GROWTH AND PUBERTAL DEVELOPMENT AMONG HIV INFECTED CHILDREN AGED 8-18 YEARS IN DAR ES SAALAM

By

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

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Muhimbili University of Allied and Health Sciences dissertation entitled **Growth and pubertal development among HIV infected children aged 8-18 years in Dar es Salaam**, as partial fulfillment of the requirement for degree of Master of Medicine (Paediatrics and Child Health) of the Muhimbili University of Health and Allied Sciences.

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DECLARATION AND COPYRIGHT

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

Signature…….. Date…….

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DEDICATION

This work is dedicated to my parents, Dr Reginald and Enike Mbwile for their love, kind and support.

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And to God, the creator of all things, who is the source of all knowledge and healing power

ABSTRACT

Background

Advances in management of HIV-infected infants and children have been remarkable, majority of infected children are now surviving into adolescence.

Several studies have shown that growth and pubertal development is often impaired among children with HIV and AIDS. Abnormalities include early deficits in height and weight, and delay in skeletal maturation. Onset of menarche and pubertal development are also delayed. The magnitudes of delayed puberty among HIV infected children in Tanzania have not been studied; however there is one unpublished study, which evaluated the growth and pubertal development parameters in the general population.

Assessment of the onset and progression of sexual maturation is important in patients with HIV because this information has immediate clinical application in the interpretation of endocrine and growth status.

Objective

To assess growth and pubertal development among HIV infected children aged 8-18 in Dar es Salaam.

Study design and Setting

This was a cross section hospital based study at Care and treatment clinics (CTC) municipal Hospitals in Dar es Salaam.

Methodology

After obtaining informed consent data was collected using a structured questionnaire. Anthropometric measurements of growth and Tanner stages of sexual development of children with HIV and AIDS aged between 8 and 18 years were assessed. Blood was taken to assess CD4 count. Both female and male were classified as having puberty when they are at Tanner stage 2 or greater for breast and genital respectively and pubic hair development in both sexes. Data was analyzed using STATA version 10 statistical packages.

Results

During the study period, 330 HIV infected children were recruited out of whom 183 (55.4 %) were female. The median age of the study populations was 12.0 years. Median weight was 32.0 (IQR 25-45) kg and girls were significantly heavier and had higher BMI than boys. All participants enrolled had HIV which was confirmed and were on ART. Median duration of ART was 48 (IQR 30-62) month. Majority of the participants were in WHO stage III and had CD4 count above 500cells/ul. HIV infected children were found in Tanner stage 2 at an advanced age compared to the reference population.

The median age at menarche was 15 (IQR 14-16) years compared to the reference population, which was 13.0 (IQR 12-15) years. Among HIV infected females there was no significant difference in weight, height and BMI compared to the reference population when they entered Tanner stage 2. Males in the reference population were in Tanner stage 2 with higher weight and BMI and they were taller compared with the reference population.

In univariable and multivariable analysis advanced age was associated with onset of Tanner stage 2 or more.

Conclusion and recommendation

Children infected with HIV and AIDS have significant delay in growth and sexual maturation. Considering these findings monitoring of growth and pubertal development should be highly emphasized in this population, it should be part of the comprehensive package. Matched case control studies of HIV-infected and uninfected children are needed to better quantify the delay in pubertal onset and to compare the pace of pubertal maturation.

ABBREVIATIONS

AIDS	-	Acquired Immunodeficiencndrome
ART	-	Antiretroviral Therapy
BMI	-	Body Mass Index
CD4	-	Cluster of Differentiation 4
CDC	-	Centre for Disease and Control
CTC	-	Care and Treatment Clinic
GH	-	Growth Hormone
HAART	-	Highly Active Antiretroviral Therapy
HIV	-	Human Immunodeficiency Virus
IDC	-	Infectious Disease Center
IGF-I	-	Insulin Growth Factor 1
IQR		Interquartile Range
MDH	-	Management Development for Health
MUHAS	-	Muhimbili University of Health and Allied Sciences
PCR	-	Polymerase Chain Reaction
U.S	-	United States of America
WHO	-	World Health Organization
ICOTRA and	-	International Clinical Operation and Health on TB AIDS
UNAIDS	-	United Nations Programme on HIV/AIDS

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CHAPTER ONE

1. INTRODUCTION AND LITERATUTE REVEW

1.1 Epidemiology of HIV and AIDS

HIV and AIDS still provide a major global challenge, a major cause of morbidity and mortality in children. According to the joint United Nations Programme on HIV/AIDS (UNAIDS), 33 million people worldwide were estimated to be living with HIV or AIDS at the end of 2009[1]. Most reside in the developing world, with approximately two-thirds in sub-Saharan Africa. The World Health Organization (WHO) estimates that 1000 new pediatric HIV infections occur daily, with 67% in Africa and 30% in South and Southeast Asia[2].

Sub-Saharan Africa remains the region most heavily affected by HIV. In 2010, about 68% of all people living with HIV resided in sub-Saharan Africa, a region with only 12% of the global population

Children are infected worldwide with HIV, with 1 in 40 adults in sub-Saharan Africa estimated to be infected with HIV, the data showing increasing in number of HIV-infected infants born to infected mothers[2]. In sub Saharan Africa 61% of people living with HIV are women, and this is associated with increase in mortality and morbidity in children under five due to HIV. In Tanzania the prevalence is higher (7.7%) among women compared to men (6.3%)[3].

Studies done before the introduction of highly active antiretroviral therapy showed that without treatment almost 50% of children with perinatally-acquired HIV infection died by age of 2 years and the majority by 5 years of age[4]. In one population of HIV infected children the median survival was shown to be 32 months from time of diagnosis without any intervention [5].The exact number of HIV infected children in sub-Saharan Africa surviving into adolescences is unknown, although it has been estimated to be 10–15 % [6]. With the increased access to antiretroviral therapy (ART) we expect that there will be a rapidly growing population of adolescences living with HIV and AIDS.

1.2 Mode of transmission

Transmission of HIV occurs via sexual contact, exposure to infected blood or vertical transmission from mother to child. The primary route of infection in the paediatric population is vertical transmission. Majority of children with HIV acquire the infection from their mothers during pregnancy, labour and delivery or after birth during breastfeeding. Vertical transmission of HIV remains the main source of paediatrics HIV infection in Africa with transmission rates as high as 25%-45% without intervention[7]. According to the Tanzania National guidelines, Mother to child transmission is responsible for over 90% of new infection in infants and young children[3].

1.3 Normal Development

Puberty is the period of transition from childhood to attainment of mature reproductive function; it is characterized by accelerated growth, development of secondary sexual characteristics, and psychological changes. Activation of the hypothalamic–pituitary–gonadal axis, which manifests as increasing pulsatile secretion of gonadotropin-releasing hormone (GnRH), this then initiates puberty.

The precise mechanisms responsible for pubertal onset are not fully understood, but it has been suggested that body mass and nutritional status as well as psychosocial health, genetic factors, and neuroendocrine inputs to the hypothalamus are all important determinants [8, 9].

There are two processes that contribute to the physical manifestations of puberty: adrenarche and gonadarche. Adrenarche normally occurs between six and eight years of age with increased adrenal androgen secretion; its exact biologic role is not well understood. It is accompanied by changes in pilosebaceous units, a transient growth spurt and the appearance of axillary and pubic hair in some children, but no sexual development[9].

Gonadarche is initiated by the macroneurons of the hypothalamus that secrete gonadotropin-releasing hormone (GnRH) that regulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the anterior pituitary[10].

In boys, LH stimulates testosterone production by the Leydig cells and, after spermarche; FSH supports the maturation of spermatozoa. In girls, FSH stimulates oestrogen production and follicle formation and, after ovulation, LH stimulates the development of the corpus luteum.

Initially, boys have testicular enlargement followed by the appearance of pubic hair, enlargement of the penis and spermarche. Skeletal and muscle growth are late events in male puberty. The age at which pubertal milestones are attained varies among the population studied and is influenced by activity level of hormone and nutritional status.

Pubertal maturation in girls begins with the acceration of growth followed by onset of breast development (thelache) between the ages of 8 and 13 years. Later signs of pubertal maturation include development of pubic and axillary hair (adrenache),which generally occurs after age 8 years (mean age 12.5 years range 10-15) and onset of menses (menarche) between 9 to 17 (median 12.8).

1.4 Pubertal changes

Adolescence is defined as a period of development, and is the biologic process in which a child becomes an adult. These changes include appearance of the secondary sexual characteristics, increase to adult size, and development of reproductive capacity. During this period of growth adolescents experience several types of maturation, including cognitive (the development of formal operational thought), psychosocial (the stages of adolescence), and biologic. The complex series of biologic transitions are known as puberty, and these changes may impact psychosocial factors.

The most visible changes during puberty are growth in stature and development of secondary sexual characteristics. Pubertal maturation occurs in sequence and timing. The staging system utilized most frequently is that published by Marshall and Tanner and the sequence of changes is commonly referred to as "Tanner stages" or sexual maturity ratings (SMR)[11]. Tanner stages are related to secondary sexual characteristics, with development of breast in females, pubic hair changes in both males and females, and genital changes in males.

Male genital and female breast development are manifestations of puberty (elevation in sex steroids and gonadotropins), whereas appearance of pubic hair is indicative of adrenarche (elevation in adrenal hormones) and may be considered a surrogate for puberty.The Tanner stages of puberty in boys are based on the development of the genitalia and pubic hair distribution while in girls it is based on breast size and shape and pubic hair distribution

1.5 Effect of HIV on puberty

Clinical interest in the impact of HIV disease and its associated Immunosuppression on the skeletal growth and pubertal maturation among HIV infected children is important. Studies have shown that Immunosuppression is associated with delayed pubertal onset among HIV-infected children [12-15].

Delayed puberty can be defined as the lack of pubertal development at an age of 2 SD above the mean, which corresponds to an age of approximately 14 years for males and 13 years for females, taking both sex and ethnic origin into consideration[16].

Pubertal delay associated with chronic illness is accompanied by a delay in growth and the pubertal growth spurt. The degree to which growth and pubertal development are affected in chronic illness depends upon the type of disease and individual factors, as well as on the age at illness onset, its duration and severity. It has been shown that the earlier the onset and the longer and more severe the illness, the greater the effect on growth and pubertal development[16].Virtually every child with any chronic disease could present with delayed puberty (due to recurrent infections, immunodeficiency, gastrointestinal disease, renal disturbances, respiratory illnesses, chronic anaemia, endocrine disease, eating disorders, exercise and a number of miscellaneous abnormalities)[16].

Delayed sexual maturation and impaired linear growth usually accompany childhood chronic illnesses such as cystic fibrosis, chronic renal failure, diabetes, cardiac diseases and inflammatory bowel diseases [12].

Infection with HIV-1 causes impaired linear growth and is likely to cause delayed sexual maturation, owing to HIV-1 itself, secondary infections, malnutrition and the host's

cytokine response [17]. Children with HIV infection have delay in both in the age of onset of puberty and in their progression through Tanner stages.

The treatment and prognosis of pediatric HIV infection in the developed world has been transformed with the introduction of highly active antiretroviral therapy. Perinatally infected children are now living into adulthood, changing the face of this epidemic from one of pessimism to one of hope. However, this transformation has brought with it many unforeseen challenges.

Growth retardation and pubertal delay are always seen in children with advanced HIV infection and are often related to the proinflammatory conditions found in advanced AIDS. . Rapid progressors have the highest incidence of growth failure. Growth and pubertal impairment are markers of advanced disease and require proper evaluation[18]

A study done in Italy showed that delayed sexual maturation is associated with acquired HIV-1 infection, particularly in girls. As a median, the onset of puberty is delayed by about 2 years in girls and 1 year in boys. This means that entry into the late Tanner stages is delayed by about 2.5 years in girls and 1.5 years in boys [15].

The results were in line with other findings described for other chronic childhood diseases, including HIV-1 infection in hemophiliac's boys. These studies showed that HIV-1 itself causes delayed pubertal development. Infected haemophiliac boys have a delayed sexual maturation compared with uninfected hemophilia's boys. It has been also shown that the delay in development of HIV-1-infected hemophiliac boys is not directly related to the disease progression[14, 19].

There was a study done to examine whether greater severity of HIV infection is associated with delayed initiation of pubertal development among perinatally HIV-infected children in US population,it was found that girls with severe Immunosuppression (CD4% <15) were significantly less likely to enter adrenarche and puberty compared with girls who were not severely immunosuppressed (CD4% > or =25). For boys, those with severe immunosuppression were significantly less likely to enter adrenarche and tended to be less likely to begin puberty compared with boys who were not immunosuppressed[12].

In Haemophilia Growth and Development Study (HGDS), they investigated the relationship between HIV-associated immune dysfunction and delayed pubertal development in a cohort of 333 boys and adolescents with moderate or severe haemophilia who were between the ages of 6 and 19 years at study entry in 1989. The study revealed statistically significant delays in pubertal development associated with increasing levels of immune dysfunction. The study emphasize the importance of following pubertal development in HIV-infected adolescent since delays in maturation may reflect underlying disease progression[14].

A study done in Uganda to assess the impact of ART on growth and sexual maturation among HIV adolescent found that 63% had delayed sexual maturity at baseline and after 12 month of treatment 60% had delayed maturation[20].

1.5.1 Mechanisms of delay in puberty in HIV

The mechanisms that lead to the delay of physiological puberty among HIV acquired infection remain unknown. Although malnutrition is probably the most important mechanism responsible for delayed puberty, emotional deprivation, toxic substances, stress and the side effects of chronic therapy, among others, have been implicated in the pathophysiology of delayed puberty. Growth dysregulation is quite common in HIV-infected children and growth failure is one of the most sensitive indicators of disease progression[18]. Beginning at birth, HIV-infected infants often have smaller size and lower birth weight than uninfected children born to HIV-infected women. The causes of growth dysregulation are varied, and can be due to alterations in gastrointestinal function, chronic or repetitive infections, and alterations in metabolic and endocrine function[18].

Children with perinatally acquired HIV infection may present with clinical features of endocrine dysfunction such as growth failure and pubertal delay. Growth and pubertal delay can be exacerbated by a variety of treatable infectious, endocrine, nutritional, and immunological disorders. Timely diagnosis and appropriate treatment of these conditions may lead to improvement or even normalization of growth and puberty.

There is scarcity of knowledge regarding the association between HIV infection and endocrine dysfunction in children. It has been suggested that HIV-infected children with advanced disease has endocrine abnormalities and therefore they should undergo periodic growth evaluation, including GH levels, IGF-I, IGF binding protein 3 and androgens, in order to identify subclinical endocrine dysfunction[17, 21]. The metabolic and endocrine effects may be the consequence of the primary infection or secondary to the use of any of the medications required to treat HIV infection and its complications.

HAART may also be associated with endocrine dysfunction with consequences on growth and puberty. A dysregulation of the hypothalamic-pituitary axis, toxic or allergic drug reactions may play a role in growth and pubertal delay of HIV-infected children. These dysfunctions require careful monitoring; in order to assess metabolic alterations that may be important in regulation of growth among HIV infected children. Better understanding of the mechanisms leading to impairment of growth and puberty in children with perinatally acqured HIV-1 infection might lead to appropriate treatment when required[22]. Pubertal delay, especially among boys, is common, and may contribute to the overall growth failure associated with HIV infection. If the basis for growth failure resides in metabolic and regulatory abnormalities, then interventions beyond increasing caloric intake will be necessary to increase linear growth rate and reverse growth failure in HIV-infected children. HIV disease affects production or secretion of hormones that regulate or directly control pubertal development. The exact triggers of puberty are not known. However, secretion of adrenal androgenic hormones and their precursors increases at approximately age of 7 years in healthy boys and girls [23]

In addition, in uninfected children, appearance of pubic hair and secondary sexual characteristics is accompanied by the elevation in gonadotropins and sex steroids, particularly testosterone in boys and estradiol in girls[24]. Mahoney et al reported that HIV-infected boys with haemophilia who had depressed immunologic status based on CD4 T-cell counts experienced delayed pubertal development compared with HIV-infected boys who had good immunologic status[14]. Other studies found that perinatally HIV-infected boys and girls also had reduced height, weight and linear growth compared with their uninfected peers[25]

1.5.2 Effect of delay in puberty

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. Delay in pubertal development may influence adult height and psychosocial development of a child, and even add to social stigma of HIV disease[26, 27]. Thus, potential delays in the onset of puberty among HIV-infected children and contributing factors need to be explored further.

Preadolescents and adolescents with HIV face additional unique complexities related to the impact of HIV on health, mental health, and normative developmental process such as school functioning, puberty, growth, peer relationships and sexuality[28]. As these children reach adolescence, suboptimal adherence to complex medication regimens also becomes a prominent issue, which can lead to viral resistance and treatment failure.[29] Additionally, adolescence is a time of increased experimentation with sexual behavior and drug use, which provides opportunities for transmission of HIV to others. Children are greatly concerned about the physical changes they undergo during puberty and often compare their own bodies with those of their peers[30].

HIV-infected children and adolescents may be more distressed about their actual or perceived maturational delay than their underlying chronic illness. Therefore HIV-infected children who attain Tanner stages at older ages but within the range of normal variability needs to be assured of their proper development. Those who are suspected to have abnormal delay in pubertal maturation may need psychological support and may benefit from slow doses of gonadal steroid hormones or growth hormone[31] or other management strategies such as nutritional support [30].

1.5.3 Puberty in normal population

In Tanzania unpublished data from a cross sectional study done by Muze et al (April 2009) to assess growth and pubertal development parameters among 3384 urban Tanzanian Children (1814 females and 1570 males) aged between 6-18 years, the mean age at onset of pubic hair was 12.3 years and breast (females) was 11.8 years. The genital development

in males was 12.2 years and pubic hair was 12.8 years. As 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 can explain for the later pubertal development observed in Tanzanian children.

1.6 Sexual maturation Assessment methods

Skeletal maturation and Tanner staging system can be used to assess sexual maturation. Skeletal maturation (or bone age) is the second method for assessment of physical maturity. Bone age is assessed by a left-hand-wrist radiograph and scored using the standards developed by Greulich[32]. Bone age provides a measure of "how far a given individual has progressed along his or her road to full maturity regardless of chronologic age. The most common method used to assess sexual maturation is by Tanner staging. Assessment of sexual maturity stages usually is done by a well-trained physician using the Marshall and Tanner method [33, 34].

There was study done in Black South African adolescents aged between 10 and 18 years to validate pubertal assessment using the sexual maturation scale developed by Tanner. It was found that Tanner staging appears to be a reasonably valid instrument to use among black South African youths[35].

1.7 Definition of terms

For purpose of this study growth was determined by assessing nutrition status by using anthropometric measurement.

HIV infected children- Children aged more than 18month with positive test antibodies using Enzyme immunoassays**.**
Antiretroviral drugs -Drugs that inhibit replication of HIV

Puberty-is the period of human development during which physical growth and sexual maturation occurs.

Adolescent- refers to a child aged 10 to 18 years.

Menarche- refers to establishment or beginning of the menstrual function or the first menstrual period.

1.8 PROBLEM STATEMENT

The introduction of highly active antiretroviral therapy (HAART) for treatment of acquired immunodeficiency syndrome (AIDS) has resulted in long survival of children infected with the human immunodeficiency virus (HIV)[36]. Previously few of the perinatally infected children would survive to adolescence as majority of them would not see their second birthday. Studies have showed that Immunosuppression is associated with delayed pubertal onset in perinatally HIV-infected children[12].

ART is known to suppress viral replication and hence slow the disease progression and improve immune status of HIV infected patients. However there are several gaps of knowledge regarding the impact of HIV on physical and sexual maturation in adolescents with longstanding HIV disease. In Tanzania so far there no published studies that have assessed physical growth and sexual maturation among HIV infected children. The current study will determine the effect of HIV infection in children surviving into adolescence.

1.9 .RATIONALE

Understanding the impact of HIV infection and its treatment on the growth and pubertal development of this cohort among HIV infected children is important for proper management of these children. Unfortunately this information is not exactly known in Tanzania.

Therefore pubertal assessment in these children and adolescents will provide information that will help in better understanding of their growth pattern and sexual maturation as well as informing the evidence based development strategies to address these problems in this cohort.

This Information is of clinical relevance; in this era where combined antiretroviral therapies have changed the course of HIV infected children from a fatal condition into a chronic condition, thereby allowing most children to survive to adolescence with satisfactory clinical status [21],

1.10 NULL HYPOTHESIS

Growth and puberty development in children with HIV and AIDS is similar to normal children in the general population.

1.11 OBJECTIVES

1.11.1 Broad Objective

To assess growth and pubertal development among HIV infected children aged 8-18 in Dar es Salaam.

1.11.2 Specific objectives

1. To determine median age at puberty among HIV infected children by sex
2. To determine the median age at menarche among girls with HIV infection
3. To determine factors (e.g. nutrition status, level of Immunosuppression, WHO stage) associated with pubertal development among children infected with HIV by sex.

CHAPTER TWO

2. METHODOLOGY

2.1 Study design

This was a cross-sectional hospital based descriptive study with historical control.

2.2 Study area

The study was conducted within the care and treatment centers (CTC) of Ilala, and Mwananyamala municipality by in Dar es Salaam region. These sites are supported by Management and Development for Health (MDH) which is a Tanzanian-based non-for profit organization. Dar es Salaam is selected conveniently and is one of the 28 regions of Tanzania with estimated population of 4 million people. It has three municipals namely Ilala, Temeke and Kinondoni.

The MDH program supports four (4) major HIV clinics, of which three are based at municipal hospitals of Mwanayamala, Amana and Temeke and another at the town center, IDC. The program also has several other minor centers situated at the health centers and dispensaries. The program offers a comprehensive care for all patients infected with HIV/AIDS and work in close collaboration with the government in provision of care to these patients. Clinics are conducted every day at all sites except on Sundays and public

holiday. Paediatric clinic is usually on Fridays only at all sites except IDC which is every day except on public holidays.

2.3. Study Population

HIV infected children aged 8-18 attending care and treatment clinics at Ilala and Mwananyamala. The minimal age was selected to reflect the minimum age of puberty in normal male and female children reported to be 8 and 9 years, respectively[37].

2.4. Sample size

Sample size was calculated using the following formula

$$N = \frac{4\sigma^2}{e^2}$$

where **σ** corresponds to standard deviation of mean age at menarche of Uganda among HIV infected adolescent which is 1.3 [38].

e corresponds to maximum likely error, which is 0.1

Therefore the minimum sample size is N was 676.

Adjusting with the finite population correction factor as population to be sampled was finite, sample size was adjusted as $n^2 = n/(1+n/N)$

Where n is the sample size calculated, N is the finite population which was 1000 HIV infected children aged between 6 to 18 years attending CTC clinic at Dar es Salaam. Therefore minimum sample size after adjustment is 400.

2.5. Sampling method

All eligible study participants scheduled for Fridays HIV care and treatment clinics whose parents, caregivers or guardians consented to participate were consecutively enrolled in the study. The study was conducted only on Friday because the clinic for HIV infected children is usually only on Friday at these sites.

2.6. Study duration

This study was conducted for a total period of 6 month from August 2011 to February 2012**.**

2.7. Recruitment of study subjects

HIV infected children were consecutively recruited from the CTC clinics in Amana and Mwananyamala after obtaining an informed written consent.

2. 8. Inclusion criteria and exclusion criteria

2.8.1 Inclusion criteria

1. Children and adolescent with HIV aged 8-18 years attending CTC at MDH
2. Children whose parents and guardian gave written informed consent to participate in the study
3. Children and adolescent whom assent was obtained.

2.8.2. Exclusion criteria

1. Children with physical disability, which prevented accurate anthropometric measurements.
2. Children with dysmorphic features suggestive of syndromic diseases or bone dysplasia known to affect height of the child and/or sexual maturation
3. Very sick child in which it was difficult to obtained consent and not able to take anthropometric measurement
4. Unwillingness of the parent or guardian to participate into the study

2.9. Study Procedure

Data and information from Patients files were used to obtain information regarding HIV status and WHO clinical stage. Structured questionnaires were used to collect information from children and adolescents regarding social demographic data, anthropometric measurement and sexual maturation.

Before conducting the study, a paediatrician with previous experience of assessment of growth and sexual maturation, trained the author and research assistant to ensure consistency of methods during data collection. Investigator and the trained research assistant performed all anthropometric measurements and assessed sexual maturation by Tanner stage.

2.9.1. Anthropometric measurements

Anthropometric measurements included weight and height. These measurements were performed after the participants had removed their shoes and with minimal clothing.

Weight was measured to the nearest 0.1 kg using weighing scale, TANITA® UM 075, which was periodically checked for accuracy and calibrated as necessary. Height was measured to the nearest 1 mm with a portable Leicester® stadiometer**.** BMI (kg/m^2) was computed using weight (in kilogram) divided by height (in meters squared).

2.9.2. Sexual maturation Staging

The sexual maturation staging includes breast development in females, genital development in males, and pubic hair development in both females and males. The sexual maturation staging was done using the Tanner staging scale [11]. The Tanner scale (also known as the Tanner stages) is a scale of physical development in children, adolescents and adults. The scale defines physical measurements of development based on external primary and secondary sexual characteristics, such as the size of the breasts, genitalia, and development of pubic hair, and was first identified by James Tanner, a British pediatrician and thus bears his name[11].

Pubertal staging criteria and definitions based on the recommendations of Marshall and Tanner known as Tanner staging 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. Tanner stage of male genitalia, female breast ,male and female pubic hair development was determined by visual inspection with aid of schematic diagrams[39].

Each maturity indicator has 5 stages that can be assigned from stage 1, representing immaturity, to stage 5, and indicating full maturity. Refer to Appendix III for Tanner staging in girls and Boys.

Girls were asked when they attained menarche. This information was used in this study to define menarche status and the proportion of having attained menarche at the time of examination for girls of various ages. The notation for each maturity indicator contains the initial letters as follows: pubic hair (PH), breast development (B), and genital development (G)

According to Tanner and Whitehouse criteria pubertal stages were defined as follows in girls: P1, preadolescent pubic hair; P2, sparse, lightly pigmented, straight pubic hair at the medial border of labia; P3, darker and thicker pubic hair that is slightly curly; P4, coarse, curly and abundant pubic hair; P5, adult feminine triangle spreading to medial surface of the inner thighs; B1, preadolescent breasts; B2, breast and papilla elevated as small mound and areola diameter increased; B3, breast and areola enlarged and no contour separation; B4, areola and papilla form secondary mound; B5, mature breasts, nipple projects, areola part of general contour.

In boys puberty stages will be defined as P1, no pubic hair; P2, scanty, long, slightly pigmented pubic hair; P3, pubic hair darker and slightly curly; P4, coarse and curly pubic hair resembling that of adults but scantier; P5, pubic hair spreading out to the medial surface of the thighs, similar to adults; G1, preadolescent penis and testes; G2, slight enlargement of penis and enlarged scrotum with a slight alteration in color and texture; G3, longer penis and larger testes; G4, larger penis and glans as well as increase in breadth; G5, size of penis and testes similar to adult.

Girls were classified as having begun puberty if they were at Tanner stage 2 or greater for breast and/or pubic hair development and to have completed puberty when they were 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 was referred to as elevation of breast and papilla at least as small mounds.

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

Pubic hair was reported as present when either sparse growth of long, slightly pigmented downy hair or straight or only slightly curled hair will be seen along the labia for girls and at the base of penis for boys.

Presence/absence of the pubertal milestone, age at examination (recorded in years) and self-reported age at menarche (recalled in whole integers or years and months) were the primary variables of interest.

2.10. Clinical status and immunological status

The WHO clinical status of these children was obtained from patient file. Clinical stages were categorized from I through IV, progressing from primary HIV infection to advanced HIV and AIDS. These stages were defined by specific clinical conditions or symptoms according to the WHO criteria. Staging was done at the time of initiation of ART. Refer appendix V.

Absolute CD4 cell count value was obtained from participant's clinical notes to determine the immunology status of these children. Refer Appendix IV. If the CD4 count was taken more than three month before the study under aseptic technique a sample of blood of about 2mls was taken from the venopuncture in an empty bottle and was processed within 24 hours for CD4 cell count at the study site

Processing of CD 4 count was done at the appropriate laboratories of the MDH program of the centers mentioned earlier using FACS count machine and result was recorded.

2.11. Data processing and analysis

Structured questionnaires were coded, and checked for consistency before double entry into the Excel computer database then transferred to STATA® IC statistical package version 10. Data cleaning was done in terms of consistence to checks for outliers and missing data.

Data analyses were done using STATA® 10 IC and results were presented as median, interquatile range and proportions as appropriate. BMI, height for age and weight for age Z scores were calculated by using Zanthro command. P value of < 0.05 was considered statistically significant.

Univarate and multivariate Logistic regression was used to determine the combined association between independent variable like nutrition status, disease severity, and age and the dependent variable of having reached puberty defined as Tanner stage two or above coded as binary variable. The statistical effect in these models was assessed using likely hood ratio tests.

2.12. Ethical clearance

Ethical and research clearence was obtained from Muhimbili University of Health and Allied Sciences (MUHAS)'s Research and Publications Committee

2.13. Ethical consideration

An informed written consent was obtained from parents and guardian prior to enrollment and assent was obtained from adolescent aged more than 12 years. Parents or guardian of the children were asked to consent for participation of their children into the study after explaining the study itself, procedures and benefit of the study or participation. They were informed that information will help to improve the quality of care and treatment of HIV infected children.

Parents or guardian were provided with opportunity to ask question prior to consenting and at the end of each data collection session. Participants were informed that participation into this study was voluntary and that they could opt out.

Examination was done in private rooms by the author and research assistant. If there were no private room at the clinic, privacy was insured by putting portable screening curtains to provide a temporary private space. A researcher of the same sex examined each child.

During examination, the author and research assistant provided proper advice and counseling when required to every child found with any abnormal condition, which needed medical attention.

For those who decided not to participate assurance was given that they will continue to receive the medical services as provided by the facility. Requesting for permission to access medical records was part of consent. At the end of the study participants continue with their care in their respective clinics as of scheduled.

2.14. Confidentiality

Confidentiality was observed and no unauthorized persons had access to the data collected. Each subject had been assigned a study identification number.

CHAPTER THREE

3. RESULTS

3.1. Demographics characteristics of study population by sex.

During the study period 330 participants were enrolled. Of these 183 (55.4%) were females and 147 (44.5%) were males. All participants enrolled had confirmed HIV infection and were on ART. Median duration of ARV was 48 (IQR 30-62) month. The median age was 12.0 (IQR 11-15) years for both females and males

Most of the participants were in WHO stage III and had absolute CD4 count above 500 cells / µL and median CD4 count of 564 cells / µL (IQR 346-917) (table 1a).

Table 1a. Demographic characteristics among HIV infected children by sex

Variable	**Female**	**Male**	**P value**
Total no (%)	183 (55.4%)	147 (44.5%)	
Growth parameters			
Median Age in Years (IQR)	12.0 (11-15)	12.0(10-14)	0.9159
Median Weight in Kg (IQR)	32.0 (25-42)	28.0(23-33.4)	0.006
Mean WAZ (SD)	0.14 (±1.0)	-0.18 (±0.9)	0.0018
Mean HAZ (SD)	0 .03 (±1.0)	-0.03 (±0.9)	0.2579
Mean BMI (SD)	0.18 (±1.1)	-0.24 (±0.7)	0.0001
Education			0.549
Primary school n (%)	147 (55.5)	118 (44.5)	
Secondary school n (%)	36 (55.4)	29 (44.6)	
Median duration on ART in month (IQR)	45 (24-48)	52 (24-60)	0.67
CDC Classification (n, %)			
A	106 (57.9)	89 (60.5)	
B	53(28.9)	45 (30.1)	
C	24(11.1)	13 (8.8)	
Median CD4 Count (IQR**)**	550 (250-875)	610 (363-978)	0.07
WHO classification (n, %)			
I	5 (2.7%)	2 (1.3%)	
II	31 (16.9%)	31 (21.0%)	
III	143 (78.1%)	107 (72.7%)	
IV	4 (2.1%)	7 (4.7%)	
Tanner stage (n,%)			
I	94(51.4%)	70(47.6%)	
II	48(26.2%)	47(32.0%)	

III	40(21.9%)	30(20.4%)
IV	1(5%)	

HIV infected Children were compared to the reference population. Among HIV infected female there were no significant difference in age compared to the reference population. However there was significant difference in weight for age, height for age and body mass index p =0.001. The reference population had higher weight and BMI as well as they were taller compared to HIV infected children. There was no significant difference in the mean age among HIV infected males. HIV infected males were underweight, shorter and low BMI compared to the reference population P=0.001 (table 1b).

Table. 1b. Comparison of the mean and confidence interval of HIV infected children and the reference population (RP) by sex.

	HIV Positive	Reference Population	P Value
	Mean (95%CI)	Mean (95%CI)	
Female			
	n=183	n=1542	
Age	12.4(12.0 - 12.8)	12.3 (12.2 - 12.5)	0.234
WAZ	0.14 (-0.01- 0.29)	-0.68(-0.7--0.6)	0.001
HAZ	0 .03 (-0.1 - 0.18)	-0.9 (-1.0 - -0.9)	0.001
BMIZ	0.18(0.02 - 0.35)	1.1 (1.1 - 1.2)	0.001

Male			
	n=147	n=1296	
Age	13.05(11.6- 14.4)	12.8 (12.7 - 13.0)	0.562
WAZ	-0.18 (-0.32 - -0.02)	-0.91(-1.0--0.9)	0.001
HAZ	-0.03 (-0.19 - 0.11)	-1.2 (-1.2 - -1.1)	0.001
BMIZ	-0.24 (-0.35 - -0.11)	1.08 (1.06 - 1.1)	0.001

3.2. Pubertal developments among HIV infected children and reference population (RP)

Female and male participants were classified as having puberty if they were in Tanner stage 2 and completed if they were in Tanner stage 4. Most of the participants in this study were up to stage Tanner stage 3 and very few in stage 4 and none in stage 5.

Among female participants 20.8% reported to have attained menarche. The median age at menarche was 15 (IQR 14-16) years while the median age at menarche for reference population was 13 (IQR 12-15) year. (P=0.001).

Age for different Tanner stages for breast and pubic hair development were compared between the study population and the reference population. HIV infected female had breast and pubic hair development at advanced age compared to the reference population, they were found at Tanner stage 2 with median age of 13.5 (IQR 12-15) years compared to the reference population with a median age of 12.0 (IQR 11-13) years. There was a difference of 1.5 years between this population and difference was statistically significant. (P <0.001) (Table 2a).

Table 2a. Ages at various Tanner stages for breast and pubic hair development among HIV infected females and the reference population (RP)

	Median Age (IQR)	**Median Age** (IQR)	**P Value**
	HIV Positive		
Tanner 1	11.0 (9-12)	9.0 (8-11)	0.001

2	13.5 (12-15)	12.0 (11-13)	0.001
3	15.0 (14-17)	13 (13-14)	0.001
4	15.0 (0)	15 (14-16)	
5	0 (0)	17 (16-18)	
Tanner 1	11.0 (11-12)	9.0 0(8-11)	0.001
2	14.0 (12-15)	12.0(11-13)	0.001
3	15.0 (14-17)	14.0 (13-14)	0.001
4	0 (0)	15.0 (14-16)	
5	0 (0)	17.0 (16-18)	

HIV infected males were found to have genital and pubic hair development at a later age as compared to the reference population. (P =0.023). They were at Tanner stage 2 with median age of 13.0 (IQR 12-14) years as compared to their reference population in which they were at median age of 12.0 (IQR 11-13) years (Table2b).

Table2b. Ages at various Tanner stages for genital and pubic hair development among HIV infected males and the reference population (RP)

	Median Age (IQR)	**Median Age** (IQR)	**P Value**
	Genital (G)		
	HIV Positive		
Tanner 1	10.0 (9-11)	10.0 (9-11)	0.936
2	13.0 (12-14)	12.0 (11-13)	0.023

3	16.0 (15-17)	14.0 (13-15)	0.001
4	16.0 (0)	16.0 (15-17)	
5	0 (0)	17 (16-18)	
	Pubic Hair (PH)		
1	11.0 (10-12)	10.0 (9-11)	0.001
2	13.0 (12-14)	13.0 (12-14)	0.137
3	16.0 (15-17)	14.2 (13-15)	0.001
4	16.0 (0)	16.0 (15-17)	
5	0 (0)	17.0 (16-18)	

3.3. Weight and pubertal development

Weight at different Tanner stages for breast and pubic hair development were compared between study population and the reference population. There was no significant difference in weight at different Tanner stages between the study population and the reference population (table 3a).

Table 3a. Weight at various Tanner stages for breast and pubic hair development among HIV infected females and the reference population (RP)

	Median Weight (IQR)	**Median Weight** (IQR)	**P Value**
	HIV Positive		

Tanner 1	25.8 (20.3-30.2)	25.6 (22.8-30.3)	0.968
2	37.2 (32.1-43.8)	35.2 (32.1-41.7)	0.613
3	46.0 (40.0-50.0)	41.6 (37.5-46.9)	0.105
4	49.0 (44.0-55.2)	49.6 (44.0-55.2)	0.945
5	0 (0)	55.9 (49.8-60.3)	
Tanner 1	28.0 (25.0-32.5)	25.6 (22.8-30.0)	0.018
2	37.2 (32.1-43.8)	35.2 (32.1-41.7)	0.613
3	46.0 (39.8-57.0)	41.6 (37.6-46.9)	0.112
4	48.0 (0)	49.6 (44.0-55.2)	
5	0 (0)	55.9 (49.6-60.3)	

Boys within the reference population (RP) were in Tanner stage 2 and completed tanner stage 4 for genital and pubic hair development with a bigger weight than HIV infected children (table 3b).

Table 3b. Weight at various Tanner stages for genital and pubic hair development among HIV infected males and the reference population (RP)

	Median Weight (IQR)	**Median Weight** (IQR)	**P Value**

	HIV Positive		
Tanner 1	23.7 (21.2-27.0)	26.8 (23.8-31.3)	0.001
2	31.0 (26.0-33.0)	34.1 (30.3-38.3)	0.002
3	41.6 (38.0-45.5)	42.3 (36.5-49.4)	0.433
4	0 (0)	50.4 (46.2-55.2)	
5	0 (0)	56.5 (51.7-61.7)	
Tanner 1	26.0 (21.1-32.1)	26.8 (23.8-31.3)	0.581
2	30.4 (26.0-33.0)	34.1 (30.3-38.3)	0.01
3	41.0 (38.8-48.5)	42.3 (36.5-49.4)	0.433
4	0 (0)	50.4 (46.2-55.2)	
5	0 (0)	56.5 (51.7-61.7)	

3.4. Height and pubertal development

Height at various Tanner stages for breast and pubic hair development of children and adolescents were between the study and the reference population. There was no significant difference in the median height for the population when they were attain breast development (Table 4a).

Table 4a. Height at various Tanner stages for breast and pubic hair development among HIV infected females and the reference population (RP)

	Median Height	**Median Height**	**P Value**

	(IQR)	(IQR)	
	HIV Positive		
Tanner 1	129.5 (122.7-138.0)	129.3 (123.2-136.4)	0.923
2	145.5 (140.0-151.0)	143.9 (140.1-144.5)	0.365
3	150.0 (145.2-153.7)	150.2 (145.7-155.2)	0.621
4	155.2 (0)	155.2 (150.8-158.6)	
5	0 (0)	157.8 (154.4-162.7)	
Tanner 1	133.0 (128.5-139.5)	129.3 (123.2-136.4)	0.07
2	145.5 (140.0-154.1)	143.9 (140.1-149.0)	0.365
3	150.0 (145.0-153.2)	150.2 (145.7-155.2)	0.448
4	0 (0)	155.2 (150.8-158.6)	
5	0 (0)	157.8 (154.4-160.2)	

Males within the reference population develop pubic hair at a higher height than those with HIV (P= 0.001). However there was no significant difference in the genital development among the reference population and HIV infected children (Table 4b).

Table 4b. Height at various Tanner stages for genital and pubic hair development among HIV infected males and the reference population

	Median Height (IQR)	**Median Height** (IQR)	**P Value**

	HIV Positive		
Tanner 1	128.0 (123.0-132.0)	131.8 (126.0-137.8)	0.001
2	140.0 (132.0-149.0)	142.1 (138.0-147.1)	0.233
3	153.0 (149.5-159.5)	153.3 (146.2-158.9)	0.971
4	0 (0)	161.7 (157.5-166.1)	
5	0 (0)	166.9 (160.5-170.2)	
Tanner 1	130.0 (126.0-135.7)	131.8 (126.1-137.9)	0.581
2	139.3 (136,2-140.2)	142.7 (139.5-145.6)	0.160
3	152.7 (150.4-159 3)	150.4 (149.3.156.9)	0.971
4	0 (0)	161.7 (157.3-166.8)	
5	0 (0)	166.3 (160.5-170.2)	

3.5. BMI and pubertal development

BMI at different Tanner stages for breast and pubic hair development were compared to the reference population. There was no significant difference between breast development among the reference population and HIV infected population. (Table 5a)

Table 5a. BMI at various Tanner stages for breast and pubic hair development among HIV infected females and n the reference population

	Median BMI (IQR)	**Median BMI** (IQR)	**P Value**
	HIV Positive		
Tanner 1	15.6 (14.5-16.5)	15.7 (14.5-16.7)	0.163
2	17.6 (15.7-19.3)	17.4 (15.9-18.7)	0.293
3	20.3 (18.9-21.2)	18.6 (16.8-20.5)	0.02
4	0 (0)	20.3 (18.7-22.6)	
5	0 (0)	22.5 (21.3-24.7)	
Tanner 1	16.1 (14.5-16.7)	15.4 (14.5-16.7)	0.029
2	17.6 (15.8-19.3)	17.1 (15.9-18.7)	0.293
3	20.3(17.8-21.3)	18.3 (16.8-20.6)	0.002
4	0 (0)	20.4 (18.7-22.6)	
5	0 (0)	22.1 (20.3-24.4)	

Males in the reference population developed genital and pubic hair with a larger BMI than HIV children (table 5b).

Table 5b. BMI at various Tanner stages for genital and pubic hair development among HIV infected males and the reference population

	Median BMI (IQR)	**Median BMI** (IQR)	**P Value**
	HIV Positive		
T			
anner 1	14.9 (14.1-15.9)	15.6 (14.6-16.8)	0.002
2	15.9 (14.7-16.6)	16.6 (15.6-17.8)	0.003
3	17.7(16.1-19.4)	18.0 (16.6-19.7)	0.478
4	0 (0)	19.2 (18.0-20.9)	
5	0 (0)	20.7 (19.0-22.1)	
Tanner 1	15.2 (14.2-16.2)	16.3 (14.6-16.8)	0.567
2	15.8 (14.6-16.6)	17.1 (15.6-17.8)	0.02
3	17.7 (16.1-19.4)	18.5 (16.6-19.7)	0.478
4	0 (0)	19.5 (18.0-20.9)	
5	0 (0)	20.7 (19.0-22.7)	

3.6. Relationships of growth parameters and disease severity indices to the onset of puberty among HIV infected children with Tanner stage 2 or more.

Tanner 2 was considered in univariate and multivariate logistic regression analysis among female and male participants

In the univariate analysis advanced age, weight for age Z score and height for age Z score were positively associated with pubertal development among female and male with HIV infection. For every increase in a year the like hood for a female to enter puberty was 0.76 times.

In multivariate analysis among female and male participants advanced age was the only factor that could independently predict the puberty.

Table. 6a. Factors associated with onset of puberty (Tanner stage 2 or more) among HIV infected females

Factor	**Univariate regression**			**Multivariate regression**		
	COR	95% CI	P value	AOR	95% CI	P value
Age	0.768	1.7-2.6	0.001	0.5	1.3-2.3	0.001
WFA Z score	2.37	5.5-20.3	0.001	1.2	0.94-14	0.06
HFA Z score	1.7	3.2-9.2	0.001	-0.8	0.48-1.7	0.8
BMI Z score	1.814	3.4-10.9	0.6	0.05		
CD4 Count	-0.77	0.35-1.9	0.682	0.7		
WHO stage	0.29	0.49-2.1	0.938	0.58		

COR= crude odds ratio and AOR = adjusted odds ratio

Male participants were 1.3 times more likely to enter puberty in every increase by one year. Other factors WHO clinical stage, CD4 count and nutrition status was not associated with onset of puberty (Table 6b).

Table.6b. Factors associated with onset of puberty (Tanner stage 2 or more) among HIV infected males

Factor	Univariate regression			Multivariate analysis		
	COR	95% CI	P value	AOR	95% CI	P value
Age	1.5	2.7-7.6	0.001	1.3	2.17-7.0	0.001
WFA Z score	3.08	7.4-12	0.001	0.46	0.0-0.6	0.93
HFA Z score	2.6	5.5-37	0.001	1.3	0.05-4.4	0.68
BMI for age Z score	1.5	2.2-9.4	0.001		0.27-37	0.931
CD4 count	1.08	0.7-11.3	0.115			
WHO stage	0.2	0.5-2.6	0.611			

COR= crude odds ratio and AOR = adjusted odds ratio

CHAPTER FOUR

4. DISCUSSION

Major improvements in access to antiretroviral therapy (ART) over the years have made it possible for children infected with human immunodeficiency virus (HIV) to reach adolescent. The pubertal growth spurt and the appearance of secondary sex characteristics are the most visible manifestations of puberty[40]. It is the lack of one or both of these that bring adolescent to a health care facility. The HIV-infected adolescents, unlike their HIV negative counterparts, are more likely to be admitted with chronic complications including stunted growth or pubertal delay[41]. Assessment of growth and pubertal development is important among children infected with HIV. There are few published data in Africa on growth and sexual maturation among children with HIV infection. Moreover, in Tanzania there are no studies that have been done to assess growth and sexual maturation among HIV infected patient prior to this study.

In this study we demonstrated that Growth and pubertal development among HIV infected children is different from that of reference population of urban Tanzanian children in Dar es Salaam. In this study children infected with HIV infection had delayed sexual maturation compared to the reference population.

HIV infected girls were found in Tanner stage 2 at median age of 13.5 (IQR 12-15) years for breast while reference population entered this stage at median age of 13.0 (IQR12-14) years for breast development. Pubic hair growth occurred at median age of 14.0 (IQR 12-15) years which was one year late. A similar pattern was observed in HIV infected boys when compared to the reference population. HIV infected boys were in Tanner stage 2 at a median age of 13 (IQR 12-14) years which is also one year late.

Findings were similar to that of multicenter longitudinal study among HIV infected children aged 8 to 18 years in Italy which showed that as a median, the onset of puberty is delayed by about 2 years in girls and 1 year in boys. The delay increases when passing through subsequent Tanner stages. This means that entry into the late Tanner stages is delayed by about 2.5 years in girls and 1.5 years in boys among HIV infected children[15].

These finding are in line with other studies done in other children with chronic childhood diseases [37], reflecting that this condition is common in children with chronic illness including
HIV and AIDS. In agreement with previous studies [14, 15] this study found that HIV-infected children were in Tanner stage 2 at older ages than uninfected HIV children. HIV infected children also had significant delay in age of attaining sexual maturation at various Tanner stages for breast (B) and pubic hair (PH) development compared to the reference population of urban Tanzania children in Dar es Salaam.

In Africa there are few published data that assessed growth and sexual maturation among HIV infected children. Among these studies, one study in Uganda assessed the impact of antiretroviral therapy for HIV-1 infected adolescents on growth and sexual maturation. At baseline sixty-three percent (63%) had delayed sexual maturity which did not improve after 12 month of ART [20], this indicates that there is delayed growth and sexual maturation even with the availability of antiretroviral therapy and may be irreversible. In this study all participants were on ART despite that they had delayed growth and sexual maturation.

A study done in USA found that HIV related Immunosuppression is associated with delayed ignition of pubertal development among perinatally HIV infected children and that these children may on average enter puberty and adrenarche at older ages than children in the general USA population[12].

A study done among children with HIV and hemophilia found that there was consistent delays in skeletal maturation, pubertal progression was slower in adolescents through

Tanner stage transition, significantly so through each of the transitions from Tanner stages 1 to 4 [42].

Another finding from this study is that HIV infected children had low Z score for weight, height and BMI for age compared to the reference population. A study of growth patterns among HIV-positive children in Europe demonstrated that, even in a high-resource setting, growth faltering is apparent among HIV infected children affecting both weight and height (although weight differences were more obvious), and overall differences between HIV-positive and HIV-negative children increased with age. Children with more advanced HIV disease also had much poorer growth at all ages[43, 44]. Common nutritional problems for HIV-positive children include poor growth compared to peers and a higher risk of becoming malnourished. Reductions in length or height of HIV-positive children are common, and poor growth (slow weight gain or decreasing weight) is often apparent even before opportunistic infections or other AIDS symptoms appear[45].

Weight and BMI at Tanner stage for breast and pubic hair development among HIV infected female was compared to the reference population. There were no significant differences in weight between HIV infected female and of the reference population. This could be explained by fact that all children were on ARVs and the median duration of treatment was 48(IQR30-62) months. HAART has been found to have a positive effect on height and weight among HIV infected children and adolescents though not sufficient to achieve healthy control values[46].

However HIV infected males had a significantly lower weight and BMI at Tanner stage 2 compare to reference population in which they were found in Tanner stage 2 with greater weight and BMI. In this study boys have poor growth pattern than girls. They had low weight and BMI.

Among HIV infected females the median height at puberty assessed by breast and pubic hair development was not statistical significantly different from that of reference population. However the median age at attaining pubic hair development in males was statistically higher in HIV infected children compared to the reference population.

This study also shows that HIV infected female had menarche at a later age compared to the reference population. The median age was found to be 15 (IQR 14-16) years compared to 13.0 (IQR 12-15) years found in the reference population. This was not different from study in Uganda in which the mean age at menarche among HIV infected adolescent was 15 years[47].

Advanced age was found to be associated with onset of puberty among children infected with HIV. Other factors like WHO clinical stage and level of Immunosuppression was not associated with the development of sexual maturation. The findings was consistent with other study which found that age at onset of puberty was not related to clinical and immunological condition, antiretroviral treatment, weigh for height and age at onset of severe disease or immune suppression[15]. However these findings were different from one study in which they found that girls with severe Immunosuppression (CD4% < 15) were significantly delayed to enter adrenarche compared to girls who were not immunosuppressed (CD4% ≥ 25). For boys, those with severe immunosuppression were significantly delayed to enter adrenarche compared to boys who were not immunosuppressed[12].

Sexual maturation was attained at advanced age among HIV infected children compared to the reference population. These findings are similar to the studies done in other developing as well as developed countries [15, 20]. The similarities can be attributed to the similar study population as well as the chronicity of the disease[13, 14, 48].

Few studies have been done to assess the cause of pubertal delay among HIV infected children. Endocrine dysfunction is maybe more complex in perinatally infected children and includes an euthyroid sick syndrome, accompanied by increased basal thyrotrophin levels, reduced free thyroxine levels[49], and low levels of insulin growth factor 1 (IGF-1) and IGF-binding protein [17]. HIV-1-induced immune dysfunction could be a mechanism causing a delayed sexual maturation through altered neural control of puberty[13].

There was an intensive endocrine evaluation among HIV infected children with Haemophilia to find the causes of delay in sexual maturation. It was found that the abnormalities in growth and pubertal development in HIV-infected boys with hemophilia

appeared was due to diminished androgen production and subsequent growth hormone secretion as a result of HIV infection, but did not appear to be due to altered thyroid function, reduced caloric intake, or severe chronic illness [48]. Another study in Kigali found that children with HIV have short stature due to low growth hormone secretion rather than malnutrition[50].

Children with perinatally acquired HIV infection may present with clinical features of endocrine dysfunction such as growth failure and pubertal delay.. Growth and pubertal delay can be exacerbated by a variety of treatable infectious, endocrine, nutritional, and immunological disorders[22]. HIV infection has been linked to cause diminished growth hormone production or release and decreased androgen secretion, even before the development of AIDS and Immunosuppression.

Findings from the Haemophillia growth and development study also demonstrated that HIV itself causes delayed pubertal development [14, 48, 51]. The earlier the onset and severity of the disease the greater the negative effect on pubertal growth. Also HIV has been associated with hypothalamic-pituitary axis dysfunction (HPG). The HPG axis is greatly affected in calorie protein energy malnutrition which is a feature of HIV infection and for this reason there is reduced growth and subsequent puberty[22]. The timing of sexual maturation is determined by the interaction of inheritance with several environmental factors. Another factor could be due to direct genetic effect.

Children with HIV infection have delays both in the age of onset of puberty and in their progression through the pubertal stages Pediatric care providers and pediatric endocrinologists should put into action appropriate preventive, screening, and therapeutic strategies to maximize survival and quality of life in these children.

Although perinatally acquired HIV infection appears to have delayed the onset of puberty, many HIV infected children may not necessarily experience abnormal pubertal delay. Tanner first reported that there was considerable individual variation among boys and girls, both in the age at which they reach any certain stage of development of genitalia or pubic hair and in the time they take to pass from one stage to another or through the whole sequence leading to sexual maturity [33, 34].

4.1 STUDY LIMITATIONS AND STRENGTHS

4.1. Limitations of the study

The sample size calculated was not reached however more than 80% of the sample size was reached which gives adequate power to detect the difference.

This was a cross sectional study and therefore not be able to establish exactly when one started or completed a particular pubertal Tanner stage. Another limitation is that this was not a matched case control study and hence we had to use historical control.

The timing of sexual maturation is determined by the interaction of inheritance with several environmental factors. The information concerning the timing of sexual maturation of the parents of HIV-1- infected children could not validated because in most cases either one or both parents had already died or were unidentifiable. The anticipated difference in age at the onset of puberty due to these environmental factors is approximately 3 months[37] and this was overcomed by the large numbers of HIV children and this reduces bias.

4.2. Strengths of the study

There is no epidemiological study done in our setting to assess the secondary sexual characteristics in children and adolescent with HIV and AIDS. Therefore this cross section study can be used as a baseline data.

CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

Children with HIV and AIDS have significant delay in growth and sexual maturation. Growth and sexual maturation occurred at advanced age. Considering these findings monitoring of growth and pubertal development in this population should be great emphasized.

5.2 Recommendation

Growth and pubertal development in children and adolescent with HIV and AIDS should be part of the comprehensive package. Matched case control study needs to be conducted to further assess the impact of HIV and AIDS among children and adolescent with long standing HIV infection.

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APPENDIX – I. INFORMED CONSENT FORM (English version).
Study No……

- **Title:** Growth and pubertal development among children aged 8-18 years with HIV infection in Dar es Salaam
- **To the Parents/ Guardians of ……..**

Foreword

I am Dr. Gloria Mbwile postgraduate student at MUHAS conducting a study for children aged 6-18 years with HIV on their growth and pubertal development.

How to participate

Weight and height measurements will be taken in all eligible children with HIV then they 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.

If any problem needing medical attention is diagnosed to the child during examination, the investigator will contact the child's parents/guardians and appropriate directives will be given.

Purpose of the Study

The study will generate important statistics of our Tanzanian's children and adolescents with HIV on their nutritional status, growth and pubertal development as compared to those in the general population. It will also assist us to understand when is the proper time for our children to receive sex education depending on their growth and pubertal development. The study has the permission from Muhimbili University (MUHAS)'s ethical committee.

Confidentiality

Your child name and other particulars will not be made public in any way and so your participation will be anonymous. Only the chief investigator and dedicated laboratory staff will handle the specimen and information obtained.

Consent

I have read and understood the explanation of the study. I accept for my child to be examined and participate in the study.

Signature of the Parent/Guardian.....

Relationship to the child.....

Date.....

Child assent to participate? YES. NO.

For more information or clarification you may contact one of the Doctors mentioned below,

Dr. Gloria Mbwile 0712488300

APPENDIX –II: FOMU YA RIDHAA (Swahili version).

Namba ya utafiti. ……

Kichwa cha Habari: Ukuaji na maendeleo ya balehe kwa watoto wenye ugonjwa wa VVU Muhimbili.

Kwa Mzazi/Mlezi wa……

Utangulizi

Mimi Dr.Gloria Mbwile mwanafunzi wa udhamili Chuo Kikuu cha Sayansi za Afya ya Muhimbili nafanya tathmini ya hiari kwa watoto wenye matatizo ya VVU kuangalia maendeleo ya ukuaji kwa watoto wa umri wa miaka 8-18 na kuangalia maendeleo yao ya balehe.

Taratibu za kushiriki

Watoto wa umri kati ya miaka 6-18 watapimwa uzito, urefu, 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 klinic. Upimaji wa watoto pamoja na tathmini hii inakadiriwa kuchukua si zaidi ya dakika kumi tu kwa kila mtotoTathmini 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 VVU. Na pia itatuwezesha kujua ni wakati gani muafaka mtoto wa Kitanzania mwenye VVU 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

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

Kama kuna swali lolote linalohusu utafiti huu au unahitaji ufafanuzi au maelezo zaidi waweza kuwasiliana na mmoja kati i huu tafadhari wasililiana na mtafiti mkuu ya madaktari wafuatao.

Dr. Gloria Mbwile 0712488300

Kama itatokea ukawa na swli kuhusu haki zako kama mshiriki wasiliana na

Prof.E.Lyamuya , Mwenyekiti wa kamati ya utafiti wa chuo

S.L.P 65001, Dar es salaam Simu namba 2150302-6

APPENDIX – III: QUESTIONNAIRES

- Informed consent (N/Y.....

- Today's Date (DD-MM-YY).....

DEMOGRAPHIC HISTORY

- Name…….
- Sex (M/F).....
- Date of birth.....
- Respondent (Self/Parent/Guardian/Other).....

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

RESIDENCE INFORMATION

If NO Region_____

If YES District_____

GENERAL EXAMINATION

Height……..

Weight (kg)…….

BMI….

FAMILY AND SOCIAL HISTORY

Education (N/Y – Grade: Nursery/STD 1-7/FRM 1-6/Tertiary/Not applicable) .....

MEDICAL HISTORY

- Is the child on ARV
  (a) YES
  (b) NO

How long has the child being on ARVs…….

CD4 count…….

Date specimen taken

Clinical and Immunological stage…….

- Have you/your child ever been admitted to hospital (N/Y).....

Reproductive history for female

- Menarche (N/Y – age)……..
- **SEXUAL DEVELOPMENT FOR GIRLS**

	I	II	III	IV	V
Pubic hair	**Preadolescent**	**Sparse, downy**	**Pigmented, coarse**	**Adult type, sparse**	**Adult**
Breast	**Preadolescent**	**Budding**	**Enlargement, no separation**	**Areola and papilla form 2º mound**	**Mature**

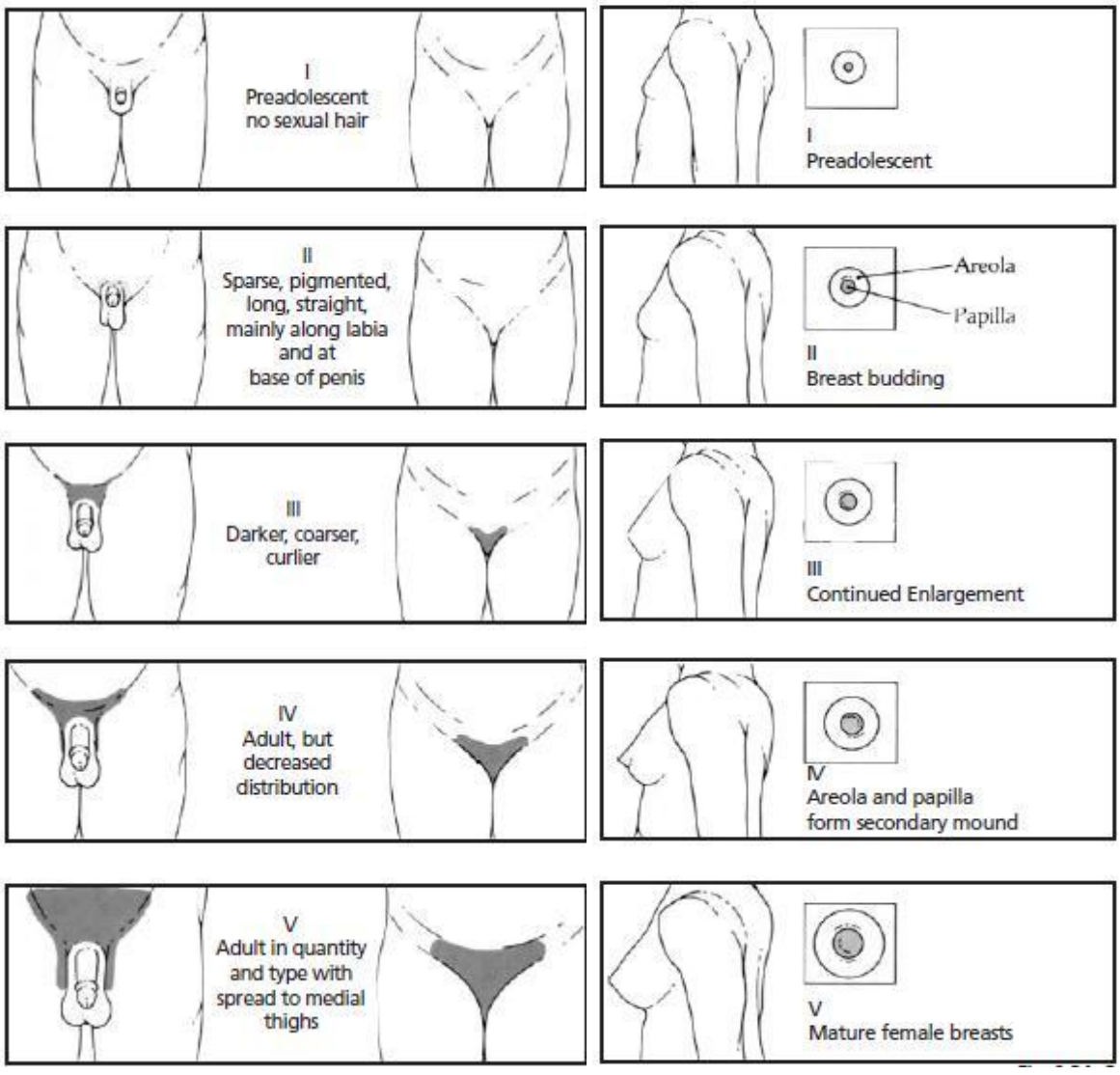
SEXUAL DEVELOPMENT FOR BOYS

	I	II	III	IV	V
Pubic hair	**Preadolescent**	**Sparse, downy**	**Pigmented, coarse**	**Adult type, sparse**	**Adult**
Scrotum/testes	**Preadolescent**	**Slight enlargement**	**Increased length**	**Increased breadth,**	**Adult size/ shape**

Comments ……..

Doctor's Name……..

APPENDIX – IV: TANNER STAGES IN BOYS AND GIRLS



APPENDEX V: IMMUNUNOLOGICAL CLASSIFICATION FOR HIV INFECTED CHILDREN

Immunological category	CD4 count (cells/µL)
No evidence of Immunosuppression	≥500
Evidence of Immunosuppression	200-499
Severe Immunosuppression	<200

APPENDIX VI: WHO CLINICAL STAGING OF ESTABLISHED HIV INFECTION

HIV-associated symptoms	WHO clinical stage
Asymptomatic	I
Mild symptoms	II
Advanced symptoms	III
Severe symptoms	IV