

Pubertal Development and its Determinants in Adolescents With Transfusion-Dependent Thalassemia

PREETI SINGH, SUKLA SAMADDAR, NUPUR PARAKH, JAGDISH CHANDRA AND ANJU SETH*

From Department of Pediatrics, Lady Hardinge Medical College and Kalawati Saran Children's Hospital, New Delhi.

Correspondence to: Dr Anju Seth,
Director Professor, Department of
Pediatrics, Lady Hardinge Medical
College and Kalawati Saran
Children's Hospital, New Delhi 110
001, India. anju_seth@yahoo.com

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Objective: To assess pubertal development and its determinants in adolescents with transfusion-dependent thalassemia (TDT). **Methods:** In this cross-sectional study from a tertiary teaching hospital in Delhi, records of adolescents aged 17-19 years with TDT on regular transfusion at thalassemia day-care centre were reviewed. Pubertal development and its determinants were assessed. **Results:** Records of 58 (33 male) adolescents with TDT were reviewed. Among them, 42 (72.4%) had normal/delayed onset with spontaneous progression of puberty, while 16 (27.6%) had pubertal arrest/failure and received hormonal replacement therapy (HRT). Short stature was observed in all adolescents on HRT. Amongst other endocrinopathies, only hypoparathyroidism was found to be significantly higher in the HRT group. On multivariate analysis, serum ferritin (OR-1.005, 95% CI 1.002, 1.009) was observed to be the only significant determinant of pubertal arrest/failure. **Conclusion:** A significant proportion of adolescents with TDT continue to have pubertal arrest/failure. High systemic iron load is the key determinant for this.

Keywords: Delayed puberty, Growth failure, Hypogonadotropic hypogonadism, Ferritin.

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With the advent of intensive transfusion and robust chelation regimes, life expectancy has increased significantly in patients with transfusion-dependent thalassemia (TDT). Concurrently, endocrine dysfunction has emerged as an important cause of morbidity in adolescents and adults with TDT. Deranged pubertal development is amongst the commonest endocrinopathies observed in adolescents with thalassemia. Early recognition and timely management of these adolescents can not only help in optimizing growth and pubertal development but also improve their bone mineral density, quality of life and preserve fertility potential.

Children with TDT experience poor growth predominantly during the peri-pubertal phase or puberty. This is primarily due to transfusion associated iron overload affecting the growth hormone-insulin like growth factor axis (GH-IGF axis) and the hypothalamic-pituitary-gonadal (HPG) axis. Despite regular transfusions and optimal chelation therapy, the prevalence of pubertal disturbances in TDT still ranges between 30-70% [1-3]. In a previous study from our center, delayed puberty and/or hypogonadism were reported in 54.1% of children with TDT [4]. The key contributory factor is transfusion-mediated hemosiderosis in HPG axis that leads to hypogonadotropic hypogonadism (HH).

With improvement in the management of children with TDT, the prevalence of growth failure and pubertal

disturbances have decreased. However, information on the prevalence and manifestations of pubertal disturbances in adolescents with TDT from India is scarce. We report the spectrum of pubertal disturbances and their determinants in adolescents with TDT.

Invited Commentary: Pages 609-10

METHODS

This cross-sectional study was conducted at the thalassemia day-care centre and pediatric endocrine clinic of a tertiary level teaching hospital in Delhi over a period of 3 months from June, 2019 to August, 2019. The records of all adolescents with TDT in the age group 17 to 19 years attending the center and on regular transfusion and chelation therapy (for at least previous 7 years) were reviewed. Information on annual transfusion requirement (ATR) and average ferritin (done at least biannually) levels over last 7 years were recorded. Anthropometric SD scores (SDS) were calculated according to age and sex-specific norms using IAP 2015 growth reference charts [5]. Mid-parental height (MPH) was calculated and the child's height within $\pm 2SD$ of MPH was considered appropriate for the genetic potential. Children with height for age below 2 SDS were considered to have short stature. Pubertal development and progression over the previous years as assessed by sexual maturity rating (SMR) according to Tanner criteria was noted.

As a part of usual care protocol at our center, children with TDT after 10 years age undergo regular growth and pubertal assessment and an annual endocrine screen. The latter consists of thyroid function tests, fasting plasma glucose, oral glucose tolerance test (if indicated), serum calcium, phosphate, alkaline phosphatase, 25-hydroxy vitamin D and parathyroid hormone (if indicated by clinical or biochemical features). Bone age, luteinizing hormone (LH), follicle stimulating hormone (FSH), and sex steroids [estradiol (female) and testosterone (male)] are assessed in adolescents with delayed/arrested/failed puberty. Low FSH, LH and sex steroids for age, sex and pubertal stage indicate hypogonadotropic hypogonadism. In those with equivocal results, gonadotropin-releasing hormone (GnRH) analog stimulation test is performed to evaluate the pituitary's ability to synthesize and secrete gonadotropins. In cases with arrested/delayed puberty, incremental age appropriate hormone replacement therapy (HRT) is started as per standard protocol. Screening for Hepatitis B, C, HIV and liver function is done in all children annually. ECG, echocardiography and MRI T2* imaging for cardiac and hepatic dysfunction is also performed to look for transfusional iron overload. A baseline DEXA (dual-energy X-ray absorptiometry) scan followed by annual/biennial screening of bone mineral density (in case of fragility fractures) is done on case to case basis.

Based on the onset and progression of puberty, the children recruited in this study were categorized into 4 groups: Group 1: Normal onset and progression of puberty, Group 2: Delayed onset and spontaneous progression of puberty after priming with low dose sex steroids for 3-6 months, Group 3: Normal/delayed onset with pubertal arrest, and Group 4: No spontaneous onset of puberty – pubertal failure.

Delayed puberty was defined as absence of gonadarche (testicular volume <4 mL) in males by 14 years and thelarche (appearance of breast bud) in females by 13 years. The failure of pubertal progression from one Tanner stage to the next over a period of 1 year was considered as pubertal arrest. Failure to achieve menarche by 16 years

was defined as primary amenorrhea while the absence of menstrual cycles for >12 months after attaining menarche was considered secondary amenorrhea.

The study was approved by the institutional ethics committee and a written informed consent of the parent/guardian of the participants was taken. Consent/assent of the participants was also taken.

Statistical analysis: These were done using SPSS version 20.0. Categorical variables were analyzed using chi square test and continuous variables, using independent *t* test. Multivariate logistic regression was done to study factors determining pubertal failure and arrest.

RESULTS

The records of 58 adolescents with TDT were reviewed (**Table I**). Thirty four (58.6%) children in group 1 and 8 (13.8%) in group 2 did not require HRT i.e., non HRT group (*n*=42; 72.4%). Sixteen (27.6%) children [group 3; 7 (12.1%) and group 4; 9 (15.5%)] had pubertal arrest/failure and received HRT. All girls in HRT group had primary amenorrhea, except for one girl in group 3 with secondary amenorrhea. All children in the HRT group had short stature while in the non HRT group, 14/23 (60.9%) boys and 7/19 (43.8%) girls were short. When compared with the respective MPH, two-third of children fell short of their genetic potential in the HRT group. All children with delayed, failed and arrested puberty had delayed bone age i.e. < -2SD from chronological age.

The baseline and stimulated GnRH levels of LH, FSH, and estradiol (female) and testosterone (male) were assessed for subjects in group 2,3 and 4 (**Table II**). Both baseline and peak values of gonadotropins and sex steroids were significantly lower in group 3 and 4 as compared to group 2. No subject in this study had evidence of hypergonadotropic hypogonadism. There was no significant difference in endocrinopathies between HRT and non-HRT groups except for hypoparathyroidism, which was significantly higher in the former group (**Table III**). On multivariate analysis of factors associated with failed/arrested puberty, the mean serum ferritin

Table I Anthropometric and Pubertal Characteristics of Children With Transfusion-Dependent Thalassemia

Characteristics	Males (n=33)		Females (n=25)	
	No HRT (n=23)	HRT (n=10)	No HRT (n=19)	HRT (n=6)
Age pubertal onset, y ^a	13.5 (1)	16.5 (1)	12 (1)	15.5 (1)
Menarche, y ^b	-	-	14.5 (1)	17.5 (1)
Height z-score ^c	-2.09 (0.90)	-3.29 (0.79)	-1.57 (1.52)	-4.25 (2.21)
Weight z-score ^d	-1.81 (0.74)	-2.76 (0.87)	-1.87 (0.78)	-2.70 (0.90)
BMI z-score	-1.06 (0.88)	-1.53 (0.85)	-0.51 (0.94)	-0.80 (0.81)
Final height - MPH, cm ^e	-6.9 (5.9)	-14.7 (8.6)	-4.1 (9.5)	-13.7 (6.5)

^a*P*<0.001 for both sexes; ^b*P*=0.002; ^c*P*<0.01 for both boys and girls; ^d*P*=0.003 for boys and 0.036 for girls; ^e*P*=0.005 for boys and 0.04 for girls. MPH: mid parental height; BMI: body mass index; HRT: hormone replacement therapy.

(OR=1.005, 95% CI, 1.002, 1.009; $P<0.05$) remained the only significant predictor while mean ATR, gender and other co-morbidities like liver dysfunction, hypothyroidism, type I diabetes mellitus, hepatitis B/C failed to show any significant association.

DISCUSSION

This study describes the spectrum of pubertal disturbances in adolescents with TDT under regular follow-up. While nearly 60% subjects had spontaneous onset and progression of puberty, more than a quarter required HRT due to pubertal arrest/failure. All adolescents requiring HRT had short stature. Hypoparathyroidism and liver dysfunction were reported more in the HRT group. High iron overload was the only significant predictor of pubertal failure/arrest.

The main limitation of this study was that the dynamics of the genotype-phenotype interaction in the development of pubertal failure was not assessed. $\beta^0\beta^0$ compared to $\beta^0\beta^+$ and $\beta^+\beta^+$ phenotypes have been shown to have increased need of blood transfusions and thereby, higher iron overload and endocrinopathies [6]. Further, the evaluation of growth axis and its relative contribution to pubertal failure was not performed.

Growth failure is one of the most common complications observed in children with TDT. This study found short stature in all children on HRT. The pubertal failure/arrest significantly contributed to the reduced final heights in these children. This is in agreement with studies on growth failure in children with TDT reported from India [7,8] and globally [9].

Hypogonadism was found in 27.6% subjects which is lower than that reported in a previous study from our center [4] and literature published before 2005 [10]. The

Table III Clinical Characteristics and Co-morbidities in Children With Transfusion-Dependent Thalassemia

Variables	Non-HRT, n=42	HRT, n=16
Males, n (%)	23 (54.8)	10 (62.5)
Pretransfusion hemoglobin, mg/dL	9.4 (0.4)	9.3 (0.5)
Annual transfusion requirement, mL/kg ^b	135.6 (11.3)	146.4 (17.2)
Serum ferritin, ng/mL ^c	2752.4 (1082)	5084.9 (1640.5)
SGOT, IU/L ^c	40.6 (15.7)	73.4 (30.7)
SGPT, IU/L	49.9 (30.2)	107.2 (63.7)
Hypothyroidism, n (%) ^d	5 (11.7)	4 (25)
Hypoparathyroidism, n (%) ^b	1 (2.3)	4 (25)
Impaired glucose tolerance/ Type I DM, n (%)	16 (38.1)	8 (50)
HCV positive, n (%)	4 (9.5)	2 (12.5)
MRI T2* liver score, ms	8.5 (7.2)	9.3 (7.3)
MRI T2* cardiac score, ms ^a	19.4 (11.2)	13.0 (5.6)
DEXA spine; SD scores ^b	-1.54 (1.64)	-3.06 (1.50)
DEXA hip; SD scores ^c	-1.38 (1.11)	-2.70 (0.96)

^a $P<0.05$, ^b $P<0.01$, ^c $P<0.001$. 1 child in non-HRT group was hepatitis B positive, and 1 in HRT group was HIV-positive. SGOT: serum glutamic-oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; DM: diabetes mellitus; MRI: magnetic resonance imaging; DEXA: dual energy x-ray absorptiometry; HRT: hormone replacement therapy. ^dsub-clinical/avert.

likely cause for this observation is use of regular transfusions, better chelation and monitoring in the current era. The variability in worldwide prevalence of hypogonadism [1-3] may be explained by discrepancy in definitions of pubertal failure, differences in study cohort

Table II Gonadotropin and Sex Hormone Profile on Gonadotropin Releasing Hormone (GnRH) Analog Stimulation Test in Children With Transfusion-Dependent Thalassemia (N=24)

	Male, n=15		Female, n=9	
	Group 3 & 4, n=10	Group 2, n=5	Group 3 & 4, n=6	Group 2, n=3
<i>LH (mIU/mL)</i>				
Basal	0.36 (0.19)	0.80 (0.48)	0.45 (0.02)	0.90 (1.15)
Peak	0.92 (0.45)	2.50 (0.55)	1.10 (0.13)	2.22 (1.45)
<i>FSH (mIU/mL)</i>				
Basal	0.61(0.56)	1.3 (1.12)	0.85 (0.63)	2.23 (1.89)
Peak	0.94 (0.14)	3.8 (1.55)	1.5 (1.18)	4.35 (2.65)
<i>Estradiol (pg/mL)</i>				
Basal	-	-	2.75 (0.65)	9.80 (6.68)
Peak	-	-	4.23 (1.13)	26.33(8.55)
<i>Total testosterone (ng/dL)</i>				
Basal	2.24 (0.66)	23.80 (5.77)	-	-
Peak	3.51 (0.68)	59.44 (6.50)	-	-

$P<0.001$ for all comparisons between Group 2 and Group 3 and 4. GnRH analog stimulation test was not performed in group 1 children who underwent spontaneous onset and progression of puberty.

WHAT THIS STUDY ADDS?

- Despite regular transfusion and intensive chelation, a significant proportion of adolescents with transfusion-dependent thalassemia continue to have pubertal arrest and failure.
- High systemic iron load is an important predictor of pubertal disturbances in these children.

and their genetic variability. Also, the design of this study would miss subjects who may develop hypogonadism and secondary amenorrhea as an adult.

The mechanism of hypogonadotropic hypogonadism is postulated to be pituitary iron deposition resulting in volume loss and failure of HPG axis [11]. Priming with low dose sex steroids improves the responsiveness of the pituitary to gonadotropin releasing hormones. It is an effective method of inducing physiological puberty, feasible even in low resource countries [12]. Therefore, it was used in adolescents in group 2 to jump-start the puberty.

The prevalence of other endocrinopathies in this study was consistent with the published literature [13]. Previous studies indicate male sex, high serum and tissue iron overload, genotype-phenotype interaction, severe clinical endocrinopathy as predictors of HH in children with TDT [14,15]. Our study showed no correlation with gender or endocrine dysfunction, while high serum ferritin levels had a significant association with pubertal failure/arrest. Further studies are required to assess the genotype - phenotype correlation in TDT, leading to a higher transfusion requirement and resultant increased systemic iron load. This will help design strategies to intensify chelation in subset of vulnerable children for the prevention of endocrinopathies and other comorbidities.

Ethics Clearance: Institutional Ethical Committee for Human Research, Lady Hardinge Medical College and associated hospitals; No. LHMC/ECHR/2018/29, dated 10 May, 2018.

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