association has been advocating the use of glycosylated hemoglobin levels (HbA1c) as the preferable test to diagnose and monitor Diabetes mellitus. Its benefits over the fasting glucose (or 2-hour oral glucose tolerance test) is its ability to be carried-out during any time of the day (7,8). WHO report of 2011 has also recommended the use of HbA1c levels in diagnosing Diabetes Mellitus (9).

1.1.2 IMPROVEMENT IN THE MANAGEMENT OF CHILDREN WITH SICKLE CELL DISEASE

Sickle cell disease is more prevalent in Africa. 75% of the global births of sickle cell disease happening every year, occur in Africa (10). Tanzania is one of the 5 countries in the world with the highest number of newborns with sickle cell disease per year, others being Nigeria, Democratic Republic of Congo, India and Uganda (11,12). Prevalence of sickle cell disease in Tanzania was found to be 6 in every 1000 births which is about 11,000 births per year (13).

According to Tluway et al, Tanzania like many other countries, is undergoing major transition in the management of children with sickle cell disease as evidenced by increase in their lifespan (14). The decreased mortality in children with sickle cell disease was brought about by improved hygiene, good nutrition and public health interventions introduced to manage children with sickle cell disease. These public health interventions include the use of Penicillin V prophylaxis, folic acid and the use of Hydroxyurea.

Before introduction of above interventions in children with sickle cell disease, 50-90% of children would die in childhood (14). However recent study done at emergency department at MNH showed mortality rate among children with sickle cell disease to be 2.1%(13). Overall the median survival of patients with sickle cell anemia in Tanzania was found to be 33 years (13)

1.1.3 SICKLE CELL DISEASE AND DIABETES MELLITUS

The increase in the lifespan of patients with sickle cell disease has increased the risk of developing various endocrinopathies including diabetes mellitus (15). Diabetes mellitus in patients with SCD, is thought to be caused by iron overload from chronic blood transfusion whereby the excess iron damages pancreatic beta cells and hence reduces insulin production (16). However in other studies, Diabetes mellitus was linked with vaso-occlusive and ischemic events occurring in patients with SCD (15)

A study by Kengne et al in Cameroon, showed prevalence of sickle cell trait in patients with type 2 Diabetes Mellitus to be 19% (17). This is supported by another study carried out by Zhou et al in USA which showed the prevalence of type 2 Diabetes Mellitus in patients with sickle cell disease to increase from 15.7% to 16.5% from 2009 to 2014 (18). In their study, Zhou et al,

revealed that patients with sickle cell disease and diabetes mellitus had increased risk of developing complications such as nephropathy and neuropathy with P value < 0.001 compared to sickle cell patients without Diabetes mellitus. It is therefore important to have proper tests to diagnose and monitor diabetes mellitus in patients with sickle cell disease as early as possible.

However, in a study by Bariha et al conducted in India, the proportion of sickle cell disease patients with Diabetes mellitus was found to be 2 out of 137 although there were more sickle cell disease patients in prediabetic stage (7 out of 137) (19). The lower incidence of Diabetes mellitus among Asian population compared to previous studies involving African-Americans could be explained by less severe disease observed in Asian population (19).

Even though most of these studies were involving adult population, they could be an alarming sign for healthcare workers attending children with sickle cell anemia (which is part of the spectrum of sickle cell disease) to start screening for diabetes mellitus so as to be able to diagnose it earlier and initiate early treatments. In a study by Mandese et al, 11.5% of 52 children with sickle cell disease were found to have insulin resistance

1.1.4 GLYCOSYLATED HEMOGLOBIN LEVELS IN CHILDREN WITH SICKLE CELL DISEASE

The use of HbA1c levels to screen and monitor for Diabetes mellitus in people with sickle cell disease has been questioned in some studies. This is due to relatively lower levels of HbA1c observed in people with sickle cell hemoglobinopathies compared to those without sickle cell hemoglobinopathies. In a systematic review by Gordon DK, it was shown that sickle cell trait had the ability to compromise the accuracy of HbA1c levels (20). Similarly, in a study by Lacy et al involving African-Americans, it was observed that the ability of HbA1c to identify presence of prediabetes and diabetes was statistically lower in people with sickle cell trait compared to those without sickle cell trait (21). There have been different explanations for the observed decline in the HbA1c levels in people with sickle cell disease compared to those without sickle cell disease.

One explanation was the decreased lifespan of the red blood cells in people with sickle cell disease and other hemolytic anemia in general, which means less time for glycosylation (5) and

hence lower values. This phenomenon was used in earlier studies such as a study conducted by Atabani et al in Sudan in 1989, whereby it was found out that HbA1c levels in children with sickle cell anemia were lower compared to children without sickle cell anemia. HbA1c levels in sickle cell anemia children were 81% of children without sickle cell anemia. This was also seen among African-American population whereby the levels of HbA1c were lower in population with sickle cell anemia with HbA1c levels of 58% of population without sickle cell anemia (22).

Another explanation of false lower HbA1c levels according to study by Grossman was the recent blood transfusion (23). In that study, there was statistical difference between HbA1c levels 28 days before transfusion and 14 days after transfusion with p-value of 0.00124. According to Radin et al in 2013, the ability of blood transfusion to lower HbA1c levels may be caused by dilution effect (5). That is red blood cells from a person with normal glucose levels can lower HbA1c levels in a person with actually high glucose levels. Although these were observations made in adult patients, it is important to consider them when managing children with sickle cell anemia due to recurrence of blood transfusions in some of them.

The other explanation of lower HbA1c levels in children with sickle cell anemia compared to children without sickle cell anemia is based on the different methods of testing for HbA1c levels. Some of these methods such as most immunoassay methods, have been shown to be interfered by hemoglobin variants hence giving lower values than it should be. However, it is the immunoassays methods which are readily available in our settings.

A study by Bleyer et al, revealed that some of the HbA1c methods were not affected by the presence of hemoglobin variants (24). This is in concurrence with a review by Little et al whereby they showed the ability of some of the HbA1c methods to be able to detect different types of hemoglobin and hence provide true levels of HbA1c (25)

Also in a study by Camargo et al in Brazil involving diabetic patients attending out-patients clinics, more than half of the diabetic patients (56%) with lower HbA1c levels had other hemoglobin variant besides normal adult hemoglobin (26). Therefore, they recommended the

use of methods which are not compromised by hemoglobin variants to screen and monitor for Diabetes mellitus in patients with sickle cell hemoglobinopathy.

Generally low hemoglobin levels in children with sickle cell anemia caused by hemolysis (hemolytic anemia) is also responsible for falsely low HbA1c levels. According to a study by Radin (5), this is due to decreased exposure of hemolyzed cells to blood glucose.

There is a need to have normal ranges of HbA1c levels corresponding to children with sickle cell anemia. This will enable healthcare workers not to miss some of the sickle cell anemia children with diabetes mellitus because of the false lower levels especially when using immunoassay methods. In this study, we also used immunoassay method to measure HbA1c levels.

1.2 PROBLEM STATEMENT

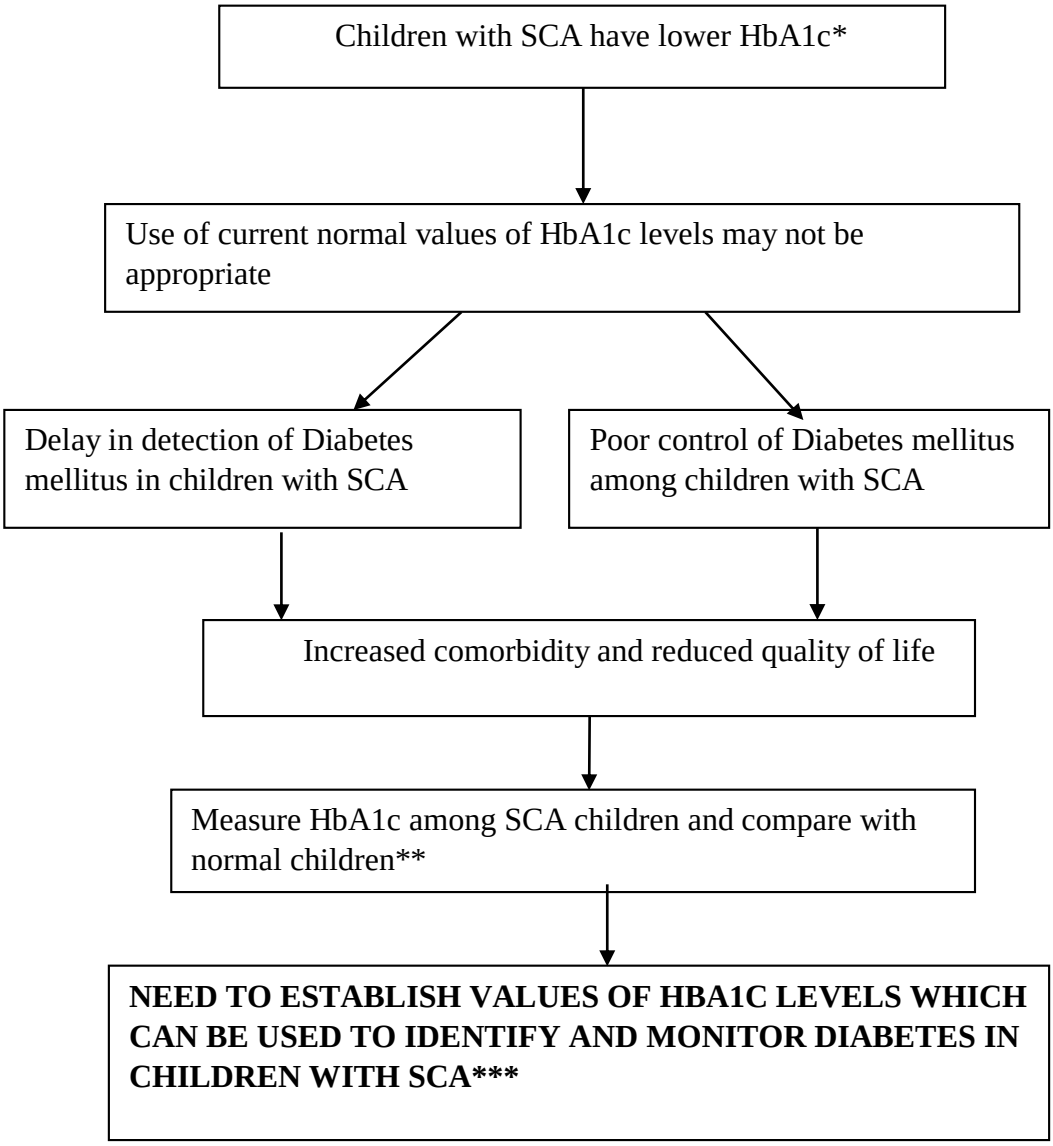
Improved quality of care in children with sickle cell anemia, has led to increase in their lifespan. This has led to the emergency of various endocrinopathies which were not obvious in the past such as Diabetes mellitus (15,27). Among the tests used worldwide to diagnose and monitor diabetes mellitus includes HbA1c levels.

The interpretation of HbA1c levels in children with sickle cell anemia has to be done cautiously. This is because of the possibility of false lower levels of HbA1c (1) in children with sickle cell anemia compared to children without sickle cell anemia. The lower levels of HbA1c seen in children with sickle cell anemia have been linked to the various methods used to measure HbA1c levels and especially the Immunoassay methods (25,28). In a study by Lacy et al, it was observed that African Americans with the sickle cell trait had lower levels of HbA1c than those without sickle cell trait (21).

Considering the wide availability of immunoassay methods in our settings, using existing HbA1c ranges which were set for children without sickle cell hemoglobinopathy to diagnose Diabetes Mellitus in children with sickle cell anemia may not be very effective. Some of the patients could be missed because of the possibly false low values (22). There are few studies focusing on HbA1c levels in children since most studies have focused on adult population. It is therefore important to establish normal levels of HbA1c that correspond to children with sickle cell anemia. The lack of normal levels of glycosylated hemoglobin corresponding to non-diabetic children with sickle cell anemia in Tanzania facilitated the choice of this study.

1.3 CONCEPTUAL FRAMEWORK

Figure 1: Conceptual Framework



1. * Hypothesis

2. ** Process

3. *** Expected outcome

1.4. RATIONALE

Using standard cut-offs of HbA1c levels (that is HbA1c level ≥ 6.5% according to American Diabetes Association) to diagnose diabetes mellitus in children with sickle cell anemia can cause missed opportunity for interventions. For example, in a study by Lacy et al, use of standard HbA1c cut-offs identified less people with diabetes mellitus among those with sickle cell trait (SCT) compared to those without sickle cell trait (21)**.**

This study aimed at establishing normal levels of HbA1c among non-diabetic children with sickle cell anemia attending sickle cell clinic at Muhimbili National Hospital. It also aimed at comparing HbA1c levels between children with sickle cell anemia and those without sickle cell anemia. Knowing the normal range of HbA1c levels, especially in a setting where the immunoassay methods are widely available or used, will provide a baseline that can be used to detect Diabetes mellitus earlier in children with sickle cell anemia. It will also allow proper monitoring of sickle cell anemia children with Diabetes mellitus hence preventing or delaying diabetic complications.

1.5 RESEARCH QUESTION

-What is the normal glycosylated hemoglobin level among non-diabetic children with sickle cell anemia attending sickle cell clinic at MNH?

-What is the comparison of glycosylated hemoglobin levels among non-diabetic children with sickle cell anemia and those without sickle cell anemia attending clinics at MNH?

1.6 OBJECTIVES

a) Broad Objective

-To determine normal levels of glycosylated hemoglobin among non-diabetic children with sickle cell anemia attending clinics at Muhimbili National Hospital

b) Specific Objectives

1. To determine reference range of glycosylated hemoglobin level among non-diabetic children with sickle cell anemia attending sickle cell clinic at Muhimbili National Hospital

2. To compare median glycosylated hemoglobin levels between non-diabetic children **with** sickle cell anemia and non-diabetic children **without** sickle cell anemia attending clinics at Muhimbili National Hospital

CHAPTER TWO

2. METHODOLOGY

2.1 Study design

This was a hospital-based cross-sectional study

2.2 Study duration

The study was conducted for a period of 6 months from October 2020 to April 2021

2.3 Study area

This study was conducted at Paediatric clinics including Sickle cell clinic held at Muhimbili National Hospital in Dar-es-salaam, Tanzania.

2.4 Study population

Study population included both children with sickle cell anemia and children without sickle cell anemia attending clinics at Muhimbili National Hospital.

2.5 Inclusion and Exclusion criteria

2.5.1 Inclusion criteria

- - Children from 9 months to 14 years

- Children whose parent(s) or guardians gave consent

2.5.2 Exclusion criteria

- Known children with Diabetes Mellitus

- Children who were overweight or obese

- Children who were on hydroxyurea (refer to appendix 5.A.1)

2.6 Sample size estimation

Sample size was calculated using the formula below:

$$N = \frac{(K x\ \boldsymbol{\sigma}^2_1 + \boldsymbol{\sigma}^2_2)\ (\mathbf{z}_{1-\alpha/2} + \mathbf{z}_{1-\beta})^2}{\underline{\Lambda}^2}$$

Where;

$K = n_1/n_2$ (n1/n2 is the ratio of children with SCA over children without SCA)

In this study, we used **K of 3** because it gave a value close to the minimum required sample size of 120 which was needed to establish the reference range of HbA1c levels in children with sickle cell anemia. This is according to The International Federation of Clinical Chemistry and Laboratory Medicine (30)

N was the total number for children with SCA

$\boldsymbol{\sigma}_1$ was the standard deviation for mean HbA1c levels in children with SCA

=**1.3** (from Atabani et al, 1989)

$\boldsymbol{\sigma}_2$ was the standard deviation for mean HbA1c levels in children without SCA

=**0.2** (from Atabani et al, 1989)

$\mathbf{z}_{1-\alpha/2}$ was the two-sided Z-value

=**1.96** (assuming 95% confidence interval)

$\mathbf{z}_{1-\beta}$ was the power of the study

=**0.84** assuming power of study to be 80%

$\underline{\boldsymbol{\Delta}}$= difference between two mean glycosylated hemoglobin levels (between children with SCA and children without SCA respectively)

= 4.9 - 5.6

= -0.7 (from Atabani et al, 1989)

Therefore;

$$N = \frac{(K \times \boldsymbol{\sigma}^2_1 + \boldsymbol{\sigma}^2_2)\,(\mathbf{z}_{1-\alpha/2} + \mathbf{z}_{1-\beta})^2}{\Lambda^2}$$

$$N = \frac{[(3 \times 1.3^2) + 0.2^2]\,[1.96 + 0.84]^2}{[-0.7]^2}$$

N = 81.76

N = 82

Adjusting for non-response rate, assuming response rate of 80% which is 1.25 then

N = 82 x 1.25

= 10 2.5

= 103 (This is a number close to the required sample size of 120)

Since the number of children with sickle cell anemia was assumed to be 3 times the children without sickle cell anemia, therefore, the sample size was estimated to be;

- 120 children with sickle cell anemia
- 40 children without sickle cell anemia

2.7 Sampling strategy

Children were selected consecutively from the sickle cell clinic and other Pediatric clinics at MNH, by the researcher and researcher assistant until the required sample size was reached. The other Paediatric clinics were Neurology, Ophthalmology, ENT (Ear, Nose and Throat) and Dermatology clinics. 120 children with sickle cell anemia who were not on hydroxyurea, were recruited from sickle cell clinic based on their hemoglobin electrophoresis results of HbSS. Children without sickle cell anemia were recruited based on the checklist developed for this study. The checklist was tested for validity by picking randomly five children without sickle cell anemia obtained in the study and conducting hemoglobin electrophoresis whereby their results were HbAA. To determine how many children were to be selected from the remaining clinics, total number of children in each of these clinics per week was obtained from the registry. There were about 170 children attending neurology clinic, 110 children attending eye clinic, 80 children attending ENT clinic and 20 children attending skin clinic per week hence a total of **380 children** in above clinics per week

Thereafter proportion of children without sickle cell anemia in each clinic was obtained by dividing number of children in individual clinics by total number of children in all clinics which was 380.

Proportion of children without sickle cell anemia in individual clinic was;

CLINIC	NEUROLOGY	OPHTHALMOLOGY	ENT	DERMATOLOGY
PROPORTION	170/380 =0.45	110/380 =0.3	80/380 =0.2	20/380 =0.05

These proportions were then multiplied by 40 which was the required sample size for children without sickle cell anemia, so as to obtain how many children should be recruited for the study from each clinic

Number of children without sickle cell anemia to be recruited in the study from each clinic

CLINIC	NEUROLOGY	OPHTHALMOLOGY	ENT	DERMATOLOGY
NUMBER OF CHILDREN	0.45 X 40 =18	0.3 X 40 =12	0.2 X 40 =8	0.05 X 40 =2

Therefore, number of children without sickle cell anemia recruited from other clinics were;

- 18 children from Neurology clinic
- 12 children from Ophthalmology clinic
- 8 children from ENT clinic
- 2 children from Dermatology clinic

2.8 Study variables

Study variables were glycosylated hemoglobin levels which was the dependent variable and sickle cell anemia status which was the independent variable

2.9 Investigation tools

Structured questionnaires initially made in English and then translated to Kiswahili were used.

2.10 Data collection

Data was collected by the researcher and researcher assistant. The researcher assistant was an intern doctor. Pre-tested structured questionnaires were used to collect information from the parents/guardians of children attending clinics at MNH who gave consent. For children with 7 years and above, they were asked for assent. The questionnaire had 2 main parts; first part which was completed through an interview and the second part which was for laboratory investigations

2.10.1 Interview

The interview was used to obtain sociodemographic information of the child as well as details of the child's sickle cell disease. These interviews involved parents/guardians of these children who had given us the consent to proceed with the study.

2.10.2 Laboratory investigations

A blood sample was drawn from the anterior cubital fossa region of the child's elbow. This region was first cleaned with 70% methylated spirit and allowed to dry. Then a 5cc syringe was used to draw 3 milliliters of blood from that area. This blood sample was then kept in 2 separate EDTA tubes. Out of the 3 milliliters of blood collected, 2 milliliters were kept in one EDTA tube and used to test for HbA1c level and the remaining 1 milliliter was kept in another EDTA tube and used to test for hemoglobin levels. The blood samples were kept at room temperature while waiting to be taken to the laboratory.

-HbA1c levels

HbA1c levels were measured using COBAS INTEGRA MACHINE which uses immunoturbidometric method. This is among the many immunoassay methods. First, the EDTA tube containing 2 milliliters of blood sample was kept in the blood roller mixer for about 3 to 5 minutes. Then the tubes were kept in the machine whereby 500 microliters of blood were drawn from the EDTA tube by the machine and used for analysis of HbA1c levels. The results of HbA1c levels were expressed in percentage.

-Hemoglobin levels

To measure hemoglobin levels, the EDTA tubes containing 1 milliliter of blood were also kept in the blood roller mixer for 3 to 5 minutes. Then these tubes were kept in the Abbott CEL-DYN RUBY hematology machine, to measure complete blood counts. From the results of complete blood counts, we were able to obtain hemoglobin levels as it is among the parameters tested in the complete blood counts. Hemoglobin levels were expressed in g/dl.

2.10.3 Quality Control

Both machines were checked daily for internal quality control before running the study samples. And there was also monthly checking for external quality control. The quality control was ensured throughout the study period.

2.11 Validity and reliability issues

Few questionnaires were pre-tested to some of the consenting parent(s) or guardians whose children were attending clinics at MNH in order to assess for validity and reliability.

2.12 Data analysis plan

Data was cleaned and coded, before entering into the SPSS version 26. Then it was tested for skewness and kurtosis to check if it followed normal distribution curve. After checking for skewness and kurtosis, 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 sickle cell anemia status.

Qualitative data was reported in terms of frequencies and percentages while quantitative data was reported in terms of median and interquartile range (IQR), and mean ± standard deviation. Categorical data were compared by using chi-square test. Continuous data were compared with independent t-test for parametric data and Mann Whitney test for non-parametric data. P-value of less than 0.05 was considered statistically significant.

2.13 Ethical clearance

Ethical clearance was obtained from MUHAS Institutional Review Board and permission was asked from the MNH administration

2.14 Ethical consideration

An informed consent was obtained from the parent/guardian of a child before recruitment into the study. Also, assent was obtained for children from 7 years and above.

The results of the laboratory investigations were given to the parents/ guardians. For those with deranged values, the attending doctor was informed so as to give proper management. Also, children with sickle cell crises were initiated on hydroxyurea.

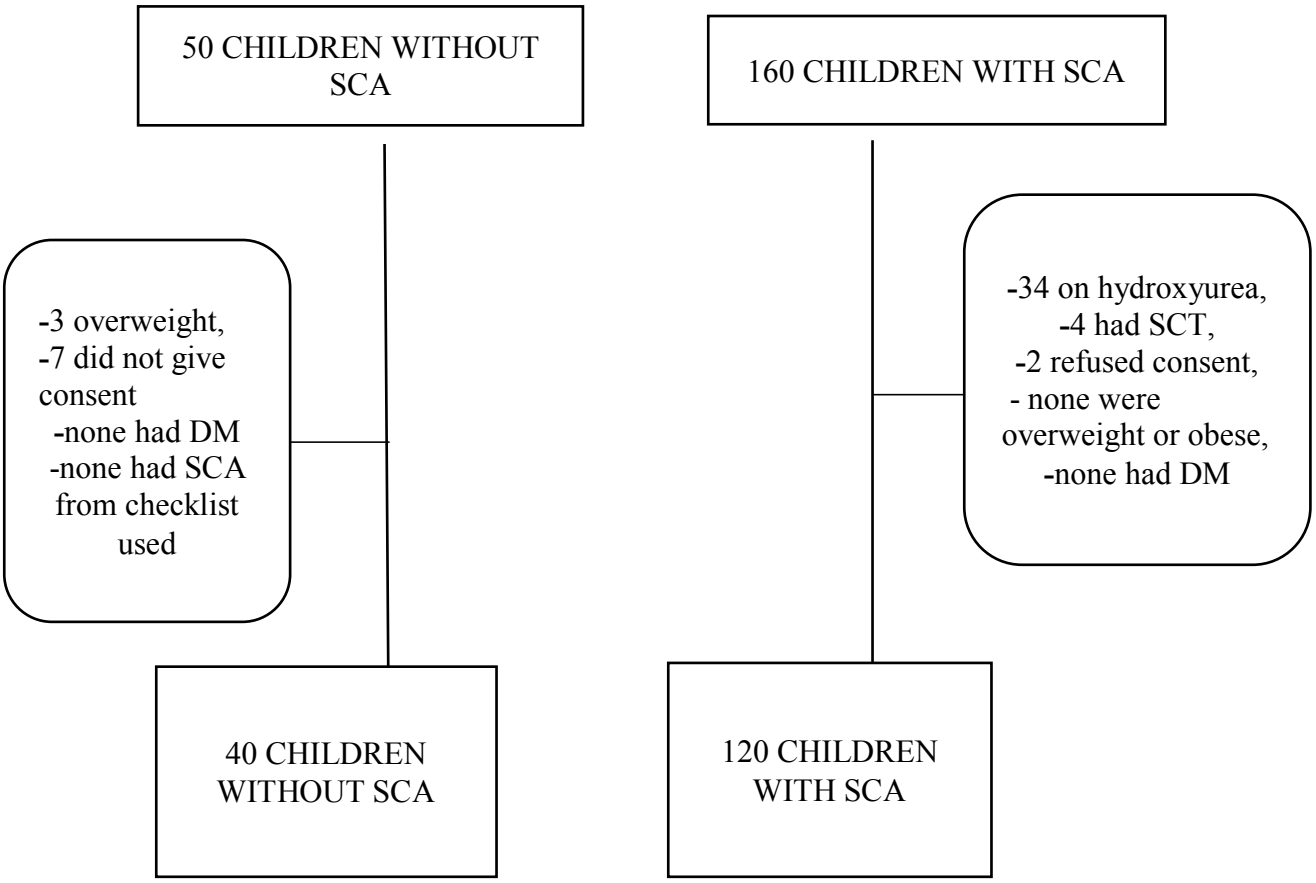
Confidentiality was observed. The children in the study were assigned numbers. No unauthorized person could access the information about children in the study.

CHAPTER THREE

3. RESULTS

A total of 160 children with SCA and 50 children without SCA were enrolled at the baseline. Out of 160 children with SCA, 34 children were excluded because of using hydroxyurea, 4 children were excluded because of having sickle cell trait (HbAS) and 2 children did not give consent hence remaining with 120 children with SCA. Of the 50 children without SCA, 3 children were excluded because of overweight and 7 children did not give consent hence remaining with 40 children without SCA.

Figure 2: Flow Chart showing enrollment of children with SCA and children without SCA



Of the 120 children with SCA, there were 68 males and 52 females. Of the 40 children without SCA, there were 16 males and 24 females.

The median age for children with SCA was 4 years with IOR of (2 to 7) years while for children without SCA was 5 years with IQR of (3 to 8) years.

There were children from different areas of Dar-es-salaam. There were also 16 children with SCA and 4 children without SCA who came from outside Dar-es-salaam.

Mean hemoglobin level was 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 of < 0.001 (Table 1)

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

	Hemoglobin Status		
Variable	**HbSS n (%)**	**HbAA n (%)**	**P – value**
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)	
Mean Hb ± SD (g/dL)	8.26 (± 1.22)	11.55 (± 1.31)	**< 0.001**

Before determining the reference range, data was tested for skewness and kurtosis to see if it followed normal distribution

Table 2: Test for Skewness and Kurtosis

	Statistic (1)	Std. Error (2)	Dividing 1 by 2
Skewness	.110	.221	0.110/0.221= **0.498**
Kurtosis	.754	.438	0.754/0.438= **1.72**

As it is seen in table 2 above, the result for skewness (0.498) was within upper limit of normal distribution which is < 0.5, however, there was excess kurtosis (1.72) as it was above > +1. This indicated that the data were non- parametric.

Normality was further tested by using Kolmogorov-Smirnov and Shapiro-Wilk tests as can be seen in table 3 below

Table 3: Tests of Normality

	Kolmogorov-Smirnov[a] test			Shapiro-Wilk test		
	Statistic	Df	Significa.	Statistic	Df	Significa.
HBA1C %	.117	120	**.000**	.976	120	**.030**

a- Lilliefors Significance Correction

From the above 2 tests in table 3, it was shown that the data did not follow normal distribution since results of Kolmogorov test had **p-value of 0.000** and result of Shapiro- Wilk test had **p-value of 0.03**. Both these p-values were < 0.05 indicating statistical difference from the normal distribution.

Data was again tested for normality by plotting a histogram superimposed with a curve and by a Q-Q plot which also showed non-parametric distribution as seen in figures 2 and 3 below

Figure 3: Histogram superimposed with a curve

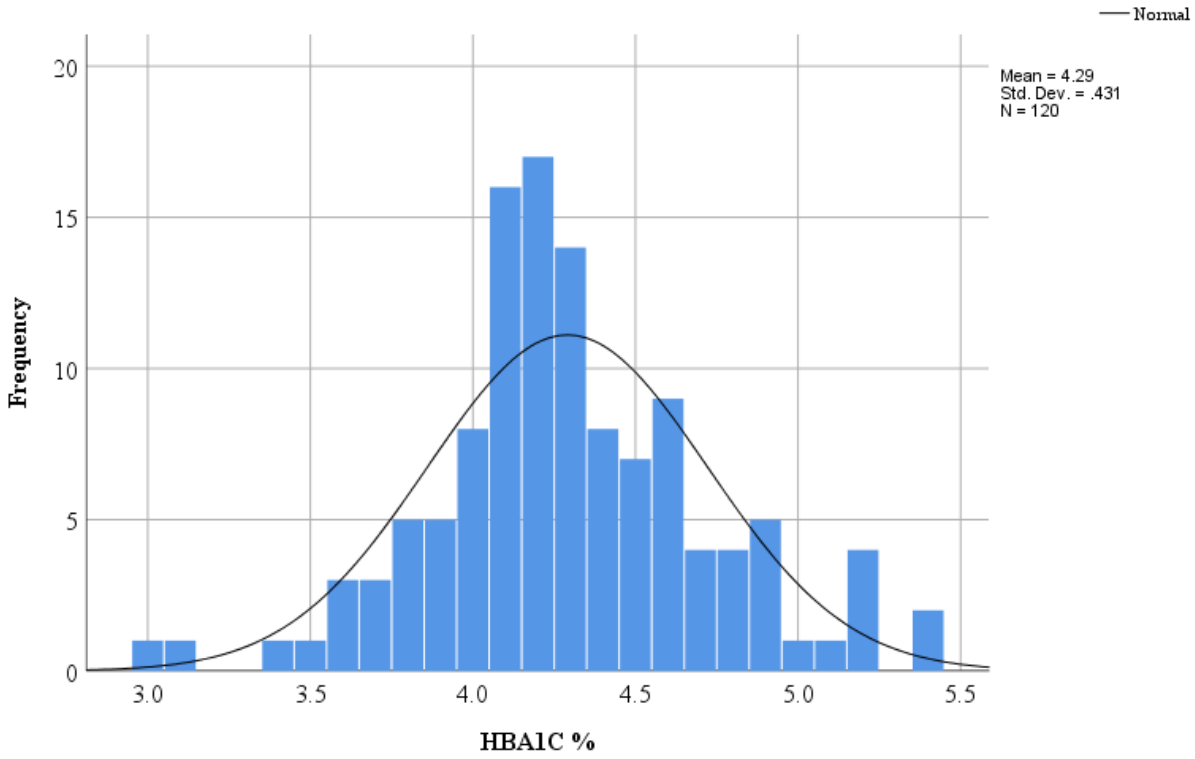
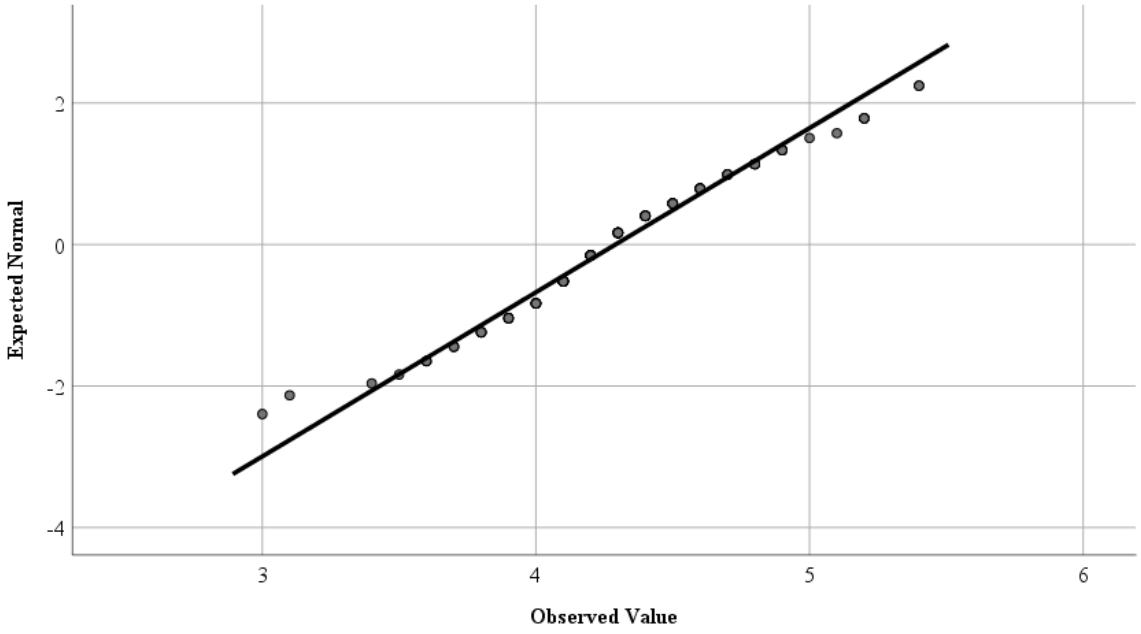
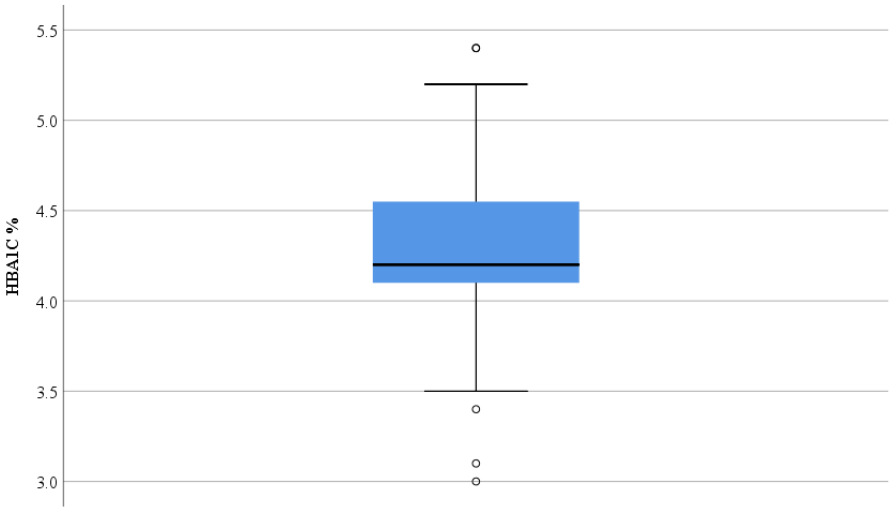


Figure 4: Q-Q plot to check for distribution



Outliers among children with sickle cell anemia were also checked by box-plot test as can be seen from the figure below;

Figure 5: Box plot chart of HbA1c levels in children with sickle cell anemia (HbSS)



HbSS

From the above tests and figures, it was revealed that HbA1c levels were not normally distributed among children with sickle cell anemia. Hence, we calculated the reference range of HbA1c levels in children with SCA by using median and IQR. By using SPSS, the reference range was found to be from **3.4%** to **5.2%** when the data was arranged in ascending order. These values were obtained from the 2.5^{th} percentile to 97.5^{th} percentile

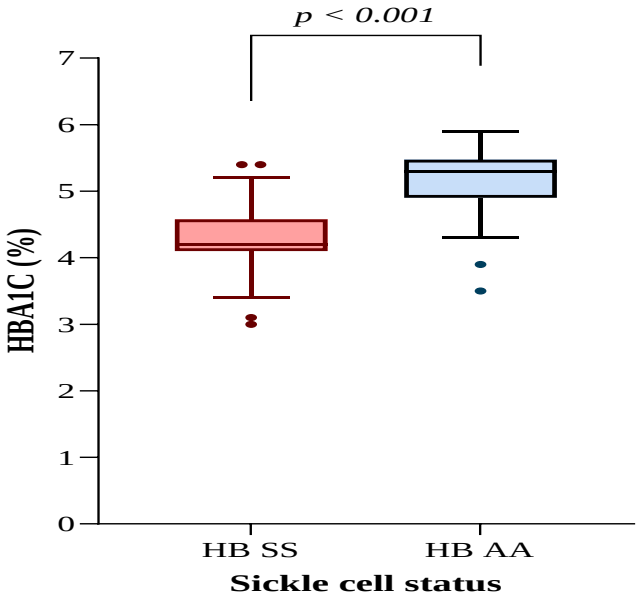
Median HbA1c levels for children with SCA was 4.2% (IQR 4.1% - 4.6%) while median HbA1c levels for children without SCA was 5.3% (IQR 4.9% - 5.5%). This can be seen from table 4 below;

Table 4 : HbA1c values between children with SCA (HbSS) and children without SCA (HbAA)

	HbSS	HbAA
Minimum	3.0%	3.5%
25^{th} percentile	4.1%	4.9%
Median	**4.2%**	**5.3%**
75^{th} percentile	4.6%	5.5%
Maximum	5.4%	5.9%

To compare the median HbA1c levels between children with SCA and those without SCA, Mann Whitney test was used. There was statistical difference between the median HbA1c levels of the children with SCA compared to children without SCA with p-value of < 0.001. This can be seen from the figure 3 below;

Figure 6: Comparison of median HbA1c levels between children with SCA and children without SCA



CHAPTER FOUR

4.1 DISCUSSION OF RESULTS

The reference range of HbA1c levels in children with sickle cell anemia in our study, was found to be from 3.4% to 5.2%. There is limited information about reference ranges of HbA1c levels in children with sickle cell anemia. Most studies were focusing in reference ranges of HbA1c levels in children and adults without sickle cell anemia. For example, in a study by Meredith et al involving healthy children from 11 months to 13 months, the normal range for HbA1c levels was from 4.2% to 5.5% (31). According to the American Diabetes Association, HbA1c levels of 6.5% and above is diagnostic for Diabetes mellitus (Appendix 6). However, from our study, the upper limit of normal HbA1c levels in children with sickle cell anemia was 5.2% which was lower than 6.5%.

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 developed countries. For instance, a study done in Sudan by Atabani et al (22) also revealed lower levels of HbA1c in children with SCA compared to children without SCA. Another study involving African-Americans by Lacy et al (21) also revealed lower levels of HbA1c in patients with SCT compared to those without SCT.

As explained earlier, there are two possibilities that have been mentioned in previous studies as to why children with SCA appeared to have lower HbA1c levels compared to children without SCA. First possibility is the different methods used 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. This means using Immunoassay method in children with SCA, was more likely to give lower levels of HbA1c compared to the actual levels in a given child. In our study, we used the immunoassay method due to its wide availability in our setting.

The second possibility was the reduced lifespan of red blood cells in children with SCA. It is known that the lifespan of normal red blood cells is about 120 days compared to lifespan of sickled red blood cells which is about 10 to 20 days. This means with decreased lifespan of red

blood cells, there is less time for glycosylation process of the sickled red blood cells resulting in lower levels of HbA1c among children with SCA(21).

Tanzania is among the top 5 countries globally with highest prevalence of SCA (10). Reviews of the sickle cell program in Tanzania, revealed that there was great improvement in the management of children with SCA (10,14) which has contributed to decrease in their morbidity and mortality. However, with increased lifespan in SCA children, there is also increased risk of developing various endocrinopathies such as Diabetes mellitus by Mandese et al (15). Therefore, there is a need for regular screening of these endocrinopathies as more children with sickle cell anemia are now able to live to their adulthood. The increase in patients of Diabetes mellitus has also been shown among children without sickle cell anemia according to Muze et al (32) in Tanzania.

The use of HbA1c levels in diagnosing and monitoring Diabetes mellitus is common worldwide. However, the challenge of using HbA1c test in children with SCA is the seemingly lower levels of HbA1c in children with SCA compared to children without SCA. This was also seen in our study whereby the median HbA1c levels in children with sickle cell anemia was lower compared to children without sickle cell anemia. The lower levels of HbA1c in SCA children can be misleading when using normal ranges for children without SCA giving the impression of no diabetes mellitus.

It is therefore important to remember this decrease in HbA1c levels, during screening and monitoring for Diabetes mellitus in children with SCA. Improper interpretation of HbA1c levels in children with SCA, could group some of the diabetic children into non-diabetic group. This can delay their diagnosis of Diabetes mellitus leading to poor control of their diabetic state and adding more comorbidity to their lives. Poor control of Diabetes mellitus is a common problem even in children without SCA. This was shown by Nooran et al in their study involving Tanzanian children (33).

The findings of this study are different from a study by Bleyer et al (24), whereby it was found out that presence of sickled hemoglobin in patients with sickle cell trait did not have impact on the levels of HbA1c. In their study, changes in HbA1c levels, were attributed to the method used

to measure HbA1c level rather than the decreased lifespan of sickled red blood cells. However, in our settings, there is wide use of immunoassay method in measuring HbA1c levels which has lower capacity to detect other hemoglobin variants. This is probably because other methods are more expensive and hence not easily affordable. Therefore, there is still a need of careful interpretation of HbA1c levels when handling children with SCA in our settings due to wide use of Immunoassay methods.

As mentioned above, the use of HbA1c ranges which are normal for people without sickle cell hemoglobinopathy, to diagnose and monitor Diabetes mellitus in children with SCA, can be misleading. This is especially when the method employed to measure HbA1c levels is affected by hemoglobin variants such as the immunoassay methods commonly found in our settings.

The inability to detect these hemoglobin variants may lead to lower levels of HbA1c than it should be hence delaying diagnosis and required intervention. Such interventions include various preventive measures for children in pre-diabetic stage and treatment for children already in diabetic stage. Delay in any of these interventions could result in increased cost of managing Diabetes mellitus because of failure to prevent or control it in earlier stages. Several complications can occur when there is delay in diagnosis or poor control of Diabetes mellitus such as retinopathy and neuropathy as shown by Majaliwa et al (34). These complications have already been seen in adults with sickle cell anemia (18). It is therefore important to have proper means of screening for Diabetes mellitus in children with SCA so as to decrease risk of complications during their adulthood.

4.2 STUDY STRENGTH AND LIMITATIONS

4.2.1 Study Strength

It gives highlight about the lower HbA1c levels in children with sickle cell anemia compared to children without sickle cell anemia especially when using Immunoassay method.

It also provides reference range of HbA1c levels which can be used to screen for Diabetes mellitus in children with sickle cell anemia.

4.2.2 Study Limitations

The use of Immunoassay method to measure HbA1c levels in children with sickle cell anemia could have accounted for lower levels of HbA1c.

The use of a checklist to screen for children with normal hemoglobin (HbAA) could have included some of the asymptomatic children with sickle cell trait as well as asymptomatic children with sickle cell anemia.

The use of simple glucometer to exclude children with Diabetes mellitus could have missed children with asymptomatic disease.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATIONS

5.1 STUDY CONCLUSION

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

Children with sickle cell anemia had lower HbA1c levels compared to children without sickle cell anemia.

5.2 STUDY RECOMMENDATION

Health personnel are advised to use HbA1c reference ranges obtained from this study when screening for Diabetes mellitus in children with sickle cell anemia.

Further studies should be done to establish reference range of HbA1c levels in non-diabetic children with sickle cell anemia on hydroxyurea.

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APPENDICES

APPENDIX 1- SWAHILI VERSION OF ABSTRACT

Muhtasari

Kumekuwa na maendeleo makubwa katika matibabu ya watoto wenye seli-mundu, ambayo yamewafanya kuishi umri mrefu zaidi kulinganisha na uko nyuma. Kuishi huku kwingi, kumewafanya wakabiliwe na magonjwa sugu kama kisukari ambayo uko nyuma hawakuwa wakisumbuliwa nayo.

Tokea Januari 2010, Jumuiya ya Kisukari ya Amerika, imekuwa ikihamasisha utumiaji wa kipimo cha Glycosylated Hemoglobin (HbA1c) katika upimaji wa ugonjwa wa Kisukari. Hata hivyo, tafiti mbalimbali zilizofanyika, zimeonyesha kuwa kuna walakini kwenye matumizi ya kipimo hiki cha HbA1c kwa watoto wenye seli-mundu. Na hii ni kutokana na viwango vidogo vya HbA1c ambavyo vimekuwa vikionekana kwa watoto wenye seli-mundu kulinganisha na watoto wasio na seli-mundu.

Kuna umuhimu wa kujua viwango halisi vya HbA1c vinavyoendana na watoto wenye seli-mundu.

Hii itawezesha ugunduaji wa ugonjwa wa kisukari kwa watoto wenye seli-mundu kwa urahisi zaidi, na hivyo kuepuka ucheleweshwaji wa matibabu ya Kisukari.

Lengo la utafiti huu

Lilikuwa kutafuta viwango halisi vya HbA1c kwa watoto wenye seli-mundu, ambao hawajapata Kisukari bado, wanaohudhuria kliniki katika Hospitali ya Taifa ya Muhimbili.

Mbinu

Utafiti huu ulifanyika katika kliniki za watoto ndani ya Hospitali ya Taifa ya Muhimbili, na ulihusisha watoto kuanzia miezi 9 mpaka miaka 14. Jumla ya watoto 120 wenye seli-mundu na watoto 40 wasio na seli-mundu walihusishwa kwenye utafiti huu. Data ziliripotiwa kama wastani na midiani. Uchambuzi wa data ulifanyika kwa kutumia “independent t-test” na “Mann Whitney test”

Matokeo ya utafiti

Umri wa kati (midiani) wa watoto wenye seli-mundu ulikuwa ni miaka 4 na watoto wasio na seli-mundu ilikuwa ni miaka 5. Viwango vya HbA1c kwa watoto wenye seli-mundu vilianzia asilimia 3.4 mpaka asilimia 5.2, Pia viwango vya HbA1c vilikuwa vidogo kwa wastani kwa watoto wenye seli-mundu (asilimia 4.2) kulinganisha na watoto wasio na seli-mundu (asilimia 5.3).

Hitimisho na Mapendekezo

Viwango halisi vya HbA1c kwa watoto wenye seli-mundu vilianzia asilimia 3.4 mpaka asilimia 5.2, Pia viwango hivi vilikuwa ni vidogo kwa watoto wenye seli-mundu kulinganisha na watoto wasio na seli-mundu.

Wataalamu wa afya wanashauriwa kutumia viwango vya HbA1c vilivyopatikana katika utafiti huu wakati wanapopima ugonjwa wa Kisukari kwa watoto wenye seli-mundu.

APPENDIX 2A: ENGLISH VERSION OF A QUESTIONNAIRE

GLYCOSYLATED HEMOGLOBIN LEVELS AMONG NON-DIABETIC CHILDREN WITH SICKLE CELL ANEMIA AT MUHIMBILI NATIONAL HOSPITAL. A CASE FOR ESTABLISHING NORMAL VALUES.

1. Questionnaire number ……..

2. Identification number…… ……...

3. Date of interview……..

A. CHILD'S BACKGROUND INFORMATION

4. Date of birth ……..……..

5. Sex a) Male

b) Female

6. Physical address (district/region) ……

B. MEDICAL HISTORY

7. SCA status

a) HbSS

b) HbAA

8. Age at diagnosis of SCA……

9. If the child has SCA, has he/she received blood transfusion?

a) Yes

b) No

10. When was the last blood transfusion?

a) 3 months or less

b) More than 3 months

C. INVESTIGATIONS

11. Hemoglobin levels……..

12. HbA1c levels…….

Thank you for participating

APPENDIX 2B: SWAHILI VERSION OF A QUESTIONNAIRE

UTAFITI KUHUSU VIWANGO VYA GLYCOSYLATED HEMOGLOBINI KWA WATOTO WENYE SELI MUNDU WASIO NA KISUKARI KATIKA HOSPITALI YA TAIFA YA MUHIMBILI.

1. Namba ya dodoso …….

2. Namba ya mshiriki…….

3. Tarehe ya kushiriki …….

A. HISTORIA YA MTOTO

4. Tarehe ya kuzaliwa…..……..

5. Jinsia a) kiume

b) kike

6. Sehemu munayoishi (wilaya/mkoa) ……

B. HISTORIA YA UGONJWA

7. Je mtoto wako ana ugonjwa wa seli-mundu?

a) ndio (HbSS)

b) hapana (HbAA)

8. Ugonjwa wa seli-mundu ulijulikana akiwa na umri gani? ……

9. Ikiwa mtoto ana seli-mundu, amewahi kuongezewa damu? (kama 'hapana' ruka swali la 10)

a) ndio

b) hapana

10. Mara ya mwisho kuongezewa damu ilikuwa lini?

a) miezi 3 au chini ya miezi 3

b) zaidi ya miezi 3

C) VIPIMO VYA MAABARA

11. Wingi wa damu…….

12. Wingi wa HbA1c……..

Nashukuru kwa ushiriki wako

APPENDIX 3A: ENGLISH VERSION OF CONSENT FORM

CONSENT FORM FOR PARENTS/GUARDIANS OF CHILDREN FROM 9 MONTHS TO 14 YEARS ATTENDING CLINICS AT MUHIMBILI NATIONAL HOSPITAL

Introduction

Greetings! I am Dr Nancy Mugyabuso. I am doing my masters in Paediatrics and Child health at Muhimbili University of Health and Allied sciences. I am conducting a research to determine normal levels of glycosylated hemoglobin among non-diabetic children with sickle cell anemia attending clinics at Muhimbili National Hospital

Purpose of the Study

The aim of this study is to determine normal levels of glycosylated hemoglobin among non-diabetic children with sickle cell anemia attending clinics at Muhimbili National Hospital aged from 9 months to 14 years

What will be done in this study?

If you allow your child to participate this study, you will be interviewed and then 3 ml of blood will be drawn from your child for the following investigations;

1. Glycosylated hemoglobin levels
2. Hemoglobin levels.

Who can participate in this study?

Children attending clinics at Muhimbili National Hospital including those with Sickle Cell Anemia aged from 9 months to 14 years

Is there any benefit to my child participating in this study?

Your participation in this study will help us to determine normal levels of glycosylated hemoglobin among non-diabetic children with sickle cell anemia attending clinics at Muhimbili National Hospital. This will help us to be able to detect Diabetes mellitus at earlier stages in this population. As for you, you will be able to know glycosylated hemoglobin levels of your child.

Is there any risk to me or my child for participating in this study?

Drawing blood sample may be painful to your child. To minimize this, we will be as gentle as possible when drawing blood sample. Also, more blood sample may be taken if your doctor in the clinic has requested other investigations, so as to avoid repeated pricks.

Right to withdraw

Involvement in this study is totally voluntarily. You can withdraw from the study at any time, even if you have already signed your consent. Refusal to participate or withdrawal from the study will not prevent your child from receiving any form of management entitled to him or her.

Confidentiality

Confidentiality of the information collected during the research is assured. All the information collected in this questionnaire forms will be entered in the computer with only the study identification number.

I have read the contents of this consent form and understood them. All my questions have been answered. I also understand that I and my child can withdraw from this study at any time and this will not interfere with my child's treatment here at MNH. I therefore agree me and my child to participate in this study.

NAME OF THE PARENT/GUARDIAN…….

Signature of the Parent/Guardian ……. Date ……

NAME OF THE INTERVIEWER…….

Signature of the Interviewer ……. Date ……

For children from 7 years and above

Hallo, I am resident in Paediatrics. I request to draw your blood sample for my research. This blood sample will also be used for other investigations ordered by your doctor today to avoid multiple pricks. I will try to be as gentle as possible. It is okay if you do not want. Thank you

NAME OF THE CHILD……. SIGN……

NAME OF THE INTERVIEWER……. Date……..

In case of any queries about this study, you can contact the researcher;

-Dr. Nancy Mugyabuso of Muhimbili University of Health and Allied Sciences, P. O. Box 65001, Dar es Salaam, mobile no. 0769 101401. Email address nancymugie@gmail.com or

-Dr. Bruno Sunguya Director of Research and Publications at Muhimbili University of Health and Allied Sciences (MUHAS). Telephone: + 255 22 2152489 email:drp@muhas.ac.tz.

APPENDIX 3B: SWAHILI VERSION OF CONSENT FORM

FOMU YA MARIDHIANO KWA WAZAZI/WALEZI WA WATOTO WENYE UMRI WA MIEZI 9 MPAKA MIAKA 14 WANAOHUDHURIA KLINIKI ZA WATOTO KATIKA HOSPITALI YA TAIFA YA MUHIMBILI

Utambulisho

Habari! Naitwa Dr. Nancy Mugyabuso. Ni mwanafunzi wa shahada ya uzamili wa udaktari wa watoto katika Chuo Kikuu cha Sayansi na Tiba Shirikishi cha Muhimbili. Ninafanya utafiti kuhusu viwango halisi vya Glycosylated Hemoglobini kwa watoto wenye seli-mundu

Lengo la utafiti

Lengo la utafiti huu ni kujua viwango vya Glycosylated Hemoglobini kwa watoto wenye seli-mundu katika Hospitali ya Taifa ya Muhimbili kwa watoto wenye umri kuanzia miezi 9 mpaka miaka 14.

Nini kinafanyika katika utafiti huu;

Ikiwa utamruhusu mwanao kushiriki katika utafiti huu, utahojiwa kuhusiana na mwanao. Halafu kiasi cha damu kadiri ya mililita tatu kitatolewa kutoka kwa mwanao kwa ajili ya kupima

1. Wingi wa Glycosylated Hemoglobini
2. Wingi wa damu

Nani anaweza kushiriki kwenye utafiti huu?

Watoto wanaohudhuria kliniki za wagonjwa wa nje katika hospitali ya taifa ya Muhimbili ikiwa ni pamoja na wale wenye seli mundu wenye umri kuanzia miezi 9 mpaka miaka 14.

Kuna faida yoyote kwa mtoto wangu kushiriki katika utafiti huu?

Ushiriki wa mwanao katika utafiti huu wa kujua viwango vya Glycosylated Hemoglobini kwa watoto wenye seli-mundu ni wa muhimu kwa sababu utatusaidia kujua viwango vya kawaida vya Glycosylated Hemoglobin kwa watoto wenye seli-mundu. Lakini pia, na wewe utaweza kujua kiwango cha glycosylated hemoglobin cha mwanao.

Je kuna madhara yoyote kwa mtoto wangu kwa kushiriki katika utafifiti huu? Kipimo cha damu kinaweza kuwa na maumivu kidogo kwa mwanao. Ila tutajitahidi kuwa makini zaidi wakati wa kuchukua damu ya vipimo hivi ili kupunguza maumivu hayo. Ikiwa daktari wako wa leo ameandikia vipimo vingine vya damu, mtoto wako atatolewa damu ya

kutosha kwa ajili ya hivyo vipimo vingine pia. Hii itasaidia kupunguza kumtoboa na sindano mwanao mara nyingi.

Ushiriki ni wa hiari;

Ushiriki wako katika utafiti huu ni wa hiari kabisa. Unaweza kujiondoa kwenye utafiti huu muda wowote hata kama utakuwa umeshasaini kukubali, bila matatizo yoyote. Kutoshiriki au kujiondoa kwako kwenye utafiti huu hakutakuathiri kwa namna yoyote ile katika matibabu ya mwanao.

Kutatokea nini kwenye taarifa itakayokusanywa?

Unahakikishiwa usiri wa taarifa za mwanao katika utafiti huu. Aidha taarifa zote kuhusu mwanao zitaingizwa katika kompyuta kwa kupitia namba maalum ambazo atapewa kila mshiriki.

Nimesoma na kuelewa vizuri kuhusu utafiti huu na maswali yangu yote yamejibiwa kwa ufasaha. Nimekubali kwa ridhaa yangu kushiriki katika utafiti huu mimi na mwanangu. Naelewa kwamba mimi na mtoto wangu tuna uhuru wa kujitoa kwenye utafiti huu muda wowote na uamuzi huu hautaathiri matibabu ya mtoto wangu katika hospitali hii.

JINA LA MSHIRIKI…….....**..**

SAHIHI YA MSHIRIKI……tarehe……

JINA LA SHAHIDI …….

SAHIHI YA SHAHIDI ……..tarehe……

-Kwa watoto wenye umri wa miaka 7 na zaidi

Habari, mimi ni mwanafunzi wa udaktari. Naomba nikutoe kipimo cha damu kwa ajili ya kupima wingi wako wa damu pamoja na sukari. Damu hii itatumika pia kupima vipimo ulivyoandikiwa na daktari wako kliniki ya leo. Hii ni kukusaidia usichomwe mara nyingi. Vipimo hivi vitanisaidia kwenye utafiti wangu. Kama usipokubali, hautalazimishwa kutolewa vipimo. Asante JINA LA MSHIRIKI ……SAHIHI…… JINA LA SHAHIDI……tarehe……..

Kama una wasiwasi kuhusiana na jinsi utafiti huu unavyoendeshwa usisite kuwasiliana na; -Dr Nancy Mugyabuso, Chuo Kikuu cha Sayansi ya Afya na Tiba Shirikishi Muhimbili S.L.P. 65001, Dar-es-salaam. Namba ya simu 0769 101401. barua pepe

nancymugie@gmail.com, au

-Dr Bruno Sunguya, Mkurugenzi wa kamati ya utafiti na uchapishaji

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APPENDIX 4A: ENGLISH VERSION OF THE CHECKLIST FOR 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.

APPENDIX 4B: SWAHILI VERSION OF THE CHECKLIST FOR SCREENING CHILDREN WITHOUT SICKLE CELL ANEMIA

A. HISTORIA YA UGONJWA WA MTOTO

1. Umri wa mtoto…….

2. Je, mtoto alishawahi kuongezewa damu?

a) Ndio

b) Hapana

3. Je mtoto amekuwa akipata maumivu ya kwenye mifupa (kwenye mikono na miguu)

a) Ndio

b) Hapana

4. Je, mtoto amekuwa akipata maumivu ya mara kwa mara, ambayo hayatulii hata akitumia dawa ya Panadol au Ibuprofen?

a) Ndio

b) Hapana

5. Je mtoto amekuwa akivimba vidole vya mikono na miguu vinavyoambatana na maumivu?

a) Ndio

b) Hapana

6. Je mtoto alishawahi kulalamikia maumivu kwenye upande wa juu kushoto kwenye tumbo (bandama)?

a) Ndio

b) Hapana

7. Je mtoto alishawahi kuwa na rangi ya manjano kwenye macho?

a) Ndio

b) Hapana

B. HISTORIA YA UGONJWA KATIKA FAMILIA YA MTOTO

8. Kuna mtu yoyote kwenye familia mwenye ugonjwa wa seli-mundu?

a) Ndio

b) Hapana

9. Je kuna ndugu yoyote wa kuzaliwa pamoja na mtoto ambaye ana historia ya kuongezewa damu mara kwa mara au kupata maumivu ya mifupa mara kwa mara?

a) Ndio

b) Hapana

MAANA YAKE

Endapo jibu lolote, kwa maswali ya hapo juu litakuwa ndio, inaashiria uwezekano mkubwa wa kuwa na seli-mundu, hivyo kutowekwa kwenye kundi la watoto wasio na seli-mundu.

APPENDIX 5: EXCLUSION AND INCLUSION CRITERIAS

5A. EXCLUSION CRITERIAS

5.A.1. Hydroxyurea

Hydroxyurea, despite being the recommended standard of care, in children with sickle cell anemia was excluded in this study since it can also interfere with the interpretation of HbA1c levels. It works by increasing production of fetal hemoglobin (HbF) over sickled hemoglobin (HbS) and adult hemoglobin (HbA). With less HbA to combine with glucose, there is less formation of HbA1c levels resulting in false low levels of HbA1c(35). Children on hydroxyurea were also excluded because of the limited sample size.

5.A.2. Overweight and Obesity

Nutrition has been shown to affect HbA1c levels. Both overweight and obesity have been associated with elevated levels of HbA1c as evidenced in a study by Bae et al 2016 (36).

5B. INCLUSION CRITERIAS

5.B.1. Age group (9 months – 14 years)

This age group was chosen for the convenience of the study. This is because Pediatric clinics at MNH attends children up to 14 years. Those above 14 years are shifted to adult clinics. And although sickle cell anemia can be detected during newborn screening, it is not a routine test in our setting. We therefore enrolled children from 9 months as most children begin to show symptoms after the age of 6 months (37).

APPENDIX 6- INTERFERENCE OF HEMOGLOBIN VARIANTS

Interference of Heterozygous Variants S, C, D, E, and Elevated HbF with Specific HbA1c Methods

Manufacturer	Method	Interference from				
		HbAS	HbAC	HbAE	HbAD	↑ HbF
Immunoassay						
Abbott	Architect/Aeroset	Yes ↑	Yes ↑	–[b]	–[b]	–[b]
Bayer (Metrika)	A1cNOW	Yes ↑	Yes ↑	No	No	–[b]
Beckman	Synchron System	No	No	No	No	–[b]
Dade	Dimension	No	No	No	No	–[b]
Olympus	AU system	Yes ↑	Yes ↑	No	No	–[b]
Ortho-Clinical	Vitros	No	No	No	No	–[b]
Point Scientific	HbA1c on Modular P	No	No	No	No	–[b]
Roche	Cobas Integra	Yes ↑	Yes ↑	–[b]	–[b]	–[b]
Roche	Cobas Integra Gen.2 (Tina Quant)	No	No	No	No	–[b]
Roche/Hitachi	Hitachi (Tina Quant)	No	No	No	No	–[b]
Siemens (Bayer)	Advia	Yes ↑	Yes ↑	–[b]	–[b]	–[b]
Siemens (Bayer)	DCA 2000	No	No	No	No	Yes[c]

Adapted from Little et al in Journal of Diabetes Science and Technology 2009

Interference of Heterozygous Variants S, C, D, E, and Elevated HbF with Specific HbA1c Methods (cont)

Manufacturer	Method	Interference from				
		HbAS	HbAC	HbAE	HbAD	↑ HbF
Ion-exchange HPLC						
Bio-Rad	D-10 (short)	No	No	No	No	–[b]
Bio-Rad	D-10 (extended)	No	No	No	No	–[b]
Bio-Rad	Variant A1c	No	No	No	Yes ↓	–[b]
Bio-Rad	Variant II A1c	No	No	No	No	No
Bio-Rad	Variant II Turbo A1c	No	No	Yes ↑	Yes ↑	–[b]
Menarini	HA8140 (diabetes mode)	Yes ↑	No	–[b]	–[b]	–[b]
Menarini	HA8160 (diabetes mode)	No	No	Yes ↓	Yes ↓	–[b]
Menarini	HA8160 (TP mode)	No	No	No	Not quantified	–[b]
Tosoh	A1c 2.2 Plus	No	No	Yes ↓	No	Yes[c]
Tosoh	G7	No	No	Yes ↓	No	No[d]
Tosoh	G8	–[b]	–[b]	Yes ↓	No	–[b]
Boronate affinity						
Axis-Shield	Afinion	No	No	No	No	–[b]
Primus	Boronate affinity HPLC	No	No	No	No	Yes[c]
Other						
Diazyme	Direct enzymatic A1c	No	No	No	No	–[b]

Adapted from Little et al in Journal of Diabetes Science and Technology 2009

APPENDIX 7: STANDARD HBA1C LEVELS ACCORDING TO AMERICAN DIABETES ASSOCIATION

With current assays, an HbA1c of less than 5.7% is considered **normal,** and an HbA1c equal or greater than 6.5% is considered **diagnostic for diabetes mellitus.** HbA1c levels from 5.7% to < 6.5% are diagnostic for **prediabetes**

HbA1c Test Results