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Pubertal Development among HIV-Infected Children aged 8-18 Years in Dar es Salaam, Tanzania: A Cross Sectional Study

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Abstract

Background
Pubertal development can be impaired among children living with HIV. This can lead to a psychosocial burden and subsequent negative self-image, social withdrawal and declining academic performance.

Objective
To assess timing of pubertal development of HIV infected children aged 8-18 years in Dar es Salaam, Tanzania.

Methods
This was a hospital-based cross-section study conducted at Amana and Mwananyamala, Care and Treatment Centres (CTC) in Dar es Salaam, Tanzania. A structured questionnaire was used to collect information on demographic and clinical characteristics. Participants were regarded to have entered puberty if they were at Tanner stage 2 or more for either breast (female) or genital (male) development and Tanner stage 2 or more for pubic hair development in both sexes. Data was analysed using STATA version 10 statistical packages. Puberty development of study participants was compared to their peers in a database of health children and adolescents attending schools in Dar es Salaam, Tanzania. Descriptive statistics were summarised as median (interquartile range), mean (standard deviation) for continuous variable and proportions for categorical variables. Differences in various groups was tested using Student t- test (means), Mann-Whitney U test (median) and chi square test (Proportions) and p value of < 0.05 was considered significant.

Results
A total of 330 HIV infected children were recruited, out of these 183 (55.4 %) were females. The median age of the study populations was 12.0 years (IQR 11-15). Median duration on ART at the time of the study was 48 months (IQR 30-62). Majority were in WHO stage III and had CD4 cell count above 500cells/µl. The median age at menarche for HIV infected adolescents was 15 years (IQR 14-16). HIV infected females and males had no significant age difference at baseline but attained puberty one or two years later than their HIV negative peers.

Conclusion
HIV infected children have delayed sexual maturation compared to their HIV negative peers. We recommend that as part of physical examination of an adolescent, sexual maturation to be emphasized and those identified to have delayed onset to be followed up and offered appropriate counselling.

Key Words: *Puberty, Sexual maturation, Tanner stage, HIV, Dar es Salaam*.

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Introduction

Global estimates show that in 2019 the number of adolescents (10-19 years) living with HIV was 1740000, and 170000 were newly infected while 34000 died from AIDS-related causes (1). During the same year About 1.8 million children aged 0-15 years were living with HIV and 150 000 were newly infected while approximately 95 000 died from AIDS related causes (1). Significant number of HIV in children and adolescents <15 years occurs through vertical transmission from mother to child accounting for over 90% of new HIV infections among this age group (2). However, horizontal transmission mainly through sexual transmission and less common injection drug use or blood transfusion has been reported to be the cause of new HIV infection among adolescents, and young people aged 15–24 year (3).

Without treatment almost half of children with perinatally-acquired HIV infection die by 5 years of age (4). However, with increased access to antiretroviral therapy (ART) a growing population of adolescences is living with HIV and AIDS (5, 6). HIV-infected adolescents, unlike their HIV negative counterparts, are more likely to present with stunted growth or pubertal delay (7-9). The onset of puberty varies among individuals, girls normally begin puberty between 8 and 13 years of age while in boys it generally occurs later, between ages 9 and 14 years (10, 11). Muze et al collected data between July 2009 to February 2010 to assess growth and pubertal development parameters of 3384 urban Tanzanian Children and adolescents aged between 6-18 years. The mean age at onset of pubic hair was 12.3 years and breast (females) was 11.8 years and the genital development in males was 12.2 years and pubic hair was 12.8 years (12).

The precise mechanisms responsible for pubertal onset in adolescents 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 (13). 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 (14-18). 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. The age at which pubertal milestones are attained varies among the population studied and is influenced by activity level of hormone and nutritional status (11). Delayed

puberty is diagnosed when there is no breast development in girls by the age of 13 years or growth of testes in boys by 14 years of age (19). Delayed puberty may complicate life due to associated stigma and discrimination related to HIV status (20). This may impose a psychosocial burden with poor body image, low self-esteem, teasing, bullying, social withdrawal, declining academic performance, and school avoidance (21).

Studies have shown that adolescents living with HIV have significant delays in pubertal onset compared to uninfected children, which is more pronounced among those with symptoms of severe immunosuppression (22). Despite high prevalence of HIV in Africa, there is limited information on growth and sexual maturation among children with HIV infection in Tanzania. This study aimed at assessing the timing of sexual maturation of HIV infected children and adolescents.

Methods

Study design and settings

This was a cross-sectional hospital based descriptive study that was conducted in two care and treatment centres (CTC) in Dar es Salaam, from August 2011 to February 2012. The study included HIV infected children aged 8-18 attending CTC of Ilala, and Mwananyamala municipal hospitals in Dar es Salaam region. The two municipal hospitals were conveniently selected. 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. (23) HIV infected children and adolescents with obvious dysmorphic features suggestive of syndromic diseases, those who were very sick, those where consent/ assent was not obtained and those who were diagnosed with HIV less than 6 months prior to the study were excluded. Eligible children and adolescents aged 8-18 years were consecutively enrolled in the study until the sample size was reached.

A structured questionnaire was used to collect participant's information on social demographic, weight and length, stages of sexual development (Tanner), HIV status, WHO disease stage and CD4 cell count.

Before conducting the study, a paediatric endocrinologist who also previously collected data used for historic cohort, trained the investigator and a research assistant to assess sexual maturation using the sexual maturity rating (Tanner staging) system.(24), Also summarized as shown in appendix 1.

The questionnaire was pilot tested at Muhimbili National Hospital CTC under supervision of two paediatricians and a paediatric endocrinologist to ensure consistency. Investigator and the trained research assistant collected data which also involved performing physical examination including assessment of sexual maturation by Tanner stage, and the process of data collection was supervised by two paediatricians.

After obtaining consent from parents and assent/consent from older children (8–17 years) aware of their HIV status, senior paediatric resident interviewed the participants and performed physical examination. Information regarding participant WHO clinical stage and CD4 count was extracted from the patient's files. The investigator also took blood samples for CD4 count test, if there were no results of recent CD4 count, taken within 3 months prior to data collection.

Ethical considerations

Ethical approval for this study was obtained from the institutional review board of Muhimbili University of Health and Allied Sciences (MUHAS) Senate Research and Publications Committee. Permission to conduct the study was obtained from the district medical offices (DMO's) offices in both Kinondoni and Ilala district, also permission was provided by the heads of the two participating hospitals.

Data processing and analysis

Data analysis was done using STATA® 10 IC, anthropometric measurements sex-adjusted Z- scores were calculated using the WHO growth standard with a STATA Macro program and interpreted according to growth reference for 5-19 years(25). Onset of puberty was defined as Tanner stage ≥2 Pubertal development of HIV infected children was compared to that of a historical cohort of HIV negative children which was assembled by Muze et al in Tanzania, comprising of children and adolescents aged 6-18 years old. The Historical cohort was assembled from July 2009-February 2010 as part of Dr. Muze's research for clinical fellowship in Paediatric endocrinology under Paediatric Endocrine Training Centres for Africa (PETCA) in Nairobi Kenya. The objective of the study was to assess the growth and pubertal development parameters of 3384 healthy children and adolescents aged 6-18 years. These children and adolescents were enrolled from 17 randomly selected primary schools and secondary schools in the 3 municipals (Temeke, Ilala and Kinondoni) of Dar es Salaam, Tanzania(12)..

Continuous variables were summarised as median and interquartile range as well as mean or standard deviation depending on distribution, while categorical variables were summarised as proportions. Differences in various groups was tested using, Student t- test (means), Mann-Whitney U test (median) and chi square test (Proportions) and p value of < 0.05 was considered significant.

Supplementary Table 1: Characteristics of the children and adolescents in a Historical cohort collected in 2009, used for comparison

Variable	**N**	**%**
Age groups		
6-<10	1,024	30.26
10-<14	1,285	37.97
14-18	1,075	31.77
Sex		
Male	1,570	46.39
Female	1,814	53.61
Socio economic status	**N**	**%**
Low	689	46.78
Middle	531	36.05
High	253	17.18
Anthropometry	**Mean (SD)**	**95% CI**
Weight	36.01 (14.01)	35.54 - 36.49
Height	140.46 (18.62)	139.83 - 141.09
BMI	17.68 (5.09)	17.50 - 17.85
Waist circumference	62.07 (8.70)	61.77 - 62.36
Age at Menarche/Adrenarche	13.20(1.14)	13.10 - 13.29
Mothers age at Menarche	14.90(1.51)	14.75 - 15.04

Results

During the study period 330 participants were enrolled, of these 183 (55.4%) were females, and the median age was 12.0 years (IQR 11-15). All participants enrolled had confirmed HIV infection and were on ART for a median duration of 48 months (IQR 30-62). Most of the participants were in WHO stage I and had a median CD4 count of 564 cells / µL (IQR 346-

917) (Table 1). Mean age and height for age z- scores were similar between male and females. There was no significant difference between male and females in relation to Tanner stage, level of education, WHO disease stage or median duration on ART. However, females were heavier than males (32 vs 28 kg) and had higher BMI Z-score (0.18 vs -0.24 kg; p=<0.001. Males had higher median absolute CD4 count (610 Vs 550 cells / µL; p=<0.001) than female (Table 2).

Table 1. Demographic and clinical characteristics of HIV infected children

Variable	**N**	**%**
Sex		
Female	147	44.5%
Male	183	55.4%
Education level		
Primary school	265	80.3
Secondary school	65	19.7
WHO disease stage		
I	7	2.1
II	62	18.8
III	250	75.8
IV	11	3.3
Tanner stage		
I	164	49.7
II	95	28.8
III	70	21.2
IV	1	0.3
	Median	**IQR**
Age	12	11-15
Weight	32.0	25-45
Age at menarche	15	14-16
CD4 count	564	346-917
Duration of ART in months	48	30-62

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Table 2. Demographic and clinical characteristics of HIV infected children in relation to 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)	0.92
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)	0.002
Mean HAZ (SD)	0 .03 (±1.0)	-0.03 (±0.9)	0.26
Mean BMIZ (SD)	0.18 (±1.1)	-0.24 (±0.7)	<0.001
Parameters related to disease			
Median CD4 Count (IQR)	550 (250-875)	610 (363-978)	0.07
Median duration on ART in month (IQR)	45 (24-48)	52 (24-60)	0.67
WHO classification; n (%)			
I	5 (2.7%)	2 (1.3%)	
II	31 (16.9%)	31 (21.0%)	0.41
III	143 (78.1%)	107 (72.7%)	
IV	4 (2.1%)	7 (4.7%)	
Education level; n (%)			
Primary school n (%)	147 (55.5)	118 (44.5)	0.55
Secondary school n (%)	36 (55.4)	29 (44.6)	
Tanner stage; n (%)			
I	94 (51.4)	70 (47.6)	0.13
II	48(26.2%)	47(32.0%)	
III	40(21.9%)	30(20.4%)	
IV	1(5%)	0(0)	

When HIV infected children were compared to their HIV negative peers in the historical cohort there was no significant age difference for both females (12.4 Vs 12.3; P=0.23) and males (13.1 Vs 12.8; P=0.56). There was a statistically significant difference (P<0.01) in relation to weight for age, height for age, and body mass index between the groups, but the

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Z scores for these anthropometries were greater than -2.0 standard deviation indicating normal nutritional status (Table 3).

Table 3. Comparison of the mean and confidence interval of HIV infected children and the children and adolescents in the historical cohort by sex

	HIV Positive cohort (n=183): Mean (95%CI)	Historical cohort (n=1542): Mean (95%CI)	P Value
	Female		
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

With regard to the pubertal development, HIV infected females and males; attained puberty at later age than their HIV negative peers. For instance, HIV infected females were more likely to be in Tanner stage II for breast development one year later (median age of 13.5 Vs 12.0 years; p= 0.01) than their HIV negative peers. In addition, HIV infected females were more likely to be in Tanner stage II for pubic hair development, two years later (median age 14.0 vs 12 years; P=0.001) compared to their HIV negative peers.

HIV infected males were more likely to be in Tanner stage II for genital development one year later (median age of 13 Vs 12.0 years; p= 0.01) than their HIV negative peers. However, there was no age difference between HIV infected males and HIV negative males in relation to being in Tanner stage II for pubic hair development (P=0.14) compared to their HIV negative peers.

None of the HIV infected children in both sexes had attained Tanner stage IV or V (Table 4).

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Table 4. Ages at various Tanner stages among HIV infected children compared to HIV negative children

Tanner stages	HIV positive	HIV Negative	P- value
	Median Age (IQR)	**Median Age** (IQR)	
Females			
Breast development			
I	11.0 (9-12)	9.0 (8-11)	0.001
II	13.5 (12-15)	12.0 (11-13)	0.001
III	15.0 (14-17)	13 (13-14)	0.001
IV	0 (0)	15 (14-16)	
V	0 (0)	17 (16-18)	
Pubic hair development			
I	11.0 (11-12)	9.0 0 (8-11)	0.001
II	14.0 (12-15)	12.0(11-13)	0.001
III	15.0 (14-17)	14.0 (13-14)	0.001
IV	0 (0)	15.0 (14-16)	
V	0 (0)	17.0 (16-18)	
Males			
Genital development			
I	10.0 (9-11)	10.0 (9-11)	0.936
II	13.0 (12-14)	12.0 (11-13)	0.023
III	16.0 (15-17)	14.0 (13-15)	0.001
IV	0 (0)	16.0 (15-17)	
V	0 (0)	17 (16-18)	
Pubic hair development			
I	11.0 (10-12)	10.0 (9-11)	0.001
II	13.0 (12-14)	13.0 (12-14)	0.137
III	16.0 (15-17)	14.2 (13-15)	0.001
IV	0 (0)	16.0 (15-17)	
V	0 (0)	17.0 (16-18)	

Discussion

This study aims to describe the timing of sexual maturation of HIV infected children and adolescents. Findings shows that HIV infected children in Dar es Salaam Tanzania have delayed pubertal development compared to their HIV negative peers. Although there was a statistically significant difference in the two groups in relation to weight for age, height for age, and body mass index, the difference was not clinically significant as the Z scores for these anthropometries were greater than -2.0 standard deviation, indicating normal nutritional status. The onset of puberty was delayed by at least one to two years for both boys and girls compared to their HIV negative peers. In study done in USA the onset of puberty was delayed by at least six months amongst HIV infected youth compared to their peers who were HIV exposed but not infected (8) (15). Similar observation of delayed onset of puberty was reported in a multicentre longitudinal study involving HIV infected children aged 8 to 18 years in Italy which showed the median, onset of puberty being delayed by about 2 years in girls and 1 year in boys(18). HIV infected children were also found to have delayed puberty by about 2.5 years in girls and 1.5 years in boys (18).

Findings from our study and other studies showing similar findings, corroborates with the existing literature, which shows that chronic diseases can affect sexual maturation (8). HIV infection among children and adolescents is acquired mainly through mother to child transmission, but horizontal transmission (through sexual transmission and less common injection drug use or blood transfusion) can occur (3). Thus, resulting in variation in duration of HIV infection, ART use and also severity of immunosuppression that can directly or indirectly affect sexual maturation. For instance, delayed sexual maturation among HIV infected children can be a result of multiple factors including; direct effect of virus, severe immunosuppression, nutritional disorders, action of cytokines and endocrine dysfunction (26). Delayed sexual maturation is also thought to be a result of HIV-1-induced immune dysfunction which alters the neural control of puberty (16). The endocrine dysfunction in perinatally HIV infected children includes an euthyroid sick syndrome, accompanied by increased basal thyrotrophin levels, reduced free thyroxine levels (27), and low levels of insulin growth factor 1 (IGF-1) and IGF-binding protein (28). The combination of under-nutrition and elevated pro-inflammatory cytokines has also been associated with reductions in insulin-like growth factor 1 and alterations in gonadotrophin-releasing hormone-secretion patterns, which may result in delayed growth and puberty (26).

In our study, despite all children being on ART for median duration of 4 years and having median CD4 of > 500 for both males and females, we observed, delayed sexual maturation. Similarly an observation from a study done in Uganda showed that almost two thirds of the patients had delayed sexual maturity, which did not improve after 12 month of ART (29). Furthermore, a delay in pubertal stages and menarche was reported form a longitudinal cohort of older children in sub-Saharan Africa, who were initiated ART at approximately 7–12 years of age and followed up for approximately 3 years on ARV (22). Findings from these studies suggest that the delay in sexual maturation is possibly a result of several factors including delayed ART and immune-suppression and is irreversible even with the use of ARVs.

Study limitations

Our study has several limitations, first it is limited by the study design (cross sectional study), we can only establish correlation, not causality. Secondly, we conveniently selected two health facilities in Dar es Salaam, which could introduce selection bias and limit generalizability. Thirdly we were unable to differentiate perinatally infected and recently infected adolescents and this could have neutralised the effect of HIV on pubertal development as those who were recently infected could still have normal pubertal development. Lastly although we ensured rigorous training, supervision and monitoring of the data collection process, we cannot exclude the possibility of interobserver error, as the two researchers each was assigned to preform data collection and assessment in one of the selected CTC.

Despite the limitations our findings are similar to that of other studies, this gives a snapshot of a possible correlation of HIV and pubertal development among children and adolescents living with HIV.

Conclusion

HIV infected children have delayed sexual maturation compared to their HIV negative peers. We recommend that assessment of sexual maturation, to be emphasized as part of comprehensive examination of adolescents during in CTCs clinic. This will help to identify those with delayed sexual maturation and may be in need of follow up and appropriate counselling.

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Authors' contributions

RK, GM, HN and CM contributed in the study conceptualization, methodology, data visualization, RK and GM drafted the manuscript, HN and CM edited the manuscript and all authors have seen and approved the final version of the manuscript.

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Potential Conflicts of Interest

The authors do not have any potential, perceived, or real conflicts of interest relating to this work.

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Abbreviations:

AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
CTC	Care and Treatment
HIV	Human Immunodeficiency Virus
IGF	Insulin Growth Factor
MUHAS	Muhimbili University of Health and Allied Sciences
WHO	World Health Organization

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Appendix 1

	Breast	Pubic Hair	Genitals	Pubic Hair
Stage 1	Small nipples. No breast. 	No pubic hair. 	No signs of puberty. Scrotum, testes, and penis as in childhood. 	No pubic hair. 
Stage 2	Breast and nipples have just started to grow. The areola has become larger. Breast tissue bud feels firm behind the nipple. 	Initial growth of long pubic hairs. These are straight, without curls, and of light color. 	Initial growth of scrotum and testes. The skin on the scrotum has become redder, thinner, and more wrinkled. The penis may have grown a little in length. 	Few hairs around the root of the penis. The hairs are straight, without curls, an of light color. 
Stage 3	Breast and nipples have grown additionally. The areola has become darker. The breast tissue bud is larger. 	The pubic hair is more widespread. The hair is darker, and curls may have appeared. 	The penis has now grown in length. Scrotum and testes have grown. The skin of the scrotum has become darker and more wrinkled. 	Hairs are darker and curlier and still sparse, mostly located at the penis root. 
Stage 4	Nipples and areolas are elevated and form an edge towards the breast. The breast has also grown a little larger. 	More dense hair growth with curls and dark hair. Still not entirely as an adult woman. 	The penis has grown in both length and width. The head of the penis has become larger. The scrotum and testes have grown. 	More dense, curly, and dark hair. The hair growth is reaching the inner thighs. 
Stage 5	Fully developed breast. Nipples are protruding, and the edge between areola and breast has disappeared. 	Adult hair growth. Dense, curly hair extending towards the inner thighs. 	Penis and scrotum as an adult. 	Pubic hair extends upwards to the umbilicus. It is dense and curly.