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Sexual maturity staging and affecting immune-hormonal factors in children aged 6-18 years living with HIV: experience from tertiary care center in North India

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Abstract

Introduction: Children living with human immunodeficiency virus (HIV) may experience growth failure and delay in sexual maturation. The aim of the study was to investigate sexual maturity rating and hormonal levels in children living with HIV aged 6-18 years.

Material and methods: In this cross-sectional observational study over 18 months, 69 subsequent children living with HIV (CLHIV), aged between 6- and 18-years, attending antiretroviral therapy (ART) clinic were enrolled. Clearance from the Institutional Ethical Committee and informed consents were obtained.

Results: Baseline data of 69 children showed male predominance (66.7%), vertical transmission (95.7%), normal body mass index (BMI) (71%), immunocompetency (3/4th), and low viral load (2/3rd). Sexual maturity staging revealed that the majority were pre-pubertal (pubic hair (PH) stage 1: boys = 54.3%, girls = 39.1%; testicular volume stage 1 = half; breast stage 1 = 39.1%). Delayed puberty was observed in 6.5% of boys and 8.6% of girls. The mean age of menarche was 13 ± 1.4 years. Hormonal levels between boys and girls showed statistically significant difference in levels of follicular stimulating hormone (FSH), luteinizing hormone (LH), prolactin, estrogen, and Free androgen index (FAI). Luteinizing hormone had high sensitivity and specificity (cut-off value = 1.06, sensitivity = 95.5%, specificity = 83.3%) for predicting stage 2 of testicular volume (gonadarche), and in girls (cut-off value = 1.74, sensitivity = 92.9%, specificity = 88.9%). For FSH in girls, cutoff value of 5.42 provided high sensitivity (92.9%) in predicting thelarche (breast stage 2). No statistically significant association was observed with CD4 categories ($p = 0.3$), viral load categories ($p = 0.2$), age at ART initiation, and BMI categories with pubertal stages. Among antiretroviral drugs, less than 50% of children on abacavir, zidovudine, and lopinavir/ritonavir had attained pubertal onset (in girls, breast stage ≥ 2; in boys, testicular volume (TV) stage ≥ 2).

Conclusions: Delayed puberty was observed in 6.5% of boys and 8.6% of girls. Median ages at thelarche and gonadarche were higher in included children when compared to healthy school going children. HIV infection may have a negative effect on the sexual maturity rating in CLHIV.

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Key words: children living with HIV, puberty, Tanner's stages, LH, FSH, testosterone.

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Introduction

Approximately 1.7 million children < 14 years are living with human immunodeficiency virus (HIV) [1]. This constitutes 5% of all people living with HIV (PLHIV) and 10% of new infections occurring in adults [2]. In South Asia, around 4% of adolescents are living with HIV [2]. In India, according to the National AIDS Control Organization (NACO), the total number of people living with HIV is 2.37 million, of which 79,000 are children [3].

With the initiation of highly active retroviral therapy (HAART), HIV infection is considered a chronic disease and hence, may affect puberty and growth spurt [4] by recurrent infections and nutritional deficiencies [5]. HIV-infected children may have a dysregulated growth pattern right from birth, with low birth weight [6]. Other mechanisms include emotional deprivation, gastrointestinal anomalies, metabolic disturbances, and hormonal disarray (GH deficiency, hypothyroidism, decreased testosterone, etc.). HIV affects puberty and its hormones due to malnutrition, growth failure, emotional stress, and chronic therapy and its toxicity. HIV-related endocrinopathy has been documented to affect the function of thyroid gland, adrenal gland, and gonads [7], with an increase in cytokine release and chronically activated immune system, which may interfere with neuronal pathways involved in gonadotropin production and secretion [8]. The decreased adrenal androgen secretion can be partly attributed to the delayed pubertal development [9].

The pubertal onset and its progression in HIV-infected children have been evaluated in few studies, and delay in the onset of puberty among HIV-infected children has been reported [10-14]. Studies evaluating the pubertal stages and hormonal analysis among CLHIV are lacking. Since there is a scarcity of studies on sexual maturation and hormone level estimation in CLHIV, we aimed to investigate the puberty and hormonal levels among CLHIV in India.

Material and methods

Study type, setting, and duration

This cross-sectional observational study was conducted in an antiretroviral therapy (ART) clinic of a tertiary hospital in India from November 2019 to April 2021 (18 months), after obtaining clearance from the Institutional Ethical Committee (approval Number: IEC 710/ 19).

Sample size

Sample size was based on Szubert *et al.* [5] study, which concluded that pubertal stage 2 (P2) in girls, P2 in boys, breast stage 2 (B2) in girls, and gonadal stage 2 (G2) in boys were observed in 15.19% of girls, 22.06% of boys, 14.61% of girls, and 21.73% of boys, respectively. With 10% margin of error and 5% level of significance, a total of 67 children were required to complete the study.

Inclusion criterion

All subsequent CLHIV, aged between 6 and 18 years, attending ART clinic, after acquiring informed written consent/assent were enrolled in the study,

Exclusion criterion

Children with known history of chronic diseases, such as type 1 diabetes, cystic fibrosis, or hepatitis B, which may affect growth and maturation, were excluded.

Data collection

After enrollment, baseline demographic details (age, gender, age at diagnosis, ART, etc.) were recorded in Performa software. Data on the routine investigations (blood counts, liver function tests, lipid profile, CD4 counts, and viral load) were obtained from previous records. World Health Organization (WHO) staging was used for classifying HIV [15]. Sexual maturity staging (SMR) examination was done in girls and boys according to standard guidelines in a separate room [16, 17]. Girls were assessed for pubic hair (PH) and breast stages, while boys were evaluated for PH stages and genitalia development based on Tanner's classification. Testicular volume (TV) in boys was measured by orchidometer (stage 1 = TV less than 4 ml, stage 2 = TV 4-8 ml, stage 3 = TV 9-12 ml, stage 4 = 15-20 ml, stage 5 = TV more than 20 ml) [18]. Stage 1 was considered as pre-adolescent stage, breast stage 2 as thelarche in girls, pubic hair stage 2 as pubarche, and TV stage 2 as gonadarche. Age at menarche was questioned in girls. Absence of sexual characters (Tanner's stage 2) by the age of 13 years in girls and 14 years in boys was considered as delayed puberty.

Hormones were evaluated as per study protocol, and included thyroid function test (free T3, free T4, and thyroid stimulating hormone (TSH)), gonadotrophins (follicular stimulating hormone (FSH) and luteinizing hormone (LH)), sex steroids (testosterone, estrogen), prolactin, and sex hormone binding globulin (SHBG)). Free androgen index (FAI) was calculated as the ratio of testosterone to SHBG. Hormone levels in collected serum samples were estimated by using fully automated immunoassay analyzer with chemiluminescence technology. FSH, LH, and estrogen levels in girls who have attained menarche were obtained on a particular phase of cycle (day, 2-7) to maintain uniformity.

Statistical analysis

Data were analyzed using SPSS software, version 17.0. Qualitative variables were presented as frequency and percentages. Quantitative variables were displayed as mean (standard deviation) or median (range). *P*-value of < 0.05 was considered statistically significant. Age at diagnosis of HIV, duration of ART, and hormonal levels were compared using Mann-Whitney test. Mean BMI and differences in bio-chemical parameters between boys and girls were

compared using Students *t* test. Differences in categorical variables (route of transmission, history of TB, CD4 categories, and viral load categories) based on staging were assessed using χ^2 test. Median age (50th percentile) at stage 2 was presented separately for boys and girls, along with interquartile range (25th and 75th percentile). Median level of all hormones (50th percentile) for each stage was reported separately for boys and girls, along with interquartile range (25th and 75th percentile). Receiver operating curve (ROC) was used to calculate area under curve (AUC) to assess the discriminatory power of hormones (LH in boys and girls, FSH in girls) for identifying stage 2. Based on cut-off values derived from ROC analysis, sensitivity and specificity for hormones were calculated. Comparison of median age at stage 2 across all categories of ART duration and median age at different pubertal stages in boys and girls (separately) were compared with BMI categories using Kruskal-Wallis test.

Results

Baseline characteristics

A total of 69 children were enrolled in the study, and all were in WHO stage 1. The baseline clinical and laboratory characteristics are shown in Table 1, with predominance of male (66.7%) and vertical transmission (95.7%). Nutritional assessment revealed that the majority had normal BMI (71%), 14.5% were thin, 13% were severely thin, and 1 child was overweight (BMI of > 2 SD). A total

Table 1. Baseline clinical and laboratory parameters of the enrolled children

Parameter	Boys (*n* = 46, 66.7%)	Girls (*n* = 23, 33.3%)	*p*-value
Age in years (mean ± SD)	12.5 ± 2.9	12.4 ± 2.7	> 0.05
Median age in years at diagnosis	5 (IQR: 2-9)	5 (IQR: 2-8)	0.90
Duration of ART in years	6.5 (IQR: 3-10)	5 (IQR: 2-9)	0.36
Vertical mode of transmission	44 (95.7%)	22 (95.7%)	?????
BMI, mean ± SD (kg/m^2)	15.8 ± 2.8	17.1 ± 3.2	0.09
Hemoglobin level, mean ± SD (g/dl)	12.0 ± 1.5	11.8 ± 1.4	0.63
Total leucocyte count, mean ± SD (mm^3)	6,360.9 ± 1,496	6,495.7 ± 2,240	0.77
Platelet count, mean ± SD (mm^3)	2.5 ± 0.6	2.7 ± 0.9	0.17
Urea, mean ± SD (mg/dl)	20.5 ± 4.8	18.3 ± 4.5	0.07
Creatinine, mean ± SD (mg/dl)	0.5 ± 0.2	0.6 ± 0.6	0.48
SGOT, mean ± SD (U/l)	37 ± 13.1	34.0 ± 8.4	0.32
SGPT, mean ± SD (U/l)	27.7 ± 15.9	25.2 ± 10.2	0.49
Total cholesterol, mean ± SD (mg/dl)	144.3 ± 27.4	161.5 ± 40.0	0.04
Triglycerides, mean ± SD (mg/dl)	127.2 ± 57.8	124.6 ± 42.8	0.85
Sodium, mean ± SD (mmol/l)	138.9 ± 2.9	138.3 ± 3.3	0.44
Potassium, mean ± SD (mmol/l)	4.2 ± 0.5	4.1 ± 0.4	0.40
Calcium, mean ± SD (mg/dl)	9.3 ± 0.7	9.3 ± 0.7	0.94
Phosphorous (mg/dl)	4.2 ± 0.8	4. 1± 0.7	0.48
CD4 count, mean ± SD (cells/mm^3)	682.3 ± 295.7	789.0 ± 344.6	0.18
FSH, median (IQR: 25/75) (mIU/ml)	3.6 (1.3-5.9)	7 (2.6-8.4)	0.01*
LH, median (IQR: 25/75) (mIU/ml)	1.2 (0.2-3.7)	4.3 (0.4-6.2)	0.03*
Prolactin, median (IQR: 25/75) (ng/ml)	7.7 (5.0-11.5)	11.5 (9.7-17.0)	0.003*
Free T3, median (IQR: 25/75) (pg/ml)	3.5 (3.3-3.8)	3.6 (3.3-3.9)	0.57
Free T4, median (IQR: 25/75) (ng/dl)	0.7 (0.7-0.9)	0.8 (0.7-0.8)	0.72
TSH, median (IQR: 25/75) (mIU/ml)	3.2 (2.4-4.5)	2.9 (2.0-4.4)	0.47
Estrogen, median (IQR: 25/75) (pmol/l)	47.4 (28.9-74.6)	73.4 (43.6-168.0)	0.003*
Testosterone (nmol/l)	0.5 (0.2-12.0)	0.3 (0.2-0.5)	0.09
SHBG, median (IQR: 25/75) (nmol/l)	90.7 (69.9-113.0)	83.6 (54.9-144.0)	0.64
FAI, median (IQR: 25/75)	3.5 (0.2-23.8)	0.6 (0.2-1.3)	0.03*

ART – antiretroviral therapy, BMI – basal metabolic index, IQR – interquartile range, SGOT – serum glutamic oxalacetic transaminase, SGPT – serum glutamic–pyruvic transaminase, TSH – thyroid stimulating hormone, FSH – follicular stimulating hormone, LH – luteinizing hormone, SHBG – sex hormone binding globulin, FAI – free androgen index.

Table 2. Percentage of children in different pubertal stages (breast and testicular volume stages)

Stages	Males, *n* (%)	Females, *n* (%)	*p*-value
Breast/ testicular volume stages			
1	24 (52.2)	9 (39.1)	< 0.001
2	14 (30.4)	3 (13.0)	
3	8 (17.4)	2 (8.7)	
4	0	8 (34.8)	
5	0	1 (4.3)	
Pubic hair stages			
1	25 (54.3)	9 (39.1)	0.428
2	6 (13.0)	3 (13.0)	
3	7 (15.2)	3 (13.0)	
4	8 (17.4)	8 (34.8)	

of 19.6% of boys and 21.7% of girls had a positive history of tuberculosis. Most children were immunocompetent, with CD4 counts > 500 cells/mm^3 (73.9%), and one-fourth (26.1%) with CD4 counts < 500 cells/mm^3. Viral load count was < 1,000 copies/ml in more than two-thirds (69.6%), but > 1,000 copies/ml in 30.4% of children. Retroviral drugs used were efavirenz (58%), zidovudine (56.5%), abacavir (30.4%), nevirapine (24.6%), lopinavir/ ritonavir (14.5%), and tenofovir (11.6%).

Sexual maturity

The percentage of children in different stages of puberty are shown in Table 2. Most of the boys (93.5%) had started their pubertal onset (TV or PH stage 2) before 14 years of age. In girls, 91.4% had started their pubertal onset (breast or PH stage 2 and above) before 13 years of age. Therefore, a total of 7.2% (6.5% of boys, 8.6% of girls) of the children presented with delayed puberty. Table 3 demonstrates median ages of boys and girls in different stages of pubertal development. The results of SMR staging (Tanner's charts) for PH showed that 54.3% of boys and 39.1% of girls were lagging at the time of enrolment. Almost half of boys (52.2%) had TV < 4 ml, and 39.1% of girls were in breast stage 1. Only half of girls (52.2%) had attained menarche at the time of enrolment. The mean age of menarche was 13 ± 1.4 years.

The results of hormone levels estimation showed that there was a statistically significant difference in levels of FSH, LH, prolactin, estrogen, and FAI, but not in free T3, T4, and TSH levels between boys and girls (Table 3). Median levels of hormones in boys and girls with different stages of PH, breast, and TV are shown in Figure 1. In PH stages in both boys and girls, the median FSH, LH, estradiol, testosterone, FA1, and prolactin levels indicated an increasing trend, but decline in SHBG levels. The levels of FSH, LH, prolactin, TSH, estradiol and testosterone showed an increasing trend with the testicular volume stages in males whereas the FAI showed a decreasing trend. Breast development staging in females showed an increase in the levels of LH, FSH, prolactin, and FAI, but a decrease in SHBG.

Receiver operating curve analysis revealed higher AUC for LH (boys – AUC: 0.913; 95% CI: 0.82-0.99%; girls – AUC: 0.90; 95% CI: 0.72-0.99%) than FSH with AUC of 0.88 (95% CI: 0.71-0.99%). Sensitivity and specificity for LH in boys showed that a cut-off value of 1.06 provided higher specificity and sensitivity (83.3% and 95.5%) in comparison with cut-off value of 0.89 (specificity 79.2% and sensitivity 95.5%) for predicting stage 2 of testicular volume (gonadarche). In girls, a cut-off value for LH of 1.74 provided higher sensitivity and specificity (92.9% and 88.9%) for predicting thelarche. For FSH in girls, a cut-off value of 5.42 showed

Table 3. Tanner's staging and median age of boys and girls based on pubic hair development, breast development in females, and testicular volume in males

Parameter	Boys			Girls		
	Median	P25	P75	Median	P25	P75
Breast/testicular volume staging						
1	10.5	8.5	12	10	9	12
2	14	13	15	11	11	12
3	16.5	15	17	14.5	13	16
4	0	0	0	14	12.8	15
5	0	0	0	17	17	17
Pubic hair staging						
1	11	9	12	10	9	12
2	14	12	15	12	11	14
3	15	14	16	15	13	16
4	16	14	17	14	12.8	15.5

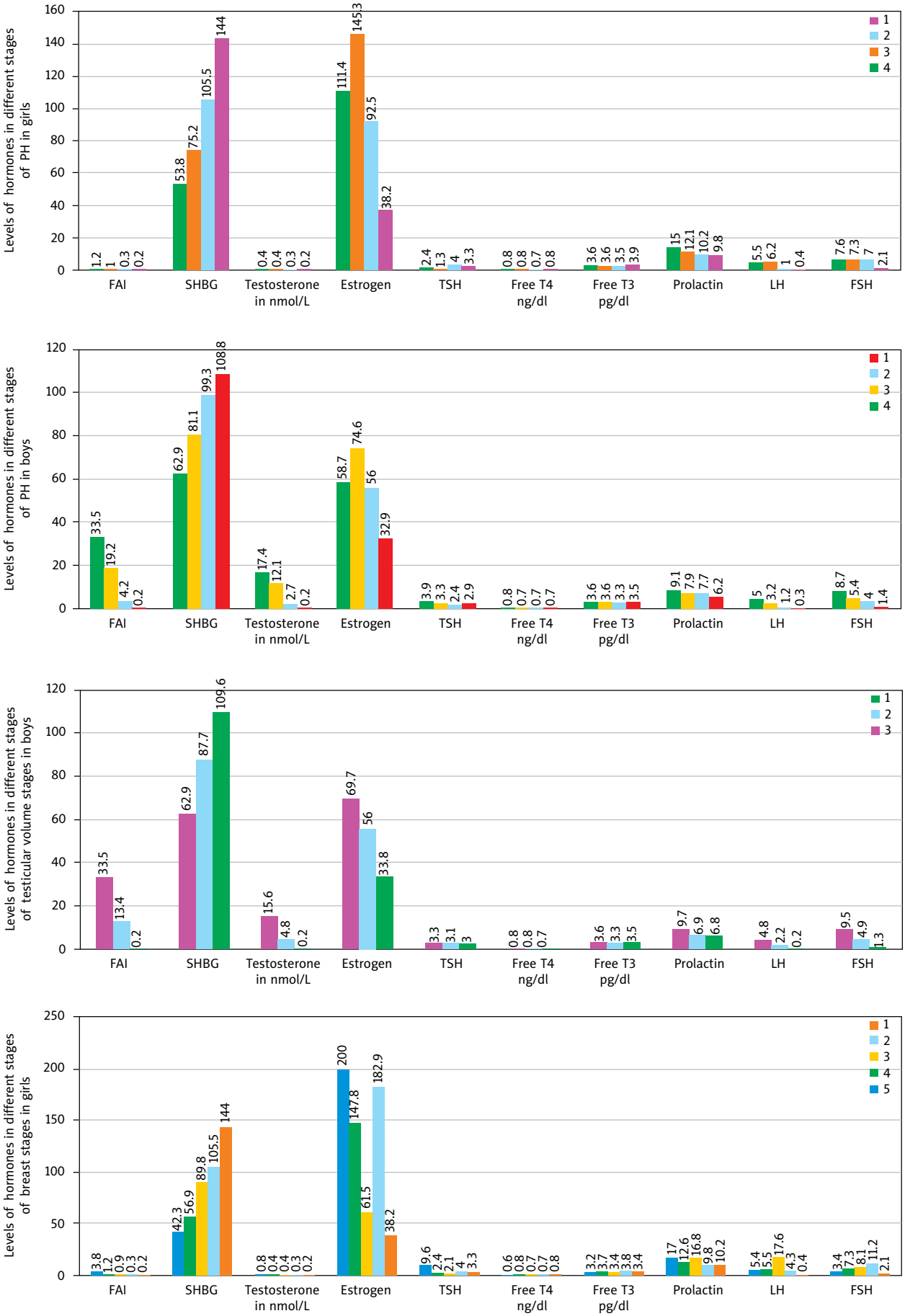

Figure 1. Median levels of hormones in different pubertal stages in boys and girls enrolled in the study

higher sensitivity (92.9%) in comparison to cut-off value of 7.2, which had higher specificity (88.9%) in predicting thelarche (breast stage 2).

No statistically significant association was observed within CD4 counts (p = 0.3), viral load (p = 0.2), and BMI categories, with pubarche and thelarche in girls or gonadarche in boys. Among antiretroviral drugs, less than 50% of children on abacavir, zidovudine, and lopinavir/ritonavir had attained pubertal onset (breast stage ≥ 2 in girls, TV stage ≥ 2 in boys). Pubertal onset in boys on tenofovir (80%) was the highest, followed by nevirapine (n = 6, 60%), efavirenz (n = 14, 50%), zidovudine (n = 13, 48.2%), abacavir (n = 4, 30.2%), and protease inhibitor (lopinavir/ritonavir). Similarly, thelarche in girls was the highest in those on tenofovir (100%), followed by nevirapine (n = 6, 85.7%), zidovudine (n = 7, 58.3%), efavirenz (n = 4, 50%), abacavir (n = 6, 50%), and protease inhibitor (lopinavir/ritonavir).

Discussion

The results of the current study among 69 children showed that only half (52.1%) of them had achieved pubertal onset (breast stage ≥ 2 in girls, and TV stage ≥ 2 in boys at the time of enrolment). These outcomes were considerably higher than the percentage of HIV-infected children attaining puberty in studies by Szubert *et al.* (18.8%) [5] and Bellavia *et al.* (16%) [19]. Mbwile [13] in Tanzania reported a significant difference in the mean age at attainment of sexual maturation between HIV subjects and controls. De Martino *et al.* [10] documented a significant delay in the onset of sexual maturation of HIV-infected girls compared with controls. The results on pubertal delay in our study were comparable to other reports. The present study showed a delay in pubertal onset in 7.2% of children (6.5% of males and 8.6% of females), which was lower than in a study by Bellavia *et al.* [19] (9.9% of boys and 10.7% of girls), but higher than the subjects of Williams *et al.* [11] (4.1%) in HIV-infected children. In boys, the median age at testicular volume stage 2 was found to be higher in our study (14 years) in comparison with studies conducted in Italy [10] (12.1 years) and Tanzania [13] (13 years). A delay of 3.6 years was noted between the median years at TV stage 2 in our study compared with a study on healthy Indian school-going boys [20]. Girls in our study attained thelarche at an earlier age (11 years) than from studies conducted in Italy [10] and Tanzania [13]. A delay of 2.4 months was observed in median age of children with breast stage 2 in our study in comparison with healthy school-going Indian girls [21]. The median age for gonadarche and pubarche in our study was comparable with Chinese and Danish studies [22, 23]. Thelarche appeared 1 year before pubarche, and similar findings were observed by Martino *et al.* [10]. The mean age of attaining menarche was 13 ± 1.4 years (median, 12.5 years; IQR: 12-14 years), and time of progression from thelarche to menarche was 1.5 years. Iloh *et al.* [24] reported comparable mean age of menarche (12.8 years), but higher age (14.3 years) was observed by Szubert *et al.* [5].

No statistically significant difference was found in pubertal stages and viral load categories and CD4 count categories in our and other studies [10]. Inconclusive results on correlation of ART with pubertal onset are present. Age at the onset of puberty was not related to age of initiation of antiretroviral therapy in our study as well as in de Martino *et al.* [10]. A study in Uganda on ART naïve HIV-infected children reported some improvement in sexual maturation after 6 and 12 months of ART therapy [25]. In African children, strong delaying effect with an older age of ART initiation on the age of attaining all Tanner's stages in boys and girls and menarche in girls was observed [5].

The strength of our study was inclusion of assessment of hormone levels, which are active in puberty. The limitations included cross-sectional design. As puberty is a continuous process, applying longitudinal study would increase the generalizability of results as well as inclusion of health control group and larger sample size for more demographic correlations.

Conclusions

We conclude that CLHIV have a considerable delay in pubertal onset, and hence require a careful evaluation in ART clinics. A perceived or actual delay in sexual maturation in adolescents can lead to a stressful environment, and poor body image and self-esteem, which in turn can lead to psychological problems, such as depression and inability to mingle with peers, school avoidance, bullying, and sub-optimal academical performance [24]. Regular assessment of CLHIV for pubertal development is important, and appropriate steps should be taken if any deviation from normal. Larger future studies are needed to validate if hormonal therapy has any beneficial role in the growth and pubertal failure in CLHIV beyond nutritional supplementation [26, 27].

What is new in this study? We evaluated the sexual maturity staging in CLHIV along with hormonal levels. High delay in pubertal onset was observed in Indian CLHIV (6.5% of boys and 8.6% of girls). Delay in median age of breast stage 2 with a lag of 2.4 months in comparison with Indian healthy school-going girls. Delay in median age at testicular volume stage 2 with a lag of 3.6 years in comparison with Indian healthy school-going boys.

Disclosures

1. Institutional review board statement: The study was approved by the Institutional Ethics Committee of the ABVIMS and Dr RML Hospital, with approval number: F. No. TP (MD/MS)(46/2019)/IEC/ABVIMS/RMLH 710/19.
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3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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