Glycosylated Hemoglobin Levels Among Non-Diabetic Children With Sickle Cell Anemia At Muhimbili National Hospital. A Case For Establishing Normal Values

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Research Article

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Abstract

Background

Improvement in the management of children with SCA, has led to increase in their lifespan. However, the increased lifespan, has been accompanied with emergence of various endocrinopathies such as Diabetes mellitus (DM).

Glycosylated Hemoglobin (HbA1c) levels have been found to be lower in children with SCA compared to children without SCA in various studies. This can lead to missed children with DM hence delaying their treatment. There is a need to establish normal ranges of HbA1c levels corresponding to children with SCA.

Objective

To determine normal range of HbA1c levels among non-diabetic children with SCA 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. The study was approved by Institutional review Board of MUHAS. A written informed consent was obtained.120 children with SCA and 40 children without SCA were recruited. Data was reported as median and IQR or as mean ± standard deviation. Chi-square was used for categorical data while independent t-test and Mann Whitney test were used for 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% with IQR of (4.1% - 4.6%) while for children without SCA median HbA1c levels was 5.3% with IQR of (4.9% - 5.5%). The median HbA1c level was significantly lower in children with SCA than children without SCA.

Conclusion and Recommendation

The reference range of HbA1c levels in children with SCA was from 3.4% to 5.2%. Children with SCA had 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 in children with SCA.

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 ≥ 11.1mmol/l

Introduction

There has been improvement in the quality of care delivered to children with sickle cell anemia (SCA) which has led to increase in their lifespan (1,2). This has led to the emergency of various endocrinopathies which were not obvious in the past such as Diabetes mellitus (DM) (3,4). Fructosamine levels are used in other countries for measuring long term control of DM in children with SCA instead of HbA1c, but this method is not available in our country so we still rely on HbA1c regardless of Sickle cell status.

HbA1c levels is among the tests used worldwide to diagnose and monitor for 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 erythrocytes due to continuous hemolysis (hemolytic anemia) found in sickle cell anemia disease (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 cause missed opportunity for interventions. 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 non-diabetic children with SCA attending sickle cell clinic at Muhimbili National Hospital. Also, to compare HbA1c levels between non-diabetic children **with** SCA and non-diabetic 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. Institutional Ethics review Board of Muhimbili Univesity of Health and allied sciences provided the ethical clearance. 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 provided written informed consent.. Exclusion criteria were children with known Diabetes Mellitus, or who were overweight or obese. Children on hydroxyurea were also excluded.

Sample size was estimated to be 120 children with SCA and 40 children without SCA by using the formula of comparison of two means. Study variables were HbA1c levels as dependent variable and SCA status as independent variable

Data was collected by the researcher and the assistant using structured questionnaires. The questionnaire had 2 main parts; first part which was completed through an interview and the second part which included results of laboratory investigations.

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. 3 milliliters of blood were drawn from that area of which 2 milliliters 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 INTEGRA MACHINE which uses **immunoassay method**. Hemoglobin levels were measured by using Abbott CEL-DYN RUBY hematology machine.

Data was analyzed using SPSS version 26. First, it was tested for skewness and kurtosis to check if it followed 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.

Data was reported as 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.

Consent for publication: being an academic institution, this was NOT APPLICABLE

Availability of Data and Materials : All the data and materials are available and can be obtained from the corresponding author .

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 SCT 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.

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 < 0.001 as shown in 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 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 both their 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;

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

From the above tests and figures, it was clear that HbA1c levels among children with sickle cell anemia were not normally distributed. Hence, the reference range of HbA1c levels in children with SCA was calculated by using median and IQR. By using SPSS, the reference range was found to be from **3.4%** to **5.2%**

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%
25th percentile	4.1%	4.9%
Median	**4.2%**	**5.3%**
75th 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 5 below;

Discussion

The reference range of HbA1c levels for children with SCA was 3.4% to 5.2%. According to the American Diabetes Association, HbA1c levels of 6.5% and above is required to diagnose Diabetes mellitus. But from our study, the upper limit of normal HbA1c levels in children with SCA was 5.2% which was lower than 6.5%. This means, there are some children with SCA that can be missed if we wait to reach HbA1c level of 6.5% to be able to diagnose DM.

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 compared to children without SCA. Another study involving adults African-Americans by Lacy et al (6) also revealed lower levels of HbA1c in patients with SCT compared to those without SCT.

As explained earlier, 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 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). 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. In our study, we used the immunoassay method due to its wide availability in our setting.

The second possibility is 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 that, 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 (6).

In a study by Bleyer et al (11), it was found out that presence of sickled hemoglobin in patients with SCT 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. 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. Hence, there is a need of using the reference range of HbA1c levels corresponding to children with SCA like the one obtained in our study.

Tanzania is one of the countries globally with highest prevalence of SCA (1). However, with improved quality of life in SCA children together with prolonged lifespan, they have increased risk of developing various endocrinopathies like DM (3). Therefore, there is a need for regular screening of these endocrinopathies as more children with SCA are now able to live to their adulthood. The increased trend in children with DM has also been observed among children without SCA (12).

It is important to consider sickle cell status, when screening and monitoring for DM with HbA1c test especially if using immunoassay methods. Improper interpretation of HbA1c levels, could group some of the SCA children with DM into non-diabetic group. This can delay their initiation of 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 also cause various complications. Such complications include retinopathy and neuropathy (14). These complications are now seen even in people with sickle cell anemia especially the adults (15). 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.

The **strength** of this study is that It gives highlight about the lower HbA1c levels in children with SCA compared to children without SCA 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. **Limitations** in this study was 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. We **recommend** health personnel 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 who are also on hydroxyurea.

Conclusion

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

Abbreviations

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

Declarations

CONFLICT OF INTEREST: None

Ethics and Consent to participate

Ethical clearance was obtained from MUHAS Institutional Review Board which allowed to administer the written informed consent to the parents of the child or the care-taker. The informed consent process and enrollment followed the Helsinki Guidelines . The parents had the autonomy to participate and understood the process. Consent for investigations and use of data for research was clearly mentioned . Permission to use the clinical data, patient records and laboratory results was granted by the Muhimbili National Hospital. They were used in a de-identified manner and strict confidentiality observed. The copies of the approvals are available on request.

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Contributions: NM : developed the concept , undertook clinical assessments and field activity, cleaned data, analysed and prepared the manuscript.

KCM : reviewed and supervised the protocol , participated in field and oversight and contributed to the manuscript.

KPM : reviewed the Concept, Proposal, Supervised field activity, initiated the first draft of manuscript , and reviewed the final draft to completion.

All authors have read and approved the manuscript

References

1. Makani J, Tluway F, Makubi A, Soka D, Nkya S, Sangeda R, et al. A ten year review of the sickle cell program in Muhimbili National Hospital, Tanzania. BMC Hematol. 2018;18(33):1–13.
2. Tluway F, Makani J. Sickle cell disease in Africa: An overview of the integrated approach to health, research, education and advocacy in Tanzania, 2004-2016. British Journal of Hematology. 2017.
3. Mandese V, Bigi E, Bruzzi P, Palazzi G, Predieri B, Lucaccioni L, et al. Endocrine and metabolic complications in children and adolescents with Sickle Cell Disease: an Italian cohort study. BMC Pediatr. 2019;1–9
4. Smiley D, Dagogo-Jack S UG. Therapy insights: Metabolic and Endocrine complications in sickle cell disease. Nat Clin Pract Endocrinol Metab. 2008;4(2):102–9
5. Gunton JE. Hemoglobinopathies and HbA1C Measurement. Diabetes Care. 2000;23(8):1197–212.
6. Lacy ME, Wellenius GA, Sumner AE, Correa A, Carnethon MR, Liem RI, et al. Association of sickle cell trait with hemoglobin A1c in African Americans. J Am Med Assoc. 2017;317(5):507–15.
7. Little RR, Roberts WL. A Review of Variant Hemoglobins Interfering with Hemoglobin A1c Measurement. J Diabetes Sci Technol. 2009;3(3):446–51.
8. Schnedl WJ, Krause R, Halwachs-Baumann G, Trinker M, Lipp RW KG. Evaluation of HbA1c determination methods in patients with hemoglobinopathies. Diabetes Care. 2000;23(3):339–44.
9. Radin MS. Pitfalls in Hemoglobin A1c Measurement: When Results may be Misleading. J Gen Intern Med. 2013;29(2):388–94.
10. Atabani GS, Hassan DA, Rahman AMA, Saeed BO. Glycosylated Hemoglobin Levels in Sudanese Sickle Cell Anemia Patients. Acta Haematol. 1989;81:140–2.
11. Bleyer AJ, Vidya S, Sujata L, Russell GB, Akinnifesi D, Hire D, et al. The impact of sickle cell trait on glycated haemoglobin in diabetes mellitus. Diabet Med. 2010;27(9):1012–6.
12. Muze KC, Majaliwa ES. Brief Communication Type 1 diabetes care updates: Tanzania. Vol. 19. 2015.
13. Noorani M, Ramaiya K, Manji K. Glycaemic control in type 1 diabetes mellitus among children and adolescents in a resource limited setting in Dar es Salaam. BMC Endocr Disord. 2016;16(1):1–8.
14. Najem S, Majaliwa ES, Ramaiya K, Swai ABM, Jasem D, Ludvigsson J. Glycemic control and complications of type 1 diabetes among children in Tanzania. J Clin Transl Endocrinol. 2021;23:1–5.
15. Zhou J, Han J, Nutescu EA, Galanter WL, Walton SM, Gordeuk VR. Similar burden of type 2 diabetes among adult patients with sickle cell disease relative to African Americans in the U. S. population : a six-year population-based cohort analysis. Br J Haematol. 2019;185(1):116–27.

Figures

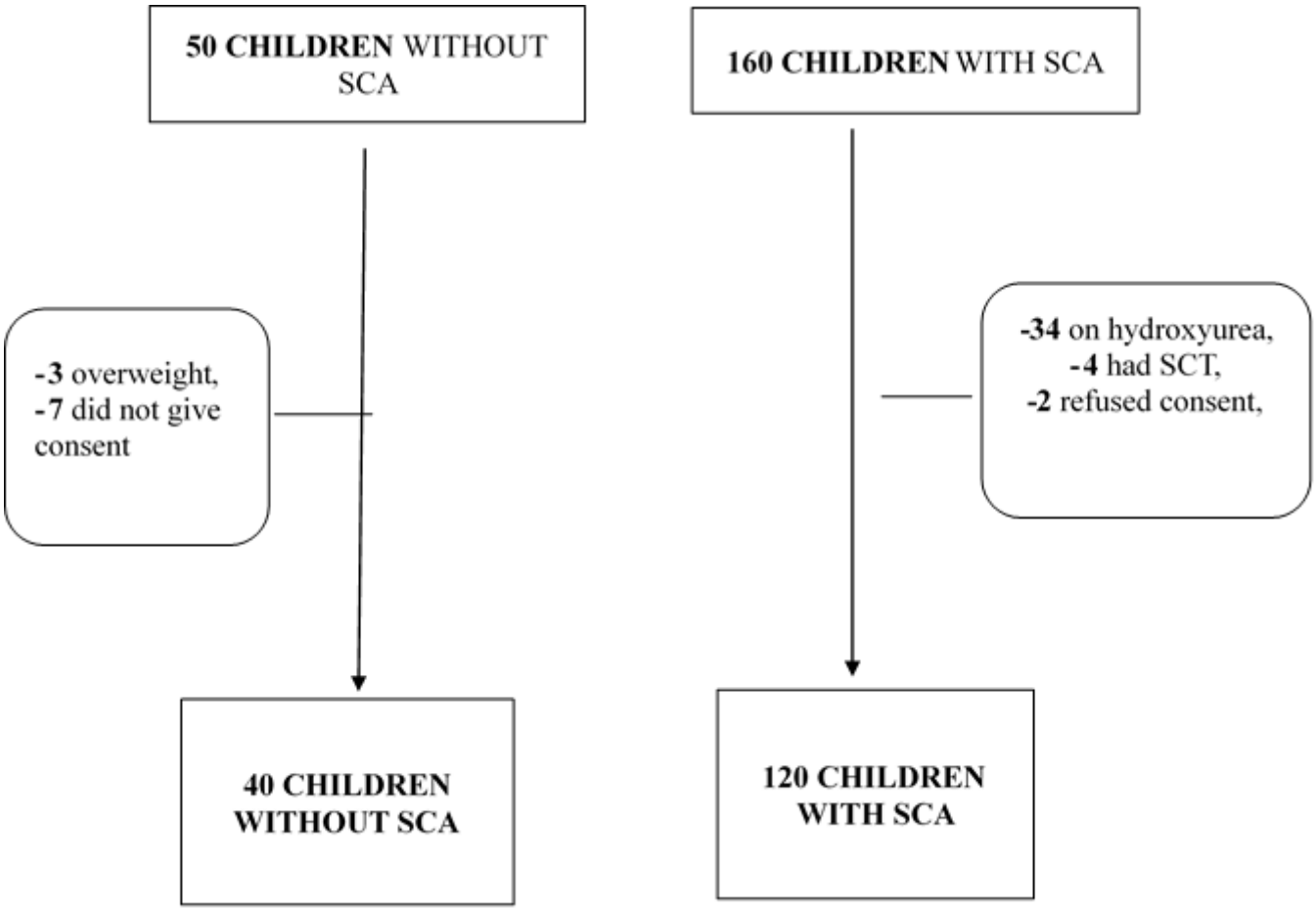

Figure 1

Flow Chart showing enrollment of children with SCA and children without SCA

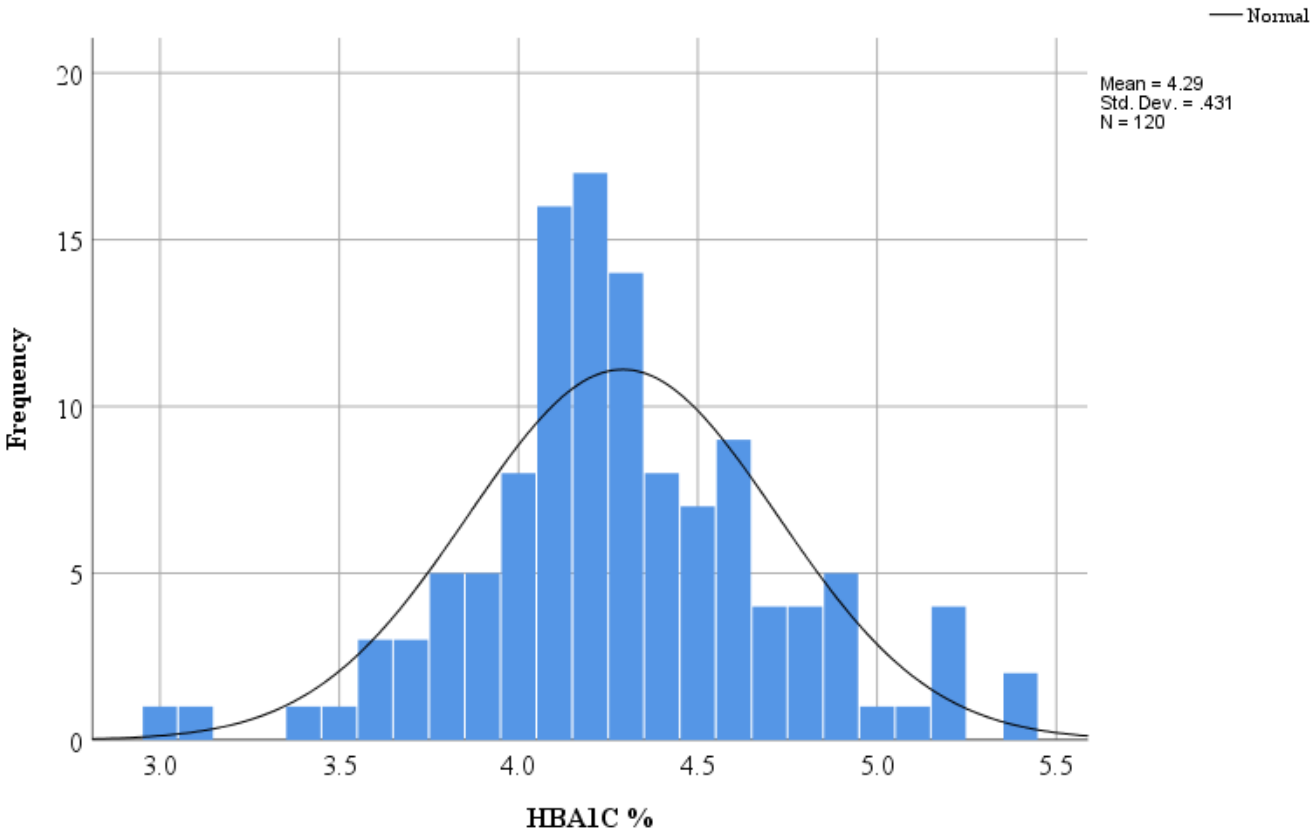

Figure 2

Histogram superimposed with a curve

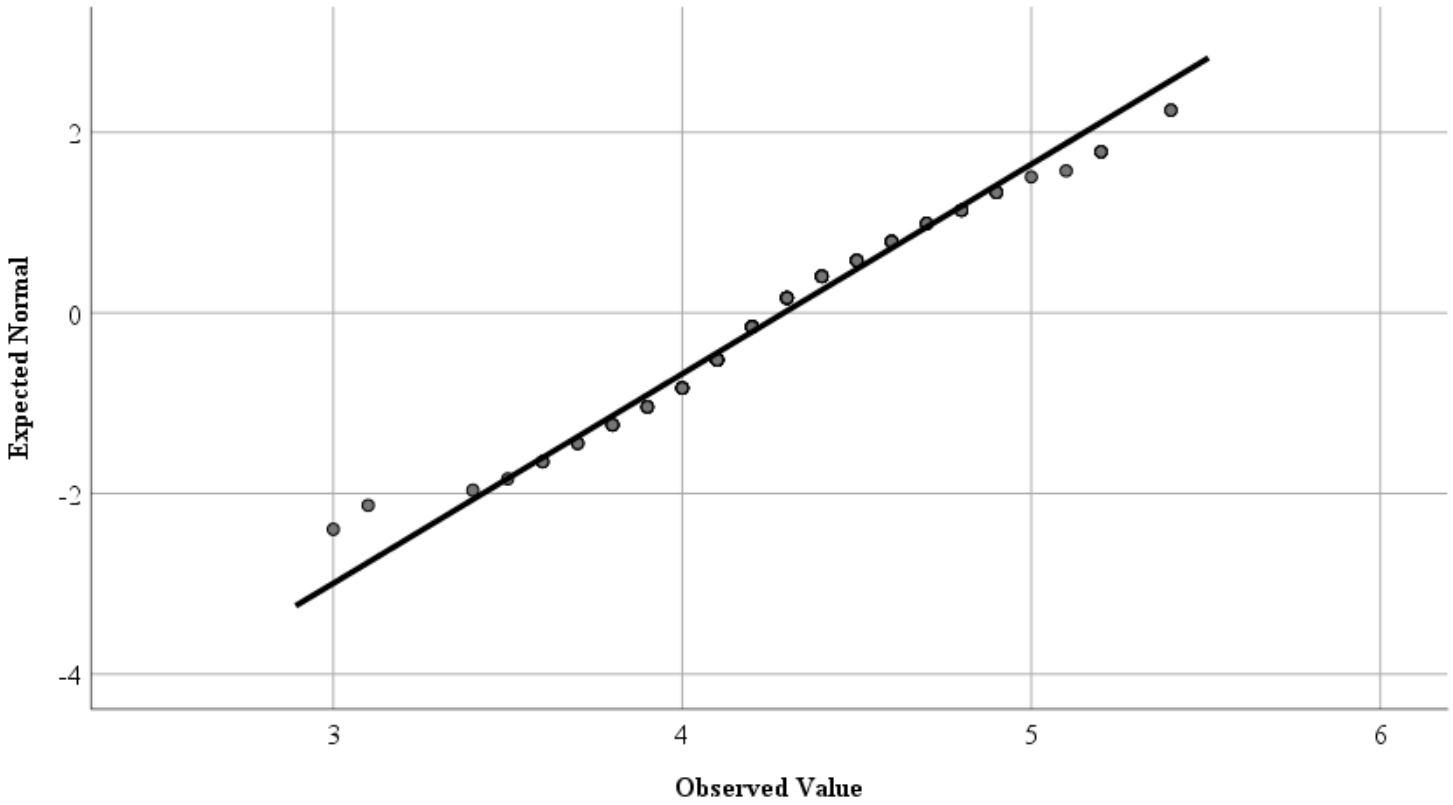

Figure 3

Q-Q plot to check for distribution

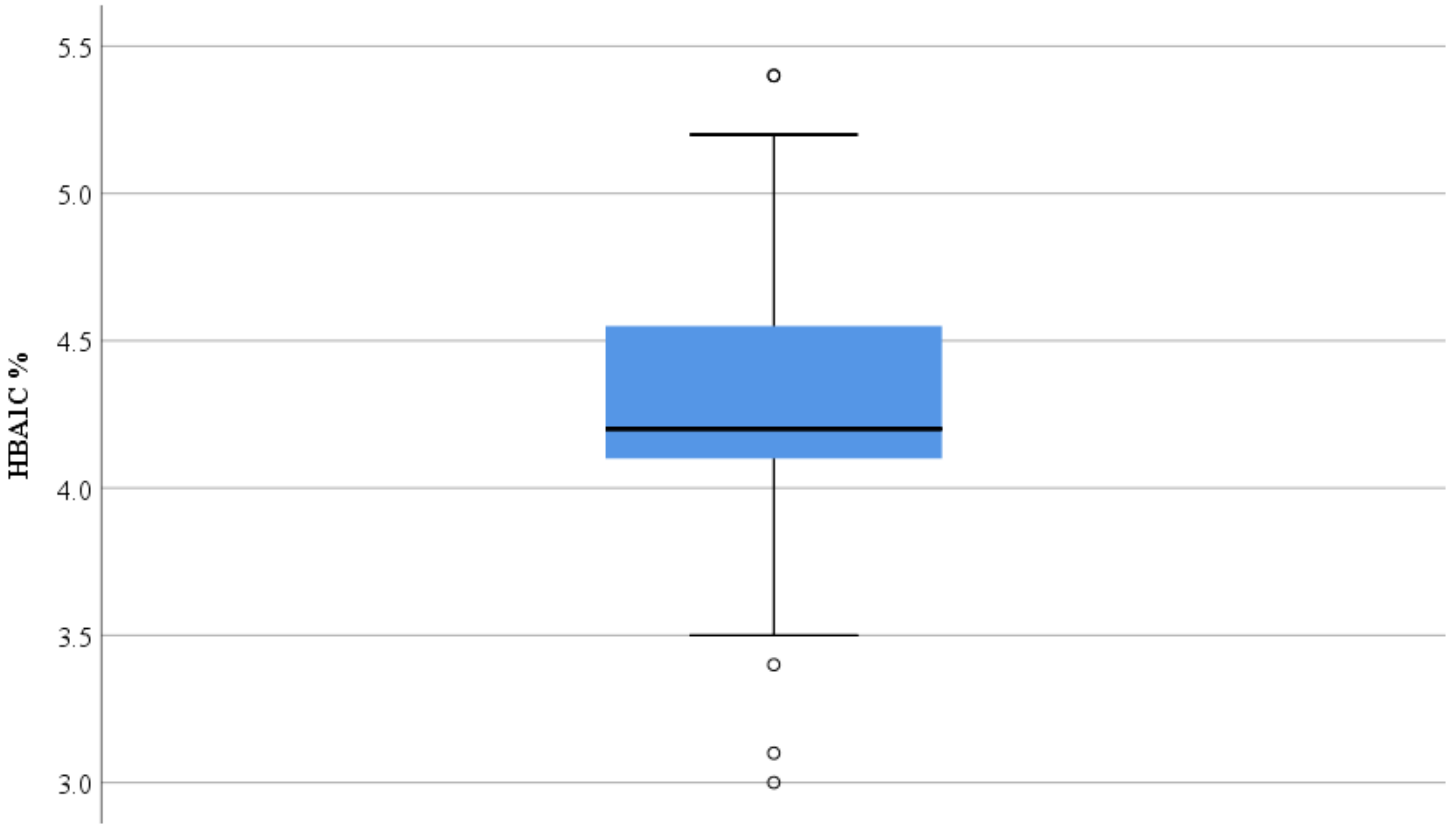

Figure 4

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

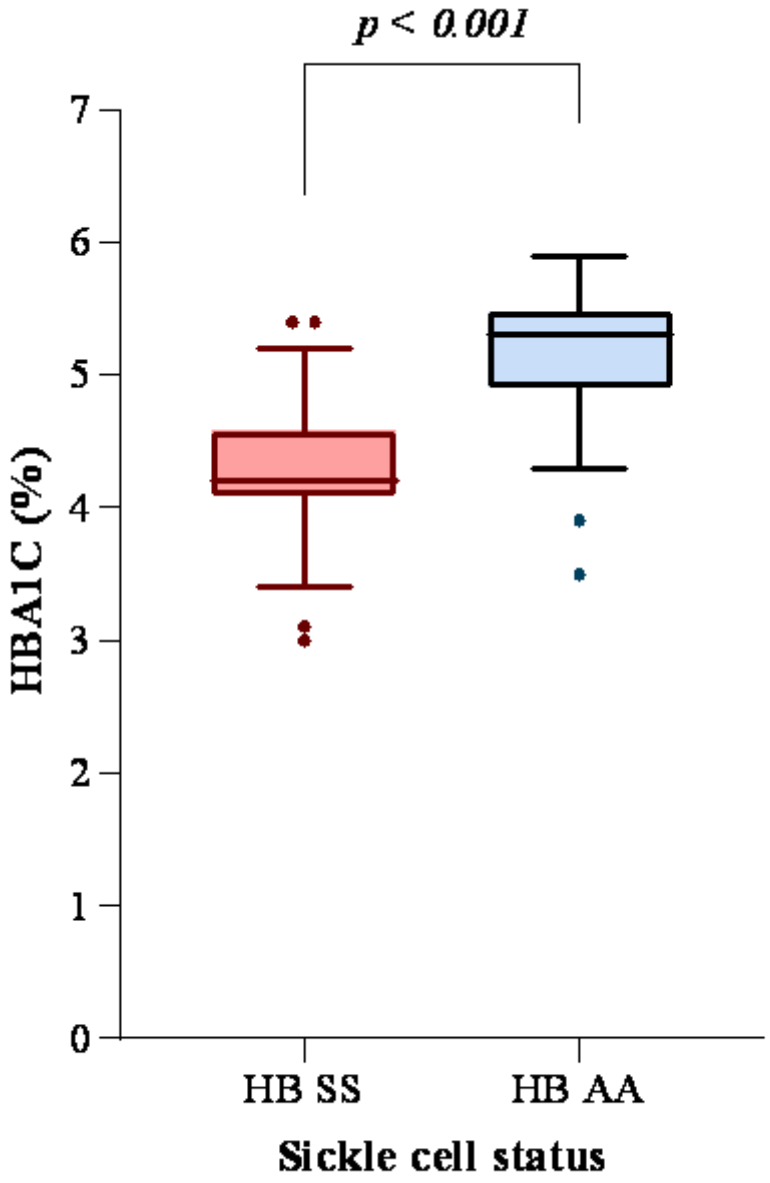

Figure 5

Comparison of median HbA1c levels between children with SCA and children without SCA