

**GROWTH AND PUBERTAL DEVELOPMENT IN CHILDREN WITH
SICKLE CELL ANAEMIA AT MUHIMBILI NATIONAL HOSPITAL 2010.**

By

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**A Dissertation Submitted in Partial Fulfilment of the Requirements for
the Degree of Master of Medicine (Paediatrics and Child Health) of
Muhimbili University of Health and Allied Sciences.**

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination of a dissertation entitled: *Growth and Pubertal development in children with sickle cell anaemia at Muhimbili national Hospital, Dar es Salaam, Tanzania in 2010*, in partial fulfilment of the requirements for the degree of Master of Medicine (Paediatrics and Child Health) of the Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam.

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I, **Theopista Jacob**, 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.

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ABSTRACT

Background: Sickle cell anaemia (SCA) is a genetic disorder with multisystem manifestations. Paediatricians and general practitioners dealing with these patients need to know the overview of the genetics, diagnosis, clinical manifestations, and treatment of sickle cell anaemia associated complications. Poor growth and delayed pubertal development is often impaired in children with SCA. Abnormalities include low Z-scores for height and weight for age, delay in skeletal and sexual maturation. Poor growth and lack of sexual development may lead to emotional and social difficulties and in some patients their consequences can persist to adulthood. The magnitudes of delayed puberty in children with SCA in Tanzania have not been studied; however there is a study which evaluated the growth and pubertal development parameters for the non sickle cell children in Dar es Salaam City. In Africa few studies on pubertal development have been published and hence, the need for baseline reference data to facilitate the interpretation of sexual maturity assessments in Tanzanian children with sickle cell anaemia.

Objective: To compare growth and pubertal development of children with SCA to normative references of urban Tanzanian children in Dar es Salaam.

Methodology: This was a hospital based cross-sectional study with historical control design. Anthropometric measurements of growth and Tanner stages of sexual development of children with sickle cell anaemia aged between 6 and 18 years were compared with normative references for growth and sexual maturity levels derived from a previous study of 3384 Dar es Salaam urban Tanzanian children aged 6-18 years. Data was analyzed using STATA IC version 9 statistical packages. In addition, z-scores were calculated based on UK population reference data which is probably currently the most comprehensive data set available for this age group.

Results: During the study period, 301 children were recruited out of whom 144 (48%) were females. The mean age (SD) of the subjects was 12.4 (± 3.5) years and the mean age by sex was not statistically significant ($P=0.108$). Girls with SCA were at puberty (breast Tanner stage 2) at a mean age (SD) of 14.8 (± 4.6) years as compared to 11.5 (± 1.5) years for non SCA controls and the difference was statistically significant ($P<0.001$). Boys with SCA were at puberty (genital Tanner stage 2) at a mean age of 13.2 (± 2.3) years as compared to 12.3 (± 1.5) years of the non SCA boys in Dar es Salaam urban population ($P<0.001$). The mean age (SD) at menarche

for girls with SCA was 14.8 (± 1.1) years and for the girls without SCA was (13.2 (± 1.3) years ($P < 0.001$).

Children with SCA had low z-scores for height for age, weight for age and Body mass index than children without SCA ($P < 0.001$). There was no statistically significant difference in weight, height, body mass index and waist circumference throughout puberty between girls with and without SCA ($P > 0.05$ throughout). Boys with SCA had low mean weight ($P = 0.001$), height ($P = 0.009$), and body mass index ($P < 0.001$) as compared to non SCA boys at puberty. Advanced sexual maturation was associated with more body fat by sex ($P < 0.001$). In multivariable logistic regression analysis, body fat percent independently predicted puberty in girls but not in boys.

Conclusion: Children with SCA have impaired growth, delayed puberty, and poor nutritional status.. Independently body fat percent predicted puberty in girls with SCA and advanced sexual maturation was associated with more body fat.

Recommendations: The findings can be used as a baseline data in the interpretation of precocity or delayed puberty in children with sickle cell anaemia population.

A longitudinal study is needed to determine exactly when one will be entering and leaving a Tanner stage also establishing the possible causes of disproportional growth between girls and boys with SCA during puberty.

LIST OF ABBREVIATIONS

BMI---Body Mass Index

DXA--- Duo energy X-ray Absorptiometry

FSH ----Follicle Stimulating Hormone

GH----Growth Hormone

HbAA---Normal adult haemoglobin A

HbAS---Sickle cell trait

HbSC ----Sickle haemoglobin C disease

HbSS beta ---Sickle cell beta thalassemia

HbSS ---Homozygous haemoglobin S

LH ----Luteinizing Hormone

MRI ---Magnetic Resonance Imaging

MNH.....Muhimbili National Hospital

MUHAS.....Muhimbili University of Health and Allied Sciences

%FM ---Percentage Fat Mass

RBC – Red Blood Cell

SCA --- Sickle Cell Anaemia

SCD --- Sickle Cell Disease

SD --- Standard Deviation

SS --- Homozygous sickle cell

T4 ---- Free thyroxin

T3 ---- Free triiodothyronine

USA ---United States of America

VOC –Vaso Occlusive Crises

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INTRODUCTION AND LITERATURE REVIEW

1.1 BACKGROUND

Sickle cell disease (SCD) is one of the most important single gene disorders in the world, and the most common form of SCD arises from the homozygous inheritance of the beta-haemoglobin S allele (HbSS) resulting in sickle cell anaemia (SCA). Long-term research programmes, particularly in Jamaica and America, have shown that simple interventions such as comprehensive care programmes, early detection and treatment of acute events, and more complex approaches such as blood transfusion and hydroxyurea therapy, have a significant direct impact on clinical course, quality of life and survival ⁽¹⁻³⁾. Despite such advances, there have been limited comparable, systematic long-term studies of SCA in Africa. This is remarkable difference considering that over 90% of SCA patients are in Africa where there may be as many as more than 200,000 children born with the disease each year compared with 1000 in the USA ⁽⁴⁾.

SCA is prevalent in most of Africa, including Tanzania ⁽⁵⁾ and Kenya ^(6, 7). Other forms of SCD such as Hb SC and HbSS β thalassemia are less common. When HbSS is deoxygenated, it forms large insoluble polymers, which affect the overall structural, functional, and rheological properties of the red blood cell (RBC). The two main pathological consequences of SCA are vasoocclusive disease (VOD) and increased haemolysis. This probably contributes to increased susceptibility to infections, disturbances of growth, pubertal development, nutrition and chronic end-organ damage. In Africa where nutrition is suboptimal for many populations, it is likely to be an important risk factor for poor growth in children with sickle cell anaemia although the causes and clinical significance of poor growth are not well understood as reviewed by Al-Saqladi et al 2008 ⁽⁸⁾.

Although SCA is due to a single point mutation in the β^S -gene, there is wide variation in clinical manifestations of the pattern and severity of the disease. The factors modifying disease phenotype include genetic determinants such as co-existent alpha thalassaemia ^(9, 10) heterocellular persistence of foetal haemoglobin ⁽¹¹⁾, beta globin haplotypes ⁽¹²⁾ and environmental factors which include infections, nutrition, socio-economic status and geographical factors ⁽¹³⁾.

Puberty refers to the process of physical changes by which a child's body becomes an adult body capable of reproduction. It is a significant physiologic event in human growth and biologic

maturation. It begins with the activation of the hypothalamic–pituitary–gonadal axis and ends with the attainment of reproductive capability and the acquisition of adult body composition and habitus⁽¹⁴⁾. The pubertal growth spurt and the appearance of secondary sex characteristics are the most visible manifestations of puberty. It is the lack of one or both of these that brings most teenagers to the health care facility. Growth accelerates fast in the first half of puberty and slowly at the completion of puberty⁽¹⁵⁾. In boys growth can continue slowly till the age of 20 to 23 years.

1.2 Effect of nutritional Status on Puberty

Studies of children with SCA from the 1960s to 1990s described poor growth, delayed skeletal and sexual maturation and demonstrated low growth percentiles in height and weight⁽¹⁶⁻²⁰⁾. However, in African countries in particular Tanzania no such studies have been done to evaluate the magnitude and levels of delayed puberty in sickle cell anaemic patients.

The aetiology of retarded growth and pubertal development in SCA is probably multi factorial with contributions from an increase in metabolic demands, suboptimal nutrition and potentially abnormal endocrine function, hypogonadism (particularly in males). A normal but delayed pattern of adolescent growth in most SS children suggests that these factors affect the timing of the adolescent growth spurt but not the final height. Children with extreme growth delay may represent an interesting subgroup that should be investigated for a possible endocrine explanation for their delayed growth and puberty⁽²¹⁾.

In a study done to determine the influence of hemoglobinopathy on growth and development by Platt, O S et al 1984⁽¹⁹⁾, the height, weight, and sexual maturation of 2115 patients 2 to 25 years old with HbSS, HbSC, sickle beta+ thalassemia (S beta+), or sickle beta O thalassemia (S beta O) were examined. They found that the curves for all hemoglobinopathy groups were significantly different from published norms for black subjects ($P \leq 0.001$), and that subjects with SS and S beta O were consistently smaller and less sexually developed than those with SC and S beta+ (P less than 0.001). For both sexes and all hemoglobinopathies, low weight was more pronounced than short height and was most apparent in subjects over the age of seven. The median age of the female subjects who had attained at least Tanner Stage V was 17.3 years for those with SS, 17.2 years for S beta O, 16.0 years for SC, and 16.5 years for S beta+; among

male subjects the corresponding values were 17.6, 18.8, 16.6, and 16.6 years. Discriminate analysis of menarche status, weight, age, and hemoglobinopathy revealed that the influences of age and weight on menarche were similar regardless of hemoglobinopathy. The author concluded that, this relationship suggests a constitutional rather than a primary endocrinology cause of sexual immaturity in patients with hemoglobinopathies.

In a study done to assess growth, nutritional status, and body composition in 36 African American children with SCA (20 females and 16 males) and 30 healthy control children (15 females and 15 males) of similar age (5-18 y) and ethnicity it was found that, relative to the control subjects and to a national sample, children with SCA had significantly lower z scores for weight, height, upper arm circumference (MUAC), and upper arm fat and muscle areas. Relative skeletal maturation was significantly delayed. After adjustment for age, children with SCA had significantly lower fat mass (prepubertal children and pubertal males only) and fat-free mass (all 3 groups ⁽²²⁾). This means that Children with SCA have impaired growth, delayed puberty, and poor nutritional status.

Nutritional status is an important marker of overall health and linear growth retardation has serious long-term physiological and economic consequences. In cross-sectional surveys to determine the prevalence and main risk groups for malnutrition and to describe the associations between age, sexual maturation and nutritional status in adolescent schoolgirls in western Kenya the overall prevalence of stunting determined by height for age and thinness (weight for height) was 12.1 and 15.6%, respectively. Of the total, 2% were severely stunted. Menarche and start of puberty were delayed by approximately 1.5-2 years compared to a US reference population⁽²³⁾.

The magnitude of moderate stunting and severe stunting in the cohort of sickle cell disease in our setting is estimated to be 23% and 13% respectively as compared to 17% and 7% in the non SCA controls who attended sickle screening clinics at MNH, but who tested as HbAS or HbAA ⁽²⁴⁾. However the control population in this report may not be representative of the general paediatric population in urban Tanzania as a proportion of these patients were referred to the clinic due to anaemia or haematological problems but they were found to be either HbAS or HbAA. The conditions triggering referral, such as iron-deficiency anaemia may also influence growth as sickle cell do. Therefore there is a need to compare the data from the MNH SCA

patients with more appropriate Tanzanian data including assessment of pubertal status, which was previously not done.

Various micronutrient deficits have been identified in SCA that may contribute to growth failure. However, there are actually relatively few of these studies for example in a study by Zemel et al ⁽²⁵⁾, found that energy, protein, and fat intake were not associated with growth failure, although a single 24-h recall of dietary intake per year is unlikely to be an accurate assessment of macronutrient intake for an individual. Nutrient deficiencies based on biomarkers have been reported for vitamins B6 ⁽²⁶⁾, D ⁽²⁷⁾, and E ⁽²⁸⁾, retinol ⁽²⁹⁾. In a 12-months trial, zinc supplementation was shown to increase linear growth in children with SCD-SS ⁽³⁰⁾.

Increased energy requirements have been reported for children, teenagers, and adults with SCD in their usual state of health ⁽³¹⁾ and increased protein turnover adding an additional nutritional burden ⁽³²⁾.

The evidence that timing of pubertal onset is related to measures of adiposity suggests that the onset of reproductive maturation among boys in populations with poor nutritional status will be delayed. This fact has been demonstrated by Campbell et al among rural Zambian boys in whom there was a delayed onset of puberty and a slow testicular development which was related to poor nutritional status ⁽³³⁾. Mounir et al ⁽³⁴⁾ did a cross-sectional study of 1606 girls in primary and preparatory schools in Alexandria to assess the mean age of menarche and the main nutritional factors affecting it. In this study every girl was subjected to anthropometric assessment including weight, height, mid upper arm circumference, waist circumference, hip circumference and triceps skin-fold thickness. BMI and body fat percentage were calculated. A 24 hours diet recall method was used to assess the dietary intake. The mean age of menarche was 11.98 \pm 0.96 years. The mean MUAC, triceps skin-fold thickness, waist circumference and hip circumference were significantly higher among menstruating girls as compared to non-menstruating girls ($p < 0.01$). Only 7.5% of the females less than the 5th percentile of BMI (thinness) were menstruating, while the corresponding figure for those at or more than 85th percentile (overweight) was 65.6% and this was statistically significant, ($P < 0.001$). Girls who attained menstruation demonstrated a higher significant mean percent of body fat (43.40 \pm 10.0) as compared to non menstruating ones (35.41 \pm 7.87), ($t = 17.09$, $P < 0.001$). These findings suggested a linear correlation between body fat percentage and the stage of sexual maturity.

Also Britton, Julie A. et al in 2004 did a cross-sectional study of 186 New York Metropolitan Area, (54 African-American, 70 Hispanic, and 62 Caucasians) to assess characteristics of pubertal development in a multi-ethnic population of nine-year-old girls ⁽³⁵⁾. Height and weight were measured and pubertal development according to Tanner stages was done. In this study it was found that African-Americans were more likely than Caucasians to have achieved puberty as determined by breast or hair development (stage 2 or higher) [age-adjusted odds ratios and 95% confidence intervals = 4.91 (2.15-11.19) and 4.25 (1.85-9.77), respectively]. Pubertal development was similar among Hispanics and Caucasians. Adiposity and height were significantly positively associated with breast or hair development. Lower energy, but higher polyunsaturated fat, consumption were suggestive of an association with breast development. The author concluded that their results were consistent with height and adiposity being associated with pubertal development.

1.3. Effect of SCD on puberty

In a study by el-Hazmi, M. A. et al ⁽³⁶⁾, on the endocrine functions in sickle cell anaemia patients found gonad hypo function. Patients with severe form of sickle cell anaemia showed more frequent abnormalities of luteinizing hormone (LH), follicle stimulating hormone (FSH), cortisol and testosterone in comparison with the patients with a mild disease. The LH, FSH, cortisol and testosterone levels were lower, while growth hormone (GH), free thyroxin (T4), and free triiodothyronine (T3) did not show significant differences between patients and the controls. This suggest that the sickle cell gene abnormality has an adverse effect on endocrine functions, therefore follow-up and appropriate management of endocrine dysfunctions are advocated in such patients

M'Pemba et al conducted a case-control study to investigate the sexual maturation of girls with homozygous sickle cell disease. The study showed a significant delay in signs of physical maturation. Puffiness of the mammary glands was at 14.4 years \pm 1 (as versus 12.4 years \pm 1.5 in controls) and puberty occurred at 14.2 years \pm 1. Menarche occurred on average at 15.2 years \pm 1.6 (as versus 13.4 years \pm 1.4 for controls). Lack of the menarche between the ages of 14 and 18 years was observed in 71% of cases as versus 10% of controls ⁽³⁷⁾.

A study by Atul Singhal et al ⁽³⁸⁾ did a study to investigate the timing and pattern of the adolescent growth spurt in Jamaican SCA patients and compared with AA controls. The onset of

the adolescent growth spurt was delayed in SS disease by 1.4 years (95% confidence interval 0.8 to 2.0) with no significant sex difference. The age at peak height velocity was delayed by 1.6 years (0.9 to 2.3) in SS compared with AA subjects. The adolescent growth of SS children was otherwise normal and there was no difference in the attained height by age 17.9 years. The age at menarche in girls with SS disease (mean (SD) 15.4 (1.3) years) was significantly later than that of girls with haemoglobin AA (13.1 (\pm 1.3) years).

In a Jamaican cohort study ⁽³⁹⁾, they followed children from birth to age 18-26.5 years with regular assessments of height, weight, pubertal stage, and haematological indices were done at a specialized Sickle Cell Clinic of the University Hospital of the West Indies. The mean age for menarche in normal controls (13.0 years) was significantly earlier than in SC disease (13.5 years) or SS disease (15.4 years). Greater weight was the only significant parameter associated with menarche across all genotype. Additional contributions occurred from high foetal haemoglobin and red cell count in SS disease. Among the anthropometric measurements taken in their study, weight appeared to be the principle determinant of age at menarche.

In Nigeria a study was done to assess the effect of homozygous sickle cell disease on the age at menarche in schoolgirls. They reported that, the average age at menarche was significantly greater among adolescents with sickle cell anaemia when compared with normal students (14.5 ± 1.13 years vs. 13.3 ± 1.09 years; with $P < 0.005$). In Nigeria, a continuing decline of the average age at menarche at a rate of about four months per decade was observed when the result from this study was compared with that from earlier southern Nigeria studies ⁽⁴⁰⁾.

1.4. Puberty in non sickle cell children

Several studies done in trying to evaluate pubertal development in different ethnic groups in US have revealed that African American girls on average enter puberty earlier than other ethnic girls. In the study by Wu Tiejian et al ⁽⁴¹⁾, Black and Mexican American girls had pubic hair and breast development and had achieved menarche at younger ages than Caucasian girls. For example, 49.4% of black girls aged 9 years had breast development compared with 24.5% of Mexican American girls and 15.8% of Caucasian girls. The mean age at onset of pubic hair, breast development, and menarche was 9.5, 9.5, and 12.1 year for black girls; 10.3, 9.8, and 12.2

years for Mexican American girls; and 10.5, 10.3, and 12.7 years for Caucasian girls. These ethnic differences remained even after adjustment for their current body mass index and several social and economic variables. The results shows a much earlier age of sexual maturity as compared to African data presented and that was related to advanced health and nutritional status in western countries.

Likewise, another study which assessed sexual maturity pattern of boys in US ⁽⁴²⁾ showed that the median ages at stage 2 for pubic hair development of Caucasian, African American and Mexican American boys were 12.0, 11.2, and 12.3 years, respectively, and at stage 2 for genital growth were 10.1, 9.5 and 10.4 years respectively. Statistically significant differences among the 3 racial/ethnic groups were found in the median ages of onset of pubic hair growth and genital development at stage 5 with and without controlling for height and weight, indicating an earlier age of pubertal attainment for the African American boys.

In Tanzania unpublished data from a study done by Muze et al (April 2009) to assess growth and pubertal development parameters are presented. Among 3384 urban Tanzanian Children (1814 females and 1570 males) aged between 6-18 years, the mean age at onset of pubic hair was 12.0 years and breast (females) was 11.5 years. The genital development in males was 12.3 years and pubic hair was 12.5 years. When compared to African American children in US, Tanzanian children (both boys and girls) start their puberty development at a later age. Furthermore Muze found that the mean age of menarche in Tanzanian girls was 13.2 years which is later than African American girls in US (menarche mean age 12.3 years). Prevalence of obesity was found to be relatively low as compared to the developed countries like US which might signify that poor nutritional status in the population might be responsible for delayed pubertal development as observed in Tanzanian children.

1.5 Percentage body fat (%FM)

BMI is a general index of adiposity. More accurate estimates of the percentage body fat or fat mass (%FM) in healthy subjects can be made indirectly by numerous techniques, including underwater weighing, total-body electrical conductivity, bioelectrical impedance, isotope dilution, potassium-40 counting, and dual-energy X-ray absorptiometry (DXA). Magnetic

resonance imaging (MRI) has recently been employed in the assessment of total abdominal fat. These methods are however time consuming and are not easily accessible ⁽⁴³⁾. However, use of skin fold-thickness measurements to estimate %FM is particularly appealing in population studies because the procedure is relatively easy to perform and non-invasive, the measurement instruments (skin fold callipers) are inexpensive and does not require electrical power to operate, and most important of all, the measurements can be done in any setting.

In a study by Freedman, David S et al in New York City, the additional information provided by skin fold thicknesses on body fatness, beyond that conveyed by BMI-for-age, among healthy 5- to 18-years old (n = 1196) was examined. They reported that information on the sum of two skin folds (triceps and sub scapular) which was used as an indicator of the overall skin fold thickness significantly ($p < 0.001$) improved the prediction of body fatness beyond that obtained with BMI-for-age. For example, the use of the skin fold sum, in addition to BMI-for-age, increased the multiple regression models for predicting body fat mass from 0.81 to 0.90 (boys), and from 0.82 to 0.89 (girls). The use of the skin fold sum also reduced the overall prediction errors (absolute value of the residuals) for %FM by 20-30%, but these reductions varied substantially by BMI-for-age. Among overweight children, defined by a BMI-for-age \geq 95th percentile, the skin fold sum reduced the predication errors for % fat mass by only 7-9% as compared to BMI-for-age. The author concluded by saying that, skin fold thicknesses, when used in addition to BMI-for-age, can substantially improve the estimation of body fatness ⁽⁴⁴⁾.

2.0 Definition of terms

For the purpose of this study, growth will be determined by assessing nutritional status using anthropometric measurements.

Adolescent refers to a child aged ten to eighteen years.

Children refer to a child aged six to nine years.

3.0 PROBLEM STATEMENT

Homozygous sickle cell disease is associated with high morbidity and mortality in early childhood in low resource countries like Tanzania ⁽⁴⁵⁾. The major causes of mortality and morbidity have been studied in settings outside Africa and markedly reduced by a range of interventions.. Therefore pubertal assessment in children and adolescents will provide information that will lead to a better understanding of the growth status and evaluation of endocrine problems. Delay of sexual development may lead to emotional and social difficulties and in some patients their consequences can persist when 'normal' height and full sexual maturation are attained ⁽⁴⁶⁾. Recent data also suggest that a delay in the 'tempo' of pubertal maturation may interfere with the normal bone accretion occurring during puberty, later causing osteoporosis. Such findings suggest that a new approach in managing delayed puberty may be necessary not only for psychological reasons but also for optimizing bone mass accretion ⁽⁴⁷⁾. Therefore knowledge of the magnitude and severity of the delayed sexual maturity will inform decision making regarding appropriate interventions as suggested by Anie, K A ⁽⁴⁸⁾ to reduce the morbidity as well as the psychosocial repercussions .

The paucity of data regarding pubertal development parameters in this cohort of patients with sickle cell anaemia in Tanzania prompted the choice of this study.

4.0 RATIONALE OF THE STUDY.

Puberty is a significant physiologic event in human growth and biologic maturation. Parameters for sexual maturity levels of Tanzanian children with sickle cell anaemia have not been studied and in Africa few studies on pubertal development have been published. This has highlighted the need for reference data to facilitate the interpretation of sexual maturity assessments in Tanzanian children with sickle cell anaemia as compared to their peer normal children.

4.1 NULL HYPOTHESIS

Growth and pubertal development in children with sickle cell anaemia is similar to normal children in the general population.

5.0 OBJECTIVES

5.1 Broad objective.

To compare growth and pubertal development among children with sickle cell anaemia to normative references of urban Tanzanian children in Dar es Salaam.

5.2 Specific objectives

- 5.2.1** To determine the mean age at puberty of children with sickle cell anaemia attending clinic at MNH by sex.
- 5.2.2** To determine the mean age of menarche in girls with sickle cell anaemia attending clinic at MNH.
- 5.2.3** To determine factors (e.g. nutritional status, disease severity) associated with pubertal development in children with sickle cell anaemia
- 5.2.4** To determine body fat percentage and its relationship to sexual maturation stages in children with sickle cell anaemia.

6.0 METHODOLOGY

6.1 Study design

This was a cross-sectional with historical control study.

6.2 Study duration

The study was conducted in a period of five months, May-September, 2010

6.3 Study area

The study was carried out at Muhimbili National Hospital sickle cell outpatient clinic. MNH is a national hospital and the largest referral hospital in Tanzania. It caters for a population of about 4 million Dar es Salaam residents and nearby Regions. It receives referrals from the 3 municipal hospitals namely Ilala, Temeke and Mwananyamala. Children with sickle cell anaemia are referred from different hospitals to MNH where they are regularly followed up at least every 6 months.

5.4 Study Population

Patients were enrolled from the ongoing prospective SCD cohort based at MNH. Active recruitment and follow up of the current cohort was initiated in April 2004.

A total of 1750 patients with sickle cell anaemia (HbSS) aged from 8 months to 49 years have been enrolled in the main SCD cohort as of end of October 2009. The mode age group being 10-19 years (36%) and another 30% aged between 6-9 years. In this study eligible participants were those with ages ranging from 6-18 yrs. The study involved about 500 patients who have confirmed HbSS status. In the age group 6-10 yrs there were about 200 patients, 11-13 yrs 130 patients, 14-16yrs 140 patients and 17-18 yrs 40 patients. In this cohort haemoglobin electrophoresis was done at enrolment and confirmed by HPLC with quantification of HbF and HbA2. Study participants are scheduled for routine outpatient visits every 6 months at which detailed clinical and laboratory data are recorded and plasma/serum samples stored. Similarly all SCA patients admitted to MNH are documented and the cause of admission classified into the broad categories of pain, anaemia, fever, pneumonia and others. The sickle cell clinic offers free health services, and is the principle source of referral for advanced care. At each clinic attendance, patients are questioned about clinical complications and hospital admissions since last seen, and relevant hospital notes are periodically reviewed. Clinic records are therefore believed to be reasonably complete and comprehensive.

5.5 Inclusion criteria

- Children and adolescent with sickle cell anaemia aged 6-18 years enrolled in the main SCD cohort study who agreed to participate.

5.6 Exclusion criteria

- Children with obvious physical disability, which prevented accurate anthropometric measurements example stroke.
- Children with obvious dysmorphic features suggestive of syndromic diseases or bone dysplasia known to affect height of the child and/or sexual maturation.

5.7 Sample size estimation.

Sample size was calculated using single mean formula to measure a variable with precision

$$N = \frac{4 \tilde{\sigma}^2}{E^2}$$

Where;

$\tilde{\sigma}$ Corresponds to standard deviation of a mean age at menarche of Nigerian school girls with SCA which was 1.13 years.

E corresponds to the maximum likely error and is 0.1

Therefore the minimum sample size N was 510.

Adjusting with the finite population correction factor as the population to be sampled was finite, the sample size was adjusted as $n' = n / (1 + n/N)$

Where n' = sample size calculated, n = sample size calculated from the formula above which was 510, and N =finite population sampled which was 480 children with SCA aged 6-18 years with complete data in the main SCD cohort. Therefore a minimum sample size after adjustment was 255 children. In this study a sample size of 301 children were recruited. This sample size was powered to detect significant difference in the pubertal development between children with sickle cell anaemia as compared to the nohealthy sickle cell children.

5.8 Sampling procedures.

All eligible children and adolescent whose parents/care givers consented to participate were consecutively enrolled and stratified by age group into the study on Thursdays and Fridays

scheduled clinic. For the purpose of this study four main age groups were described according to the pubertal development characteristics. Age ranging 6-<11yrs were taken as category one, 11-<14 yrs category two, 14-<17yrs category three and 17-18 yrs category four. At the end of the study, in each category two third of their population were recruited.

At the clinic recruitment was done by provision of information regarding the study to all parents/guardians. Those who agreed and their children met the inclusion criteria were requested to sign the consent form for participation. About 15-20 patients were recruited every week.

5.9 Procedures

This study utilized existing and prospectively collected data from the ongoing MUHAS SCD cohort. The database of the clinical cohort study was used to ascertain information regarding the SCD status. The physical measurements were taken by the author and research assistants as described below.

A structured questionnaire was used to collect information from the parents/guardian of children and adolescents. Information included social demographic data, anthropometric measurements, skin fold parameters, sexual maturation parameters and laboratory results of haemoglobin status.

5.9.1 Age calculation

Children's chronologic age was calculated as a decimal age by subtracting the measurement date from the date of birth, the latter was ascertained from the existing main SCD cohort records.

5.9.2 Anthropometric measurements

The author and one research assistant performed all anthropometric measurements that included weight, height, and waist circumference and skin folds. These measurements were performed after the participants have removed their shoes and upper garments. Weight was measured to the nearest 0.1 kg using TANITA UM 075 weighing scale, which was periodically checked for accuracy and calibrated as necessary.

Height was measured with a portable Leicester stadiometer to the nearest 1 mm; the subject was upright and the head in the Frankfurt plane. Waist circumference was measured with a flexible, non-elastic tape to the nearest 1 mm midway between the tenth rib and the iliac crest at the end

of a gentle expiration. The circumference measurement was done over the naked skin and used the mean value of two measurements for the analysis ⁽⁴⁹⁾.

BMI (kg/m^2) was computed using weight (in kilogram) divided by height (in meters squared).

Skin fold thicknesses at 2 sites (sub scapular and triceps) was measured with a Lange calliper to the nearest 1mm. All measurements were taken on the right side of the body using standard procedures as described by Slaughter MH et al, and the average of two readings at each site was recorded ⁽⁵⁰⁾. Of the prediction equations that exist for children only those developed by Slaughter et al and Dezenberg et al. are recommended for children of African ancestry. In this study, equation by Slaughter MH et al was used.

For the triceps measurements:

1. With a grease pencil, a mark point at the back of the arm midway between the tip of the elbow and the shoulder was made
2. Skin fold was picked up with thumb and forefinger of the left hand.
3. Jaws of the calliper were applied to the skin fold so that the grease mark was midway between the jaws.
4. Thumb was released from the calliper handle, so that the tips of the calliper have full exertion on the skin fold reading taken immediately after the first rapid fall.

For the sub scapular measurements

Below tip inferior angle scapula 45 degrees to vertical (back – just under shoulder blade.)

1. Skin fold was picked up just under the shoulder blade – following the natural fold of the skin.
2. With grease pencil, a mark midway the fold was made. While holding the skin fold approximately 1 inch from the mark, we proceeded with steps 3, 4, as in Triceps section

The sum of these two skin folds (SF sum) was used as an overall measure of skin fold thickness.

An estimate of percentage body fat was calculated with the use of sex-specific Slaughter equations.

The equations for boys were the following:

Percentage body fat for children with triceps and sub scapular skin folds <35 mm:

Percentage fat = $1.21(\text{triceps} + \text{sub scapular}) - 0.008(\text{triceps} + \text{sub scapular}) - 1.7$

Percentage body fat for children with triceps and sub scapular skin folds >35 mm:

Boys = $0.783(\text{sum of 2 skin folds}) - 1.7$

The equations for girls were the following:

Percentage body fat for children with triceps and sub scapular skin folds <35 mm:

Percentage fat = $1.33(\text{triceps} + \text{sub scapular}) - 0.013(\text{triceps} + \text{sub scapular}) - 2.5$.

Percentage body fat for children with triceps and sub scapular skin folds >35 mm:

Girls = $0.546(\text{sum of 2 skin folds}) + 9.7$

Intra and inter-observer correlation coefficients were assessed after training of the research assistant and the author, and it was less than 3% for any of the measurements made.

5.9.3 Sexual maturation Staging

Assessment of sexual maturity stages usually is done by a well trained physician using the Marshall and Tanner method^(51, 52). Several studies have tried to evaluate the validity of the Tanner self-assessment method with conflicting results. Chan et al estimated the reliability of pubertal self-assessment in Hong Kong Chinese children by using questionnaire with gender-specific line drawings and brief explanatory text in Chinese found that the method can reliably estimate sexual maturation status as most agreement between self and the ratter's assessments differed by only one Tanner stage.

In this study the author and one research assistant received training on assessment of sexual maturity stages from an experienced physician who conducted an earlier study of pubertal development in urban Tanzanian children. Materials prepared for the study composed of text, photographs and visual aids for both girls and boys and were the same as those used in the previous study in normal urban Tanzanian children in Dar es Salaam.

Pubertal staging criteria and definitions based on the recommendations of Marshall and Tanner known as Tanner staging^(51, 52) was assigned to each maturity indicator, that is, pubic hair in each gender, breast development in girls, and genital development (penis, testes, and scrotum) in boys. Each maturity indicator has 5 stages that can be assigned from stage 1, representing immaturity, to stage 5 indicating full maturity.

Refer to Appendix V for Tanner staging in girls and Boys.

Girls were asked about whether they had attained menarche and their age (years) at first regular menstruation. Accordingly, this information was used in this study to define menarche status both the prevalence of having attained menarche at the time of examination for girls of various ages and self-reported age at menarche.

Girls were classified as having begun puberty when they are at Tanner stage 2 or greater for breast and/or pubic hair development and to have completed puberty when they are at Tanner stage 4 or greater for breast and pubic hair development provided menarche has started.

Breast development was assessed by inspection and breast palpation. Breast development is referred to the elevation of breast and papilla at least as small mounds ⁽⁴¹⁾.

Boys were classified as having begun puberty when they were at Tanner stage 2 or greater for genital and/or pubic hair development, and to have completed puberty when they were at Tanner stage 4 or greater for genital (penis, testes and scrotum) and pubic hair development.

Pubic hair was reported as present if either sparse growth of long, slightly pigmented downy hair or straight or only slightly curled hair were seen along the labia for girls and at the base of penis for boys ⁽⁴¹⁾.

The notation for each maturity indicator contains the initial letters as follows: pubic hair (PH), breast development (B), and genital development (G). Thus, Tanner stage 2 for genital development in boys is indicated as G2 ⁽⁵⁷⁾.

Presence/absence of the pubertal milestone, age at examination (recorded in years to one decimal point) and self reported age at menarche (recalled in whole integers or years and months) were primary variables of interest. Other variables like social economic status, current BMI and waist circumference, and body fat percentage were included in the study to describe the characteristic of the sample and to serve as covariates.

5.9.4 Social economic status assessment

Each family social economic status was classified as high, middle or low social economic class as described by Oyedeji G and employed in the study of normal urban Tanzanian children ⁽⁵³⁾.

6.0 DATA PROCESSING AND ANALYSIS

6.1 Data entry and analysis

All filled questionnaires were coded, and checked for consistency before double entry into the STATA IC version 9 computer databases. Data cleaning was done in terms of consistency checks for outliers and missing data.

Data analysis was done using STATA IC version 9, and the results were presented as the mean, 95% confidence intervals and proportions.

The mean ages at puberty and menarche between the two groups were compared using t- test for independent samples, Mann Whitney ranksum test for non-parametric test for non-normally distributed data. Since male and female children experience different growth patterns, they were compared separately and a $P < 0.05$ value was considered as statistically significant.

Logistic regression was used to determine the combined association between independent variables like nutritional status, disease severity, age and fat levels and the dependent variable of having reached puberty as defined as Tanner stage two or above coded as a binary variable.

The statistical significance of the effects in these models was assessed by using likelihood ratio tests.

Z-scores for height for age, body mass index and weight for age was calculated against the UK 1990 growth reference values using the "zanthro" ado programme in STATA 9. These growth standards are the most comprehensive available for older children above 60 months and include data for individual up to 23 years. These references are proposed to be the most appropriate for use in populations who may take long to reach their final stature.

7.0 ETHICAL CONSIDERATION

In doing this study the major ethical issues have been put in consideration.

Since the examination involved exposure of some private parts of children, privacy was ensured by putting portable screening curtains to provide a temporary private space. Each child was examined by a researcher of the same sex.

No any risk to the participants was anticipated from this study. On the contrary, there was a community and scientific benefit of establishing reference age ranges of attaining sexual maturation in our Tanzanian children/ adolescent with sickle cell anaemia which forms one of the vital components in interpretation of growth status and evaluation of various endocrine problems.

During examination, the author and research assistant gave appropriate advice and counselling as necessary to every child found with any abnormal condition which needed a medical attention.

Every child had equal opportunities to be involved in the study regardless of the sexual status.

Before conducting the study permission was obtained from Muhimbili University of Health and Allied Sciences (MUHAS)'s Senate Research and Publications Committee.

In every child an informed written consent was sought from the parent or guardian after a full explanation of the purpose and nature of the study done. Where appropriate assent from the children was sought and documented on the consent form.

8. RESULTS

8.1 Demographic characteristics of study population

During the study period, 301 children were recruited out of whom 144 (48%) were female. All enrolled participants had sickle cell anaemia (HbSS) confirmed by haemoglobin electrophoresis and HPLC in the main sickle cell cohort. Most of the study participants (78%) were from low socioeconomic status, 17% middle and 5% from high socioeconomic status. In the SCA population assessed, the mean age ($p=0.1087$), height for age ($p=0.26$) and body mass index z-scores did not differ by sex ($p=0.34$). Girls were significantly heavier ($p=0.019$) and contained more body fat than boys ($p<0.001$) although there was no difference in measures of disease severity (Table 1a).

In the non SCA group, a total of 3384 children were involved in the study out of which 1818 were girls (53.7%). Mean age was significantly greater in boys ($p=0.021$) than in girls (Table 1a). Girls were significantly shorter ($p < 0.001$) and had higher mean BMI than boys ($p<0.001$). There was no gender difference in the mean weight or mean waist circumference.

Table 1a. Distribution of baseline characteristics of children with and without SCA by sex.

Variables	Females	Males	P-values
	144 (48%)	147 (52%)	
Mean Age (Years)	12.6 (12.1 - 13.2)	12.0 (11.5 -12.5)	0.1087
Mean weight (Kg)	31.3 (29.5 - 33.0)	28.7 (27.4 -30.0)	0.019‡
Mean HAZ (height for age z-score)	-1.92 (-2.1 - -1.7)	-2.07 (-2.3 - -1.9)	0.26 [†]
Mean BMIZ (BMI for age z-score)	-1.35 (-1.5 - -1.2)	-1.48 (-1.6 - -1.3)	0.34 [†]
Skin folds & % body fat			
Mean triceps Skin fold (cm)	10.8 (10.2 - 11.4)	8.0 (7.7 - 8.3)	< 0.001‡
Mean sub scapular Skin fold (cm)	7.8 (7.3 - 8.2)	5.9 (5.6 - 6.1)	< 0.001‡
Mean waist Circumference (cm)	62.0 (60.7 - 63.2)	59.6 (58.8 - 60.5)	0.002‡
Mean body fat %	21.9 (20.7 - 23.2)	15.0 (14.4 - 15.6)	<0.001‡
Disease severity (median)			
life time number of admissions	4.4 (3.7 - 5.1)	4.4 (3.7 - 5.1)	0.91‡
Previous blood transfusions	1.8 (1.4 - 2.1)	2.2 (1.7 - 2.8)	0.173‡
Characteristics of the non-SCA control population			
	1818 (53.7%)	1566 (46.3%)	
Mean age (years)	11.6 (11.5 - 11.8)	11.9 (11.7 - 12.1)	0.021‡
Mean weight (Kg)	36.0 (35.4 - 36.6)	35.9 (35.2 - 36.5)	0.737‡
Mean HAZ	-0.8 (-0.91- -0.81)	-1 (-1.1- -0.95)	< 0.001‡
Mean BMIZ (BMI for age z-score)	-0.3 (-0.4 - -0.3)	-0.5 (-0.54 - -0.4)	< 0.001‡
Mean waist Circumference (cm)	62.2 (61.7 - 62.5)	62.0 (61.6 -62.4)	0.53‡

Values are expressed as means and 95% confidence intervals, ‡Student's t-test independent sample; [†]Mann Whitney ranksum test (non-parametric test for non-normally distributed data).

Baseline characteristics for children with and without sickle cell anaemia by sex were compared. There were significant differences in age ($p<0.001$), height for age ($p<0.001$), weight for age ($p<0.001$) and Body mass index Z-scores ($p<0.001$) between children with SCA and control children. Girls with sickle cell anaemia were 1.0 year older than those without sickle cell anaemia. As well they were shorter, underweight and thinner than those without sickle cell anaemia $P < 0.001$; Table 1b.

There was no significant difference in the mean age between males with and without sickle cell anaemia ($p=0.69$). Males with sickle cell anaemia were underweight ($p<0.001$), shorter ($p<0.001$) and thinner than those without sickle cell anaemia $P < 0.001$; Table 1b.

Table 1b. Comparison of the baseline characteristics for children with and without sickle cell anaemia by sex

Variable	Girls		Boys	
	Non SCA ($n = 1818$)	SCA ($n = 144$)	Non SCA ($n = 1566$)	SCA ($n = 147$)
Age	11.6 (11.5 - 11.8)	12.6 (12.1 - 13.2)†	11.9 (11.7 - 12.8)	12.0 (11.5 - 12.5)
WAZ	-0.68 (-0.7 - -0.6)	-2.20 (-2.4 - -2.0)†	-0.91 (-1.0 - -0.9)	-2.38 (-2.6 - -2.3)†
HAZ	-0.86(-0.9 - -0.8)	-1.92 (-2.1 - -1.7)†	-1.0 (-1.1 - -1.0)	-2.1 (-2.3 - -1.9)†
BMIZ	-0.3 (-0.4 - -0.3)	-1.40 (-1.5 - -1.2)†	-0.48(-0.5 - -0.4)	-1.48 (-1.6 - -1.3)†
WC	62.2(61.7 - 62.5)	62.0 (60.7 - 63.2)	62.0 (61.6 - 62.4)	59.6 (58.8 - 60.5)†

Values are expressed as means and 95% confidence intervals. T -test independent sample, † $P < 0.001$, WAZ =Weight for age Z-score, HAZ = Height for age Z-score, BMIZ = Body mass index Z -score WC=waist circumference.

8.2 Pubertal development in SCA compared to non-SCA control population.

Mean age at menarche was significantly greater in the SCA participants with 24% having reported having reached menarche, at a mean age of 14.8y (± 1.2 y SD) compared to 31.7 % at a mean age of 13.2y (± 1.1 y SD) in the non-SCA control population ($P < 0.001$).

Age at the different Tanner stages for breast (B) and pubic hair (PH) development were compared for SCA against non-SCA populations (Tables 2a&b).

Girls with SCA entered breast and pubic hair Tanner stage 2 and completed breast and pubic hair Tanner stage 4 later than non-SCA children ($P < 0.0001$). Girls with sickle cell anaemia begun puberty (breast Tanner stage 2) at a mean age of 14.8 years as compared to their non SCA controls at 11.5years. These were 3.3years later and the difference is highly statistically significant. Below 12.7 years no breast development and below 15.2 years no pubic hair growth observed in girls with SCA. Generally there was asynchronous maturation in the development of puberty, that is, initial areolar/breast (thelarche pathway) then pubic hair (adrenarche pathway) development, without development of the other characteristic.

Table 2a. Ages at Tanner stages for breast and pubic hair development in girls with and without SCA.

Breast Stage (B)	Mean age Non SCA	Mean age SCA	Pubic hair (PH)	Mean age Non SCA	Mean age SCA
Tanner 1	8.7 (796) 8.5-8.8	9.7 (77) 9.7-10.8*	Tanner 1	9.0(921) 8.6-9.1	11.4(102) 10.7-12.1*
2	11.5 (305) 11.3-11.6	14.8(22) 12.7-16.8*	2	12.0(226) 11.6-12.1	15.7(25) 15.2-16.2*
3	13.0(148) 12.7-13.2	15.8(22) 15.3-16.3*	3	13.5(166) 13.2-13.8	16.5(9) 15.7-17.3*
4	14.7(327) 14.6-14.9	16.4(21) 15.8-17.0*	4	15.0(310) 14.8-15.2	16.9(7) 16.0-17.8*
5	16.5(242) 16.3-16.7	18.2 (1)	5	16.7(195) 16.5-16.9	18.2 (1)

* P < 0.001 (t-test independent sample), values expressed as mean, (n) and 95% confidence interval

Boys without sickle cell anaemia enter puberty (G2 and PH2) earlier than those with sickle cell anaemia $p=0.0002$. Below 14.8 years no pubic hair observed in children with sickle cell anaemia, the delay was highly statistically significant $P < 0.001$. However there is no difference in the age at which both groups completed puberty (G4 and PH4). Genital development (penis, testes, and scrotum) appears earlier than pubic hair in both groups.

Table 2b. Ages at Tanner stages for genital and pubic hair development in males with and without SCA.

Genital (G)	Mean age Non SCA	Mean age SCA	Pubic hair (PH)	Mean age Non SCA	Mean age SCA
Tanner 1	9.0(720) 8.8-9.1	9.7(72) 9.2-10.2 [†]	Tanner 1	9.1(771) 8.9-9.2	11.2(128) 10.7- 11.7*
2	12.3(327) 12.1-12.4	13.2(52) 12.5-13.8*	2	12.5(276) 12.4-12.7	15.8(16) 14.8-16.7*
3	14.1(161) 14.0-14.4	15.6(24) 14.8-16.3*	3	14.2(135) 13.9-14.4	16.5(2) 16.3-17.6*
4	16.1(193) 15.9-16.3	16.3(5) 14.8-17.7	4	16(196) 15.8-16.2	16.5(10) 15.7-16.8
5	16.8(165) 16.7-17.0	16.9(4) 15.4-17.5	5	16.8(177) 16.7-17.0	17.5(1)

* $P < 0.001$, [†] $P < 0.05-0.001$ (t-test independent sample), values expressed as mean (n) and 95% confidence interval

8.3 Weight and pubertal development

Weight at different Tanner stages for breast (B) and pubic hair (PH) development were compared for SCA against non-SCA populations (table 3a).

Girls without sickle cell anaemia started puberty with a larger weight than those without sickle cell anaemia. At tanner stage 2 for breast and pubic hair development showed a statistical significant difference ($P < 0.01$) but the mean weights at which they complete puberty in both groups had no statistical significance ($P = 0.16$).

Table 3a. Weights at Tanner stages for breast and pubic hair development in female children and adolescents with and without SCA.

Breast	Mean weight Non SCA (n) 95%CI	Mean weight SCA (n) 95%CI	P-value	Pubic hair	Mean weight Non SCA 95%CI	Mean weight SCA 95%CI	P- value
Tanner 1	24.5(796) 24.2-24.9	23.6(77) 22.5-24.6	0.123	Tanner 1	25.7 25.3-26.1	25.9 24.4 -27.2	0.788
2	34.6(305) 33.8-35.4	30.7(22) 27.4-34.0	0.0106 [†]	2	37.1 36.1 – 38.2	42.1 39.4 –44.9	0.003 [†]
3	41.3(148) 40.1-42.5	41.4(22) 39.0-43.8	0.95	3	42.4 41.2 – 43.5	42.7 39.2 –46.2	0.9057
4	48.6(327) 47.7-49.5	46.0(21) 42.9-49.1	0.1623	4	50.2 49.2 – 51.2	47.1 39.9 –54.3	0.3502
5	55.4(242) 54.3-56.5	61.1(1)		5	55.5 54.4 – 56.6	61.1	

[†]P < 0.05, t- test independent sample

Boys without sickle cell anaemia entered tanner stage 2 and completed tanner stage 4 for genital development with a bigger weight than those with SCA. At Tanner stage 2 for genital development boys without sickle cell anaemia were heavier by 2.4 kg more ($p < 0.001$) and at tanner stage 4 they were heavier by 9.4 kg than those with SCA ($P=0.01$). There was no weight difference for both groups in the development of pubic hair but significant difference was observed at the weight required to complete pubic hair development, more weight on those without sickle cell anaemia ($P < 0.001$).

Table 3b. Weights at Tanner stages for genital and pubic hair development in male children and adolescents with and without SCA.

Genital	Mean weight non SCA(n) 95%CI	Mean weight SCA(n) 95%CI	P-value	Pubic hair	Mean weight Non SCA 95%CI	Mean weight SCA 95%CI	P-value
Tanner 1	26.1 25.6 – 26.6	23.4 22.3 – 24.5	0.001	Tanner 1	26.3 25.8 – 26.8	26.0 25.2 – 27.3	0.9750
2	34.2 33.4 – 34.9	30.4 28.7 – 32.0	0.001	2	34.8 34.0 – 35.6	35.7 31.7 – 39.7	0.6060
3	43.0 41.4 – 44.6	35.7 32.9 – 38.6	0.0008	3	43.2 41.5 – 44.9	44.3 36.6 – 51.9	0.8784
4	51.3 50.1 – 52.4	42.2 34.7 – 49.6	0.0143	4	51.2 50.0 – 52.4	41.5 38.7 – 44.4	0.005
5	56.8 55.5 – 58.1	45.9 34.2 – 57.5	0.0103	5	56.7 55.5 – 57.9	55.9	

T-test independent sample

8.4 Height and pubertal development.

Height at the different Tanner stages for breast (B) and pubic hair (PH) development were compared for SCA against non-SCA populations (Tables 4a&b). There was no difference in the mean heights required for the girls to enter or complete breast development. At Tanner stage 2 for pubic hair development girls with sickle cell anaemia were taller than those without SCA ($P < 0.001$, t test independent sample).

Table 4a. Heights at Tanner stages for breast and pubic hair development in female children with and without SCA.

Breast	Mean height Non SCA (95%CI)	Mean height SCA (95%CI)	P- value	Pubic hair	Mean height Non SCA 95%CI	Mean height SCA 95%CI	P- value
Tanner 1	125.7 125.0-126.3	126.8 124.6-129.1	<0.001	Tanner 1	127.3 126.3-127.9	130.9 128.5-133.2	<0.001
2	140.9 139.9-141.8	141.6 138.3-145.0	0.68	2	144.2 143.3-145.2	151.6 149.5-153.7	<0.001
3	149.1 148.0-150.2	151.2 148.4-154.0	0.1720	3	150.4 149.3-151.5	153.1 149.3-156.8	0.2609
4	154.0 153.0-154.9	153.8 151.7-155.6	0.870	4	154.5 153.6-155.4	152.3 148.3-156.4	0.4972
5	156.6 156.0-157.2	159.2		5	157.2 156.5-157.8	159.2	

T-test independent sample

Boys without SCA started genital development at a higher height than those with SCA (P=0.009) but no significant difference in heights at completion of genital development. However those with SCA develop pubic hair at a higher height than boys without SCA (P= 0.008, t-test independent sample)

Table 4b. Height at Tanner stages for Genital and pubic hair development in male children and adolescent with and without SCA.

Genital	Mean height HbAA 95%CI	Mean height HbSS 95%CI	P- value	Pubic hair	Mean height HbAA 95%CI	Mean height HbSS 95%CI	P- value
Tanner 1	128.0 127.3-128.8	125.7 123.4-128.1	0.056	Tanner 1	128.6 127.9-129.3	131.1 129.1-133.1	0.0095
2	141.8 141.0-142.7	138.5 135.7-141.3	0.0092	2	142.4 141.5-143.3	147.6 141.7 –53.3	0.0083
3	152.7 151.0-154.0	148.1 143.9-152.3	0.048	3	152.4 150.7-154.1	158.7 158.4-187.9	0.3726
4	161.7 160.7-162.8	155.6 147.3-164.0	0.0629	4	161.6 160.6-162.6	159.9 155.3-160.5	0.1088
5	164.9 163.9-166.0	162.4 152.3-172.5	0.4559	5	165.6 164.6-166.5	171.0	

T-test independent sample

8.5 Waist circumference and pubertal development

Waist circumference at the different Tanner stages for breast (B) and pubic hair (PH) development were compared for SCA against non-SCA populations (Tables 5a&b). There was no significant difference in their waist circumference (WC) for the girls with and without SCA when they started and completed breast development. On the other hand the mean waist circumference for girls with SCA was bigger than those without SCA when they started pubic hair development ($p = 0.001$). However there was no significant difference in the mean waist circumference in the boys with and without SCA when they entered or completed puberty.

Table 5a. Waist circumference at Tanner stages for breast and pubic hair development in female children and adolescent with and without SCA.

Breast	Mean WC Non SCA 95%CI	Mean WC SCA 95%CI	P-value	Pubic hair	Mean WC Non SCA 95%CI	Mean WC SCA 95%CI	P-value
Tanner 1	55.8 55.4-56.1	56.2 54.6-57.9	0.4634	Tanner 1	56.6 56.3-57.0	58.3 56.8-59.8	0.0056
2	62.2 61.5-62.9	62.9 61.5-64.2	0.6139	2	63.5 62.5-64.4	68.4 66.4-70.5	0.0011
3	65.7 64.6-66.8	69.8 67.3-72.2	0.0088	3	66.3 65.3-67.3	67.8 66.2-69.3	0.5122
4	69.4 68.6-70.1	70.6 67.8-73.3	0.4161	4	70.2 69.3-71.1	76.0 68.1-83.7	0.0548
5	71.1 70.1-72.1	84		5	70.4 69.5-71.3	84	

t-test independent sample

Table 5b. Waist circumference at Tanner stages for genital and pubic hair development in male children and adolescent with and without SCA.

Genital	Mean WC Non SCA (95%CI)	Mean WC SCA 95%CI	P-value	Pubic hair	Mean WC Non SCA 95%CI	Mean WC SCA 95%CI	P-value
Tanner 1	57.3 56.9-57.8	56.4 55.5-57.4	0.2209	Tanner 1	57.5 57.1-57.9	58.4 57.6-59.3	0.0755
2	61.7 61.1-62.4	61.6 60.2-62.9	0.8545	2	61.9 61.2-62.6	63.3 60.8-65.8	0.3777
3	66.4 65.2-67.2	63.0 61.0-65.1	0.0432	3	66.3 65.0-67.7	71.5 65.1-77.8	0.3479
4	69.2 68.1-70.2	65.4 59.3-71.3	0.2521	4	68.7 67.7-69.7	65.2 62.4-68.0	0.1324
5	69.9 70.0-70.8	67.3 61.4-73.1	0.3799	5	70.8 69.8-71.6	72.0	

T-test independent sample

8.6 BMI and pubertal development.

BMI at the different Tanner stages for breast (B) and pubic hair (PH) development were compared for SCA against non-SCA populations (Table 6a&b). Girls without SCA started breast development with a large mean BMI as compared to those with SCA ($p=0.001$, t-test). However there was no significant difference in their mean BMI when they started pubic hair development or completed puberty in general.

Boys without SCA started genital development with a large mean BMI (fatter) than those without SCA $p < 0.001$. There was no significant difference in their mean BMI when they completed genital development in both groups. Statistical difference was observed in their mean BMI when completing pubic hair development in which boys with sickle cell anaemia were thinner than those without.

Table 6a. BMI at Tanner stages for breast and pubic hair development in female children and adolescent with and without SCA.

breast	Mean BMI		P-value	Pubic hair	Mean BMI		P-value
	Non SCA 95%CI	Mean BMI SCA 95%CI			Non SCA 95%CI	Mean BMI SCA 95%CI	
Tanner 1	15.5 15.2-15.9	14.7 14.2-15.2	0.1280	Tanner 1	15.8 15.5-16.1	15.0 14.5-15.5	0.0959
2	17.3 17.0-17.6	15.4 13.9-17.0	0.001	2	17.8 17.4-18.1	18.3 17.3-19.2	0.4299
3	18.6 18.1-19.0	18.0 17.3-18.8	0.4476	3	18.6 18.3-19.1	18.2 16.8-19.6	0.6195
4	20.4 20.1-20.7	19.4 18.3-20.6	0.1650	4	21.0 20.6-21.3	20.2 17.7-22.8	
5	22.5 22.1-22.9	24.1		5	22.4 22.0-22.8	24.1	

T-test independent sample

Table 6b. BMI at Tanner stages for Genital and pubic hair development in male children and adolescent with and without SCA.

Genital	Mean BMI Non SCA 95%CI	Mean BMI SCA 95%CI	P- value	Pubic hair	Mean BMI Non SCA 95%CI	Mean BMI SCA 95%CI	P- value
Tanner 1	15.9 15.5-16.3	14.7 14.3-15.0	0.0553	Tanner 1	15.8 15.5-16.2	15.1 14.8-15.4	0.0975
2	16.9 16.7-17.1	15.7 15.3-16.1	<0.001	2	17.1 16.8-17.4	16.2 15.4-17.0	0.1714
3	18.2 17.8-18.6	16.1 15.5-16.7	0.0002	3	18.4 17.9-19.0	17.6 16.0-17.7	0.7086
4	19.6 19.2-19.9	17.3 15.6-19.2	0.6749	4	19.5 19.2-20.0	16.6 15.9-17.3	<0.001
5	20.9 20.4-21.3	17.4 15.1-19.5	0.0151	5	20.7 20.3-21.1	19.1	

T-test independent sample

8.7 Body fat percent and its relation to sexual maturation stages in children with sickle cell anaemia

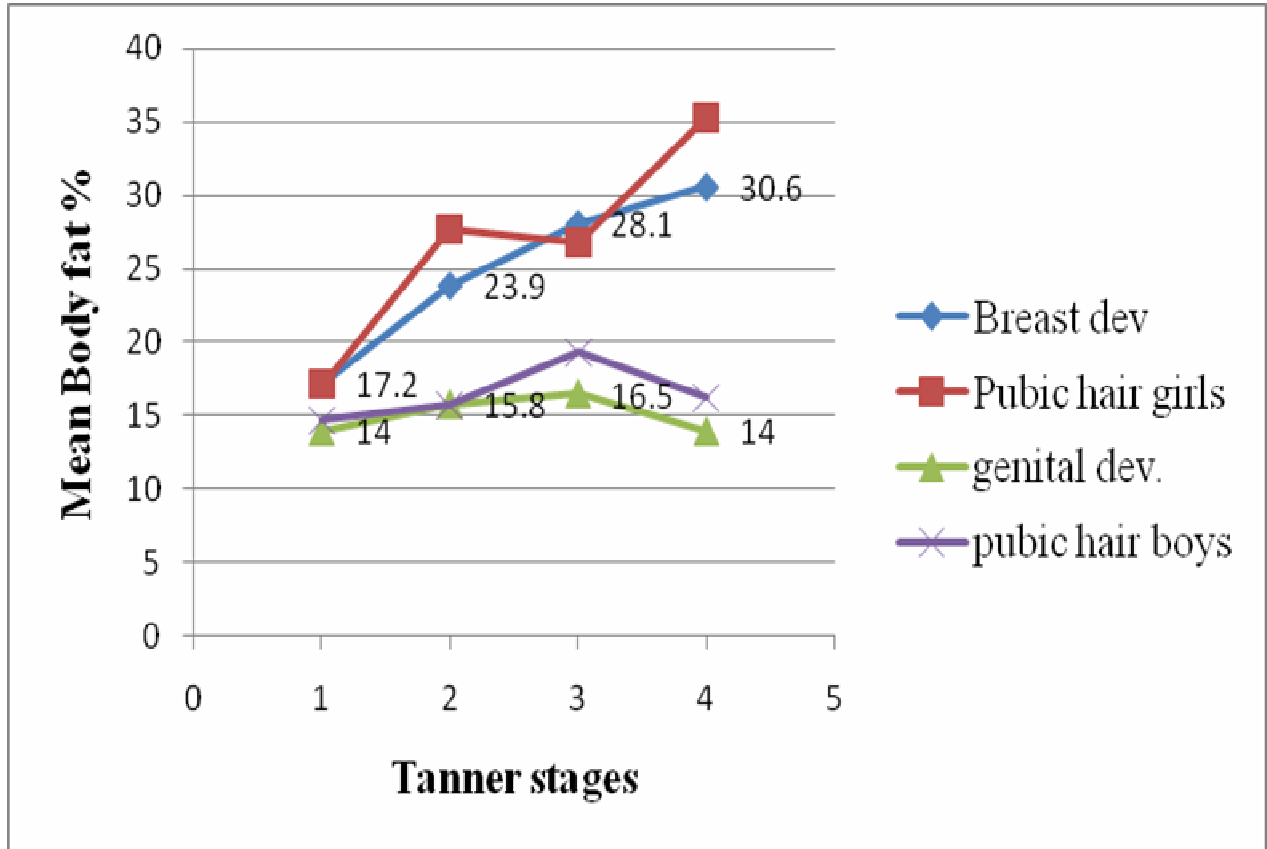
Body fat percent among children and adolescent with sickle cell anaemia was determined and compared within the pubertal Tanner stages figure 1.

In girls body fat percent increased as breast and pubic hair Tanner stage increased and at stage 3 the mean body fat for pubic hair was similar to body fat for breast development. The maximum body fat was attained at stage 4 for both breast and pubic hair development.

In the Univariate analysis of variance the pair wise comparison among the breast and pubic hair Tanner stages indicated that the estimated marginal means of body fat in Tanner stage one was highly statistically significant from stage two, three and four ($P < 0.001$).

In boys body fat increased as the Tanner stage for pubic hair and genital development increased and peaked at Tanner stage 3 thereafter there was a gradual decrease in body fat percent. The estimated marginal means of body fat in genital and pubic hair Tanner stage one was statistically significant different from stage two through four ($P < 0.001$) but the mean fat at tanner stage two was not statistically significant different from stage three ($P = 0.211$).

Figure1. Unadjusted mean body fat percent trends for breast, genital and pubic hair development from Tanner stage 1 through 5 in children with sickle cell anaemia.



8.8 Relationship of growth parameters and disease severity indices to onset of puberty (Tanner stage 2 or more) in children with sickle cell anaemia

Relationship between growth parameters, disease severity indices and pubertal development Tanner stage 2 was considered in a Univariable and multivariable logistic regression analysis as

shown in table 7a and b. When BMI adjusted for age and body fat percentages were included in a multivariable model, body fat was found to be a good predictor of puberty than BMI. In the Univariable analysis age and body fat percent were positively associated with pubertal development in girls. In every increase in a year the likely hood for a girl to enter puberty was 3times and an increase of 1% body fat the likely hood of entering puberty was 1.4 times. Same results in the multivariable analysis and therefore independently advanced age and high body fat were found to predict puberty.

Table 7a. Factors associated with onset of puberty (Tanner stage 2 or more) in girls

Term	Univariable regression			Multivariable regression		
	Odds ratio	95% CI	P-value	Odds ratio	95% CI	P-value
Socio-economic status	0.6	0.3-1.4	0.086	-	-	-
Age	3.1	2.2- 4.4	< 0.001	2.8	1.9 –4.3	< 0.001
Weight for age Z-score	1.1	0.86 – 1.4	0.389	-	-	-
Height for age Z- score	1.0	0.76 – 1.4	0.927	-	-	-
Body fat percentage	1.4	1.2 – 1.5	< 0.001	1.3	1.1 – 1.6	< 0.001
Number of admission	1.0	0.9 – 1.1	0.66	-	-	-
Number of blood transfusion	1.2	1.0 – 1.4	0.05	-	-	-

Advanced age, weight for age z- score, height for age z-score and body fat percent were positively associated with pubertal development for boys in the Univariable analysis (table 7b). Boys were 2 times more likely to enter puberty in every increase by one year. Similar results in the multivariable analysis and the results were highly statistically significant (P < 0.001).High body fat percent, high weight for age and height for age z-score were not associated with puberty

in the multivariable analysis and therefore only advanced age could predict puberty independently in boys.

Table 7b. Factors associated with onset of puberty (Tanner stage 2 or more) in boys

Term	Univariable regression			Multivariable regression		
	Odds ratio	95% CI	P-value	Odds ratio	95% CI	P-value
Socio-economic status	0.8	0.4-1.5	0.547	-	-	-
Age	2.0	1.6 – 2.4	< 0.001	2.2	1.7 – 2.8	< 0.001
Weight for age Z-score	0.7	0.5 – 0.9	0.003	1.8	0.8 -3.9	0.164
Height for age Z-score	0.6	0.4 – 0.8	0.001	0.8	0.4 – 1.8	0.59
Body fat percentage	1.04	0.9 - 1.1	0.422	-	-	-
Number of admission	1.0	1.0 – 1.1	0.258	-	-	-
Number of blood transfusions	1.1	0.9 – 1.2	0.308	-	-	-

9.0 DISCUSSION

The pubertal growth spurt and the appearance of secondary sex characteristics are the most visible manifestations of puberty ⁽¹⁴⁾. It is the lack of one or both of these that brings most teenagers to the health care facility.

Assessment of the onset and progression of sexual maturation are important in patients with sickle cell anaemia. The information has immediate clinical application in the interpretation of endocrine and growth status. In Africa few studies on pubertal development have been published. Baseline reference data is required to facilitate the interpretation of sexual maturity assessments in Tanzanian children with sickle cell anaemia. The aim of this study was to compare growth and pubertal development of children with sickle cell anaemia to normative references of urban Tanzanian children in Dar es Salaam.

In the studied sickle cell population the mean age and z-scores for height for age and body mass index were similar between girls and boys. As well the measure of disease severity by sex was similar. This means that sex was not a confounding factor for the study and that the sickle gene has affected them equally.

Girls were heavier and had a higher percentage body fat than boys. Normally during puberty rising levels of oestrogen in girls enhance fat deposition. Therefore fat tissue increases to a greater percentage of the body composition in girls than in boys. It has been reported that at the age of 10 years, the average girl has 6% more body fat than the average boy increasing steadily to 50% more body fat by the end of puberty ⁽¹⁴⁾.

Growth and Pubertal development in Tanzanian children with sickle cell anaemia was found to differ from children without sickle cell anaemia. Delayed sexual maturation in children with sickle cell anaemia was evident in this study. The mean age at menarche (14.8 ± 1.2 years) was later than that reported from girls without sickle cell anaemia in the non-SCA control population (13.2 ± 1.1 years). The delay was 1.6 years and the difference was highly statistically significant.

The delay in mean age at menarche could be due to poor nutritional status in children with sickle cell anaemia as demonstrated by low Z-score for weight, height and body mass index for age. This supported what has been observed in other studies that as a group, overweight girls tend to mature earlier than girls who are not overweight. Likewise underweight females generally experience menarche at later ages than normal weight females^(54,55).

Delay in the mean age at menarche has been reported by Modebe O. et al among Nigerian school girls⁽⁴⁰⁾. That study reported a mean age at menarche to be 14.5 ± 1.13 years for SCA girls as compared to 13.3 ± 1.09 years in non SCA girls. The Nigerian girls mature at almost same age as Tanzanian girls with and without sickle cell anaemia. This similarity in delayed menarche could be explained by the fact that both studies originate from African countries and that they may share similar genetic and environmental factors.

The delay on the mean age at menarche has also been reported by M'Pemba-Loufoua et al in Brazzaville⁽³⁷⁾. He reported age at menarche of 15.2 ± 1.6 years in SCA as versus 13.4 ± 1.4 years for controls. The mean age was later than what we found in our study. Lack of comprehensive care programmes in Congo and early detection and treatment of acute events in children with SCA could account for the delay. The author reported in another study that, Congolese adolescent with SCA were associated with increased number of hospitalization and blood transfusion⁽⁵⁶⁾. Endocrinological factors as well might account for this observation since familial short stature is common in Congo and therefore could have affected the mean age at menarche because even for the non SCA population puberty was delayed compared to Tanzanian non SCA.

G R Serjeant et al did a study to investigate the distribution of age at menarche in Jamaican girls with sickle cell anaemia as compared to controls⁽³⁹⁾. He reported mean age in normal controls (13.0 years) to be significantly earlier than in SS disease (15.4 years).

Comparing Jamaican results to this study, Tanzanian girls with sickle cell anaemia attained menarche 0.6 years earlier than Jamaican girls. Most Jamaican people are of African origin and may therefore share similar genetic factors although environmental factors may modify the age at menarche. However the differences observed between Tanzanian and Jamaican study could be due to the fact that this study relied on the ability to recall for estimating the age at menarche while G.R. Serjeant et al used a longitudinal study.

The age at different Tanner stages for breast (B) and pubic hair (PH) development in children with SCA was compared to that for non-SCA populations. There was a significant delay in the age at puberty in all sexual maturation parameters. The signs of physical maturation like puffiness of the mammary glands was observed at a mean age of 14.8 years (95% CI =12.7-16.8) as compared to 12.0 years (95% CI =11.6-12.1) in the non SCA controls. Pubic hair growth in SCA girls were observed at a mean age of 15.7 years (95%CI=15.2-16.2) as compared to 12.0 years (95%CI=11.6-12.1) of the non SCA control population.

The mean age of girls in stages 2 through 4 for breast stage and pubic hair development was 1.4 to 2.8 years delayed in comparison to their control non SCA children.

A similar pattern of delay was observed for the boys with sickle cell anaemia as compared to those without sickle cell anaemia. The causes of delayed puberty in this population are multi-factorial. One of the factors is poor nutritional status as supported by low z-scores for weight, height and BMI for age. Cox S.E et al reported a significant increase in prevalence of malnutrition in Tanzanian patients with sickle cell anaemia as compared to controls ⁽²⁴⁾. She reported that 18% of the main cohorts were <2.5th percentile for BMI and that suggested an adaptation to chronic energy/nutrient deficiency, perhaps from slowed rates of growth and stunting. Another factor could be due to direct genetic effects which account for at least 46% of the variation of timing of puberty in well-nourished populations ⁽¹⁴⁾. However the specific genes affecting timing are not defined yet although androgen receptor gene has been implicated.

Some studies have reported endocrine dysfunction in children with sickle cell anaemia ^(21, 36). Olambiwonnu N.O et al in Nigerian children with SCA reported increased gonadotropin concentrations for stage of sexual development. His findings suggested a transient impairment of gonadal function during the first decade of life. In Saudi Arabia el-Hazmi M.A et al as well reported gonadal hypo function in children with sickle cell anaemia. The endocrine abnormality described in their studies could account for the delayed puberty in our setting in addition to malnutrition.

Tanner breast stage two in Congolese girls with SCA reported to develop at 14.4 ± 1 years as versus 12.4 ± 1.5 years in controls⁽³⁷⁾. Tanzanian girls with sickle cell anaemia were at breast

development Tanner stage two 0.4 years later than Congolese girls. Tanzania and Congo as sub-Saharan countries may share similar environmental factors affecting age at puberty.

In American children with sickle cell anaemia similar delay has been reported especially the age at which they complete puberty as reported by Platt O S et al in their study⁽¹⁹⁾.

Findings from my study suggest that we need to adopt an approach in managing delayed puberty as it is necessary not only for psychological reasons but also for optimizing bone mass accretion. Therefore it is high time to use standard guideline in the treatment of complications related to sickle cell anaemia so as to reduce morbidity.

Children with sickle cell anaemia have low z-scores for weight, height and body mass index as compared to non sickle cell children in the general population. The causes of poor growth in children with sickle cell anaemia are probably multi factorial. Some of them could be due to decreased appetite and food intake during illness and increased energy expenditure due to hyperactive bone marrow and hyper metabolism.

Similar findings has been reported by Barden et al in their study to assess growth, nutritional status, and body composition in 36 African American children with sickle cell anaemia⁽²²⁾. Compared with control subjects, children with SCA had impaired growth and delayed skeletal maturation. In that report, z-scores for weight, height, arm circumference, and upper arm fat and muscle areas were significantly lower than their controls. The suboptimal growth was attributed to chronic energy deficiency observed in children with sickle cell anaemia. In Tanzania where nutrition is suboptimal for many families, nutritional factors are the strongest and most obvious environmental factors affecting growth.

Weights at Tanner stages for breast and pubic hair development in girls with and without SCA were compared. Girls with sickle cell anaemia at puberty have significantly lower weights than those without sickle cell anaemia. The growth during puberty continued normally since weights of girls with and without SCA observed at the end of puberty were similar. This finding has not been observed in other studies done in America and Brazil^(57, 58). They reported a significant lower weight throughout puberty in children with SCA as compared to non SCA control population. Cox S.E et al reported a lack of growth spurt during adolescent in Tanzanian SCA cohort as compared to UK reference data⁽²⁴⁾. She postulated that growth retardation was likely,

at least partly, an artefact from delayed and extended puberty, particularly in females which allowed for a substantial degree of catch up growth.

Boys with sickle cell anaemia in this study have low body weight, height, body mass index and waist circumference throughout puberty compared to boys without sickle cell anaemia. Boys followed the growth pattern reported in other studies from 1960s to 1990s which described low weight and height percentiles in children with SCA as compared to normal children ⁽¹⁶⁻²⁰⁾.

Combining results of girls and boys with sickle cell anaemia, boys have poor growth than girls. The reason for the difference in physical growth pattern between girls and boys with sickle cell anaemia observed in this study is not known. This calls for an investigation to identify potentially possible modifiable factors that may promote more normal development in boys as they are more affected. In the analysis of nutritional status of the main SCA cohort Cox S.E et al observed more wasting, underweight and stunting in males than females ⁽²⁴⁾. It was postulated in that study that, the disparity between the age groups in the cohort and differences in SCA effects on pubertal delay between males and females may explain the discrepancies observed between males and females with SCA.

The disproportion was similar to what was reported by Phebus C.K et al ⁽¹⁶⁾. In his study to assess growth patterns by age and sex in children with sickle cell anaemia he observed that, boys were more severely affected than girls. Also Singhal, A et al in their analysis of growth data from children in the Jamaican Cohort Study, noted retardation of adolescent growth and development was common in boys with SS disease than in girls ⁽⁵⁹⁾.

In girls the mean height at puberty through breast development pathway was not different between the groups but there was statistical significant difference in height through pubic hair pathway. As for weight in girls with sickle cell anaemia, height throughout puberty was similar to those without SCA. Tanzanian findings were different from those described in Jamaican and American studies ^(16-20, 60) where weight and height was lower in SCA girls as compared to non SCA.

Similar trend for waist circumference and mean body mass index throughout puberty has been observed between girls with and without sickle cell anaemia. Barden et al in their study to determine body composition in African – American children with sickle cell anaemia reported deficits in fat (energy) stores, and low free fat mass coupled with low upper arm muscle area indicating muscle wasting as compared to non sickle cell children ⁽²²⁾. Since Tanzania is a low

income country its children and adolescents overall growth is probably equally affected by malnutrition.

The mean body fat percent increases as breast and pubic hair Tanner stage increases in girls. This observation led to the hypothesis that a degree of body fatness is needed to trigger the neuroendocrine events that lead to the onset of menses. In this study the mean body fat percent at tanner stage two for breast was 23.9 % (CI 21.8-26.1) and for pubic hair was 27.7% (CI 24.7-30.8) . This was a critical amount of body fat implying that a particular body composition, in addition to other environmental and psychosocial factors, was important in triggering and maintaining the pubertal process as discussed by nFrisch et al ⁽⁶¹⁾. Mounir GM et al in their study among Alexandrian adolescent reported similar findings suggesting a linear correlation between body fat percentage and the stage of sexual maturity. Age and higher body fat percent were positively associated with pubertal development in girls with sickle cell anaemia in this study. A high amount of fat was required to trigger the neural hormonal system to initiate puberty and therefore needed time to accumulate. The findings are consistent with the hypothesis that suboptimal nutrition and increased metabolic demands in sickle cell anaemia may delay physical and sexual development.

The findings were not similar to what was observed from the USA Cooperative Study and the study by Serjeant G R et al among Jamaican girls with sickle cell anaemia ^(19, 39). The studies did not estimate percentage body fat instead used only weight, height and haematological indices as potential determinant of pubertal development. Age and weight were the principle determinant of age at menarche. In the multi-ethnic population study by Britton et al adiposity and height were significantly positively associated with breast or pubic hair development ⁽³⁵⁾. In Tanzanian study height was not found to be predictive of pubertal development. Poor nutritional status might be responsible for this observation since girls with sickle cell anaemia were found to have low z-scores for weight, height and BMI as compared to normal girls in the population.

The percentage of fat in boys increased from 14.0% in genital Tanner stage one to a maximum of 16.5% at stage three during aged 9.7 – 15.6years. There was a significantly decrease in percentage of fat to about 14% in the 16 - 18 age groups in Tanner stage 4. At Tanner stage one through three is the time for pubertal growth spurt and accumulation of fat as a source of energy

to maintain puberty. Thereafter the body fat changes to muscle mass in boys. By the end of puberty boys have heavier bones and nearly twice as much skeletal muscle. The dramatic decrease in body fat percent from stage three was expected in boys as a normal physiological change during puberty. The average male at Tanner stage 5 has been reported to be about 150% of the lean body mass of an average female, and about 50% of the body fat⁽¹⁴⁾.

A combination of increased resting energy expenditure and nutritional factors accounts for many of the reported changes in body composition in children and adolescents with SCA. The effects of chronic illness, anaemia, increased cardiac workload, hyperactive erythropoiesis, increased protein turnover, and inflammatory and oxidative stress all contribute to the hyper metabolic state in SCA^(31, 32). Similar result in changes in body fat has been observed by Xiao Yan-jie et al in Chinese primary and secondary school students⁽⁶²⁾.

Only age was associated with pubertal development in boys with sickle cell anaemia. In the Univariable analysis body fat percent was positively associated with pubertal development but controlling for age and other factors in the multivariable analysis no association was seen. These findings in boys with sickle cell anaemia could be explained by the fact that they were thin containing low body fat and BMI z-scores as compared to girls with sickle cell anaemia – this doesn't explain it.

10. STUDY LIMITATIONS

The study was a cross sectional with historical control and therefore will not be able to establish exactly when one will be starting or completing a pubertal Tanner stage. Therefore results need to be used cautiously to estimate when one is expected to enter puberty. Under-representation of older age groups to be able to accurately get the mean age at final Tanner stages (age at completion of puberty) and determine the prevalence of adults >18 who have not yet reached Tanner stage 4 or not.

Strengths of the study.

The epidemiology of the development of secondary sexual characteristics in children with sickle cell anaemia in our setting has not been studied before. Therefore this cross sectional data can be used as a baseline reference in the interpretation of precocious or delayed puberty in children with sickle cell anaemia population.

11. CONCLUSION

Growth and pubertal development in children and adolescents with sickle cell anaemia was significantly delayed as compared to non sickle cell children. The monitoring of growth and pubertal development in children with SCA is an essential requirement for comprehensive care, facilitating early diagnosis of growth failure and delayed puberty.

12. RECOMMENDATIONS

Growth and pubertal development monitoring should be part of the comprehensive care of children and adolescents with SCA to facilitate early diagnosis of poor growth and delayed puberty.

A longitudinal study is needed to establish the possible causes of disproportional growth between males and females with SCA during puberty. Also need to establish potentially modifiable causes of poor growth and delayed puberty in our society eg. Nutritional and hormonal.

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APPENDIX –I

CONSENT FORM FOR PARTICIPATION IN A STUDY (English version).

Study No.....

Title: Growth and pubertal development in children with SCA at Muhimbili National Hospital

To the Parents/ Guardians of

Foreword

I am Dr. Theopista Jacob a postgraduate student at MUHAS conducting a study for children aged 6-18 years with sickle cell anaemia on their growth and pubertal development.

How to participate

Weight, height, skin fold thickness and waist circumference measurements will be made in all eligible children with sickle cell anaemia, and then will be assessed on their breast and pubic hair development. Every child will be examined by an investigator of the same sex in a private room/area which will be prepared at the clinic. The whole examination is estimated to take not more than 10 minutes per each child. The evaluation is not compulsory, meaning that any parent/guardian of the child is free to accept or refuse to be involved in the study without affecting the child's clinic activities or treatment conduct.

APPENDIX –II**FOMU YA RIDHAA YA KUSHIRIKI KATIKA UTAFITI (Swahili version).****Namba ya utafiti.****Kichwa cha Habari: Ukuaji na maendeleo ya balehe kwa watoto wenye ugonjwa wa siko seli Muhimbili.**

Kwa Mzazi/Mlezi wa.....

Utangulizi

Mimi Dr.Theopista Jacob mwanafunzi wa udhamili Chuo Kikuu cha Sayansi za Afya ya Muhimbili nafanya tathmini ya hiari kwa watoto wenye matatizo ya siko seli kuangalia maendeleo ya ukuaji kwa watoto wa umri wa miaka 6-18 na kuangalia maendeleo yao ya balehe.

Taratibu za kushiriki

Watoto wa umri kati ya miaka 6-18 watapimwa uzito, urefu, mzunguko wa kiuno(waist circumference) , unene wa ngozi na kuangalia ukuaji wa vinyweleo na mandeleo ya ukuaji wa matiti kwa watoto wa kike. Kila mtoto ataangaliwa na Daktari wa jinsia yake kwenye chumba au sehemu maalumu iliyotayarishwa hapa clinic. Upimaji wa watoto pamoja na tathmini hii inakadiriwa kuchukua si zaidi ya dakika kumi tu kwa kila mtoto Tathmini hii ni ya hiari kabisa,

kila mtoto au mzazi ana hiari ya kukataa au kukubali, na hii haitaathiri shughulu za mtoto hapa kliniki. Kila mzazi/mlezi atajulishwa endapo kutapatikana tatizo lolote linalohitaji uchunguzi au matibabu zaidi.

Dhumuni la Utafiti

Utafiti huu utawezesha kupata takwimu muhimu kuhusu hali ya lishe, ukuaji na balehe kwa watoto wa Kitanzania wenye matatizo ya siko seli. Na pia itawezesha kujua ni wakati gani muafaka mtoto wa Kitanzania mwenye siko seli anapaswa apatiwe elimu ya jinsia kutegemeana na kupevuka kwake.

Utafiti huu umepata kibali kutoka kwa kamati ya jopo la madaktari wa Chuo kikuu cha Tiba cha Muhimbili

Hatari

Hakuna kipimo chochote cha damu kitakachochukuliwa. Zoezi hili si la utoaji wa chanjo au dawa yoyote ya kunywa. Hivyo hakuna hatari yoyote inayotarajiwa kutokea kwa mtoto kushiriki katika utafiti huu.

Ridhaa ya makubaliano/ kukubali

Nimesoma na kuelewa maelezo kuhusu utafiti huu. Nakubali mwanangu apimwe na kushiriki katika utafiti huu.

Sahihi ya mzazi/mlezi.....

Uhusiano na mtoto.....

Tarehe.....

Mtoto amekubali kushiriki kwenye utafiti? NDIYO HAPANA

Kwa ufafanuzi au maelezo zaidi waweza kuwasiliana na mmoja kati ya madaktari wafuatao.

Dr. Theopista Jacob , simu namba 0787364643

Dr. F.Kalokola , simu namba 0754844597

Dr. Sharon Cox , simu namba 0755406115

APPENDIX III

OYEDEJI CLASSIFICATION OF SOCIAL CLASS

Points	Occupation	Level of Education
1	Senior Public Servants, Professional, Managers, Large scale traders, Businessman contractors	University graduates and equivalent
2	Intermediate grade public servant and Senior School Teachers	Secondary School Certificate (A level) with teaching or other professional training
3	Junior School Teacher, Drivers Artisan	Secondary School Certificate (O level) or Grade II teachers Certificate holders or equivalent.

4	Petty traders, Labourers, messengers and similar grades	Primary School education
5	Unemployed	Can just read and write or illiterate

The sum of father's occupation and education scores plus mother's occupation and education scores divided by 4 gives the social class.

Scores: 1 to 2- High social economic class

2.1 to 3-Middle Social economic class

3.1 to 5- low social economic class

APPENDIX –IV

Questionnaire

SCD Study No|_|_|_|_|

PROFORMA FOR GROWTH AND PUBERTAL STUDY

SCREENING

- Informed consent (N/Y)..... |_|
- Today's Date (DD-MM-YY) |_|_|-|_|_|-|_|_| **DATETOD**

DEMOGRAPHIC HISTORY

- Name.....|_____| **NAME**
- Sex (M/F)..... |_| **SEX**
- Date of birth..... |_|_|-|_|_|-|_|_| **DOB**
- Respondent (Self/Parent/Guardian/Other)..... |_| - |_____| **RESPON**

RESIDENCE INFORMATION

Are you currently residing in Dar es Salaam? Yes |_| No |_|

If NO Region|_____| **REGION**

If YES District|_____| **DISTR**

GENERAL EXAMINATION

	Father	Mother
Education (Score)		
Occupation (Score)		
Mother's age at menarche	X	














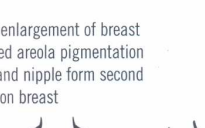
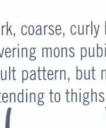



Comments

Doctor's Name.....

Signature.....

APPENDIX V

TANNER STAGING FOR BOYS AND GIRLS

BOYS			GIRLS		
	Testicular Size ¹	Pubic Hair	Breasts	Pubic Hair	
STAGE 1	Length: <2.5 cm Volume: 1,2,3 mL	None 	<ul style="list-style-type: none"> No palpable glandular tissue Areola not pigmented Breast does not project from wall 	None 	STAGE 1
STAGE 2	Length: >2.5–3.3 cm Volume: >3,4,5,6,8 mL	Sparse growth at base of penis 	<ul style="list-style-type: none"> Glandular tissue palpable, continuous to diameter of areola Nipple and breast project as single mound 	Occasional wispy strands along labia 	STAGE 2
STAGE 3	Length: >3.3–4.0 cm Volume: >10,12,15 mL	Darker, coarser, curly hair over junction of pubes 	<ul style="list-style-type: none"> Glandular tissue beyond areola Nipples are enlarged and becoming pigmented Contours of breast and areola in single plane 	Darker and coarser hairs extending superiorly over pubis 	STAGE 3
STAGE 4	Length: >4.0–4.5 cm Volume: >15,20 mL	<ul style="list-style-type: none"> Adult pattern No spread to medial surface of thigh 	<ul style="list-style-type: none"> Further enlargement of breast Increased areola pigmentation Areola and nipple form second mound on breast 	Dark, coarse, curly hair covering mons pubis in adult pattern, but not extending to thighs 	STAGE 4
STAGE 5	Length: >4.5 cm Volume: >25 mL	<ul style="list-style-type: none"> Adult in quantity and type, but in inverse triangle Spread to medial thighs, but not to linea alba 	<ul style="list-style-type: none"> Adult pattern with 	<ul style="list-style-type: none"> Adult pattern with 	STAGE 5
STAGE 6		<ul style="list-style-type: none"> Adult pattern with 	<ul style="list-style-type: none"> Adult pattern with 	<ul style="list-style-type: none"> Adult pattern with 	STAGE 6