ORIGINAL ARTICLE

Postnatal Assessment of Minipuberty in Indian Preterm and Full-term Male Infants

Vijay Sheker Reddy Danda, Krishna Reddy Thaduri, Srinivas Rao Paidipally, Madhavi Verpula, Sandeep Reddy Devireddy

Department of Endocrinology, Gandhi Medical College and Hospital, Secunderabad, Telangana, India

ABSTRACT

Objectives: To study the differences in the timing and magnitude of postnatal urinary gonadotropins and testosterone secretion during minipuberty in Indian preterm (PT) and full-term (FT) male infants.

Methods: This prospective observational study included 30 PT and 60 FT male infants. Urinary luteinizing hormone (LH), follicular stimulating hormone (FSH), and testosterone, and stretched penile length (SPL) and testicular volume (TV) were measured on day 7, first month, second month, fourth month and at six months of age.

Results: The highest elevation of mean (SD) urinary LH was observed in PT infants in comparison to FT infants [12.6 (1.4) vs 4.9 (0.6) µIU/mg, respectively; $P < 0.001$] in the first month. FSH levels were lower in PT than FT infants on day 7 ($P < 0.001$). Testosterone was significantly elevated in PT than FT infants [70.8 (5.6) vs 44.6 (3.2) ng/mg; $P < 0.001$] with a greater mean percentage increase in SPL ($P < 0.001$) and TV ($P < 0.001$) by the first month.

Conclusions: Indian PT male infants showed a greater increase in urinary LH and testosterone, with a faster increase in SPL and TV.

Keywords: *Male infants, Minipuberty, Preterm, Stretched penile length, Urinary gonadotropin*

INTRODUCTION

The hypothalamic-pituitary-gonadal (HPG) axis is transiently activated in three phases of life. The first is during the fetal period, the second is during neonatal and early infancy and the third is during puberty at adolescence [1]. At birth, the inhibitory effect of the placental estrogens on the gonadotropin-releasing hormone (GnRH) neurons is lost leading to the activation of the HPG axis during the neonatal and early infancy period [2]. This is referred to as minipuberty and was first described by Forest et al [3]. Minipuberty occurs in both sexes with peak gonadotropins between the first to third month of infancy. There is a clear sexual dimorphism, with the luteinizing hormone (LH) being higher in boys and follicle stimulating hormone (FSH) being higher in girls. LH returns to prepubertal values (<0.3 IU/L) within 6 months in both sexes. However, FSH takes 3-4 years to return to baseline in females. The surge of gonadotropins leads to an increase in testosterone in males and estradiol in females translating to penile and testicular growth in boys and breast and uterine development in females, respectively [4]. The exposure to gonadal steroids in males leads to differences in body composition with a greater fat-free mass and a higher growth velocity in comparison with females [5]. However, the importance of minipuberty in females is yet to be ascertained. Also, the consequence of HPG axis activation on future reproductive potential is not known. Minipuberty presents with a unique window of opportunity to diagnose congenital hypogonadism and disorders of sex development [6].

Data on hormonal changes in minipuberty in preterm (PT) babies are scarce. Prior studies demonstrated an exaggerated minipuberty response in PT males leading to faster penile and testicular growth [7]. Hyperandrogenism occurring as an adaptive response may adversely affect the programming during early development posing a greater risk for future cardio-metabolic disorders [5,7,8]. Minipuberty may be affected by ethnicity; studies on minipuberty from India are scant. Therefore, the main objective was to study the differences in the timing and magnitude of postnatal urinary gonadotropin and testosterone secretion in Indian PT and full-term (FT) male infants.

Correspondence to: Dr. Vijay Sheker Reddy Danda, Department of Endocrinology, Gandhi Medical College and Hospital, Musheerabad, Secunderabad, Telangana 500003, India.
drdvsreddyendo@yahoo.com
Received: May 15, 2023; Initial review: June 21, 2023;
Accepted: Nov 18, 2023.

METHODS

This prospective, observational study was conducted at a tertiary level hospital from March, 2019 to February, 2020. The procedures followed were in accordance with the ethical standards and with the Helsinki Declaration of 1964, as revised in 2013. After obtaining informed consent from their mothers, 30 PT (delivered at < 37 weeks' gestation) and 60 FT male infants were included in the study. Neonates requiring level-3 care and those with gross congenital abnormalities, ambiguous genitalia, congenital heart disease, liver disorders and renal disorders were excluded. Participants were recruited from the postnatal ward by simple random sampling and followed up for a total of five visits. Visit 1 (V1), corresponded to day 7 (D7) of life. Follow-up visit 2 (V2), visit 3 (V3), visit 4 (V4), and visit 5 (V5) were done in months 1, 2, 4, and 6, respectively. Appropriate follow-up was ensured through telephonic reminders. Basic demographic data, anthropometric measurements, and examination findings of external genitalia were collected during each visit. Measurements were taken by a trained pediatrician. Testicular volume (TV) was measured by Prader's orchidometer and stretched penile length (SPL) was measured by a transparent rigid ruler. Penis was stretched and length was measured from the pubic symphysis to the tip of the glans penis along the dorsal side of the penis to the accuracy of 1mm. The pre-pubic fat pad was pushed to the bone for accurate readings; an average of three measurements was taken.

Urinary analysis was considered for convenience as multiple samples had to be collected over a period of time. Spot urine samples were collected with the help of a condom catheter into a plastic bag and were stored at -70º C till laboratory analysis. Urine LH was measured by ELISA based kits (DRG International) with an intra-assay coefficient of variation (CV)% of 0.7% and inter-assay CV% of 9.4%. Urine FSH was measured by ELISA based kits (Calbiotech) with an intra-assay CV% of 1.4% and inter-assay CV% of 8.23%. Urine testosterone was measured by ELISA technique (Calbiotech) with an intra-assay CV% of 2.91% and inter-assay CV% of 8.65%. All the urinary samples were processed by Varioskan lux multimode plate reader for measurement of LH, FSH, and testosterone levels.

Statistical analysis: Data were analyzed with Microsoft Excel and Graph Pad Prism (version 7.0.4). The categorical variables were presented as frequency and percentages. Continuous data were presented as mean and standard deviation. Statistical analyses of the differences in the means between the groups and within the group were done by unpaired and paired student's *t*-test respectively. Differences were considered significant if the *P* value was < 0.05.

RESULTS

A total of 60 FT and 30 PT male infants were recruited in the study. Out of a total of 450 visits, 23 visits were missed (17/300 in term and 6/150 in preterm) with a success rate of 94.8%. All the infants completed the study. **Table I** shows the birth characteristics of PT and FT infants at first visit. **Table II** shows the prospective and comparative levels of urinary FSH, LH, testosterone and genital findings.

The differences in mean urinary LH levels between V1-V2, V2-V3, V3-V4, and V4-V5 were +5.2, -4.2, -4.5, and -1.8 µIU/mg among PT infants in comparison to +0.7, -1.2, -2.3 and -0.8 µIU/mg among FT infants. The differences in mean urinary FSH between V1-V2, V2-V3, V3-V4, and V4-V5 were +3,-1.7,-2.1, and -0.7 µIU/mg, in PT infants in comparison to -1, -1.1, -0.3, and -0.6 µIU/mg among FT infants. Changes in the urinary testosterone values mirrored the changes in LH values with a peak at V2 which was similar in both FT and PT (**Table II**). The average change in testosterone levels between visits were +20.2, -15.2, -31, and -17.7 ng/mg in PT infants in comparison to +24.6, -20.4,-9.4, and -7.2 ng/mg among FT infants. The sequential changes in SPL in PT and FT infants are shown in **Table II**. The increment in the testicular volume was nonlinear, with a maximum increase in size seen between V1 and V2 in both groups. PT infants showed a significant mean percentage increase in SPL (20% vs 13.3%; $P < 0.001$) and TV (60% vs 30%; $P < 0.001$) at V2 in comparison with FT infants.

DISCUSSION

This study demonstrated an earlier and exaggerated rise of urinary LH and testosterone in Indian PT male infants as

Table I Birth Characteristics in Preterm and Term Male Infants

Variables	*Preterm (n = 30)*	*Term (n = 60)*
Maternal age (y)	25.6 (3.35)	25.8 (3.85)
Mode of delivery[a]		
Vaginal	20 (66.6)	45 (75)
Elective cesarean section	6 (20)	12 (20)
Emergency cesarean section	4 (13.4)	3 (5)
Gestational age (week)[b,c]	33 (32-33)	38 (38-39)
APGAR score (5 min)[b,c]	8 (7-8)	9 (8-9)
Birth weight (kg)[c]	1.5 (0.19)	3.1 (0.19)
Birth length (cm)[c]	43 (3.25)	49.9 (1.4)

Data expressed as mean (SD), [a]n (%), [b]median (IQR). [c]P <0.001

Table II Comparison of Urinary Gonadotropins, Testosterone, and Genital Characteristics Among Preterm and Term Male Infants

Visits	*Preterm (n = 30)*					*Full-Term (n = 60)*				
	LH (μIU/mg)	*FSH (μIU/mg)*	*Testosterone (ng/mg)*	*SPL (cm)*	*TV (mL)*	*LH (μIU/mg)*	*FSH (μIU/mg)*	*Testosterone (ng/mg)*	*SPL (cm)*	*TV (mL)*
Visit 1	7.5 (1.4)[a]	4 (0.8)	50.6 (6.6)[a]	2.5 (0.1)	1.0 (0.1)[a]	4.3 (0.7)	4.9 (0.5)	20 (5.4)	3.0 (0.2)	1.3 (0.4)
Visit 2	12.7 (1.4)[a]	7 (0.6)[a]	70.8 (5.6)[a]	3 (0.1)	1.6 (0.4)	5 (0.6)	3.9 (0.5)	44.6 (3.2)	3.4 (0.3)	1.7 (0.4)
Visit 3	8.5 (1.0)[a]	5.3 (0.6)[a]	55.6 (3.1)[a]	3.3 (0.1)	2 (0.4)	3.8 (0.5)	2.8 (0.4)	24.2 (3.9)	3.7 (0.3)	2.1 (0.4)
Visit 4	4 (0.4)	3.2 (0.4)	24.6 (2.5)	3.5 (0.2)	2.3 (0.4)[a]	1.5 (0.3)	2.5 (0.3)	14.8 (2.2)	3.9 (0.3)	2.4 (0.4)
Visit 5	2.2 (0.3)	2.5 (0.2)	6.9 (1.5)	3.5 (0.1)	2.4 (0.4)	0.7 (0.1)	1.9 (0.4)	7.6 (1.4)	3.9 (0.3)	2.5 (0.2)

Data presented as mean (SD) based on 95 % completed visits. [a]P < 0.001, P > 0.05: All comparisons between PT and FT. LH Luteinizing hormone, FSH Follicle stimulating hormone, SPL Stretched penile length, TV Testicular volume

compared to FT infants during minipuberty. There was a significant increase in SPL and TV among PT infants by the end of the first month.

Urinary gonadotropins were utilized for the assessment of minipuberty in the present study as it is non-invasive and convenient for repeated measurements. A recent Indian study demonstrated a good correlation between serum and urinary gonadotropins [9]. There was a significant elevation of LH in PT infants as early as day 7; suggesting the possibility of an earlier onset of minipuberty. The highest elevation of LH was observed by the end of first month in both groups; with the magnitude being greater in PT infants (12.7 μIU/mg vs 5 μIU/mg). An earlier Australian study established reference ranges for serum LH and serum FSH in premature newborns till 43 days after birth [10]. The mean LH levels were higher in PT infants in the present study in comparison to the earlier study [10]. Different ethnicity and assays may be the reason for this discrepancy. After the first month, there was a gradual decline in LH levels till 6 months in both the groups. However, at the end of 6 months, LH levels were in the pubertal range (i.e., > 0.3 IU/mL) in PT infants suggesting a prolonged minipuberty. Testosterone declined from first month onwards to pre-pubertal values by the end of 6 months in both groups. The results of this study conform to the earlier observations of higher testosterone levels in PT than FT infants [7]. Initial urinary FSH levels were higher in FT male infants. However, from first to third month, PT infants had significantly higher FSH levels. The mean FSH levels observed at first month were significantly higher in PT males in this study as compare to another study (5 μIU/mg vs 1.1 μIU/mg) [10]. There was a steady decline in FSH levels among FT infants reaching pre-pubertal values by six months (i.e. < 0.3 IU/mL). Postnatal HPG axis activation lays an important role in the completion of genital development, the lack of which may lead to poor penile growth and cryptorchidism.

The higher LH and testosterone levels observed in PT boys may be a mechanism to complete penile growth and testicular descent. However, the mechanisms of exaggerated minipuberty in preterms are not known. An earlier Indian study, reported gestational age-wise references for SPL and TV [11]. The mean SPL of PT infants (born at 32-33 weeks gestation) was similar to the present study at V1. The maximum mean percentage increment in SPL was observed in the first month that was greater in PT infants than to FT infants. This could be attributed to an exaggerated testosterone elevation in PT infants. The mean TV at V1 in PT infants was greater in this study as compared to the earlier Indian study [11]. The difference can be attributed to the usage of plasticine ellipsoid crafted using water displacement method with smaller volumes ranging from 0.2 mL to 0.9 mL [11]. The maximum mean percentage increase in TV occured in the first month was twice greater in PT infants in comparison with FT infants in this study as also observed in an early study [7]. As the incidence of prematurity is increasing, its association with lower reproductive rates in men and women is being investigated [12].

A study from Denmark, evaluated reproductive hormones during minipuberty in healthy infants and in those with disordered sex development [13]. In the current study, PT infants had significantly higher androgen levels in comparison to FT infants. The long-term consequences of this variation are not known. A Finnish study on minipuberty found that testosterone may have an effect on neurobehavioral development during early infancy [14]. According to the Developmental Origins of Health And Disease (DoHAD) theory, many chronic adult diseases have their origin during early development. The associa-tion of exposure to higher androgens during infancy risk of future metabolic disorders in adulthood needs to be evaluated.

There are a few limitations of our study. The sample

WHAT THIS STUDY ADDS?

- There is an earlier and stronger activation of the hypothalamic-pituitary-gonadal axis in preterm male infants in comparison to full-term infants.

size was small and hormonal analysis was done on urinary samples rather than serum. ELISA was used instead of newer assays for hormonal estimation. Testicular volume was measured by Prader's Orchidometer rather than ultrasonography.

To conclude, minipuberty occurred earlier with a greater magnitude, leading to a faster increase in SPL and TV in Indian PT male infants than FT infants. Further studies with a larger sample size are needed to establish the normative data in the Indian population and evaluate the association of of hyperandrogenism and future risk of metabolic disorders during adulthood in PT infants.

Acknowledgement: Dr. Donthamala Suchitra, Associate Professor, Department of Pediatrics and Neonatology, for her help with subject recruitment and follow-up.

Ethics clearance: Institutional ethics committee of Gandhi Medical College/Hospital, Hyderabad, Telangana (No: IEC/GMC/2019/2/16); dated Mar 16, 2019.

Contributors: VSRD: Study idea, protocol development, data collection, manuscript writing and will act as guarantor for the study; KRT: Developing protocol, data collection, analysis and manuscript writing; SRP: Developing protocol and manuscript writing; MV: Data analysis and manuscript writing; SRD: Developing protocol, data analysis and manuscript writing; VSRD, KRT, SRP: Critical appraisal and revision of manuscript. All authors approved final version of manuscript

Funding: None; *Competing interest*: None stated.

REFERENCES

1. Becker M, Hesse V. Minipuberty: Why does it happen? Horm Res Paediatr. 2020;93:76.
2. Kuiri-Hänninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. Horm Res Paediatr. 2014;82:73-80.
3. Forest MG, Cathiard AM, Bertrand JA. Evidence of testicular activity in early infancy. J Clin Endocrinol Metab. 1973;37:148-51.
4. Bizzarri C, Cappa M. Ontogeny of hypothalamus pituitary gonadal axis and minipuberty: An on going debate? Front Endocrinol (Lausanne). 2020;11:187.
5. Davis SM, Kaar JL, Ringham BM, Hockett CW, Glueck DH, Dabelea D. Sex differences in infant body composition emerge in the first 5 months of life. J Pediatr Endocrinol Metab. 2019; 32:1235-9.
6. Renault CH, Aksglaede L, Wøjdemann D, Hansen AB, Jensen RB, Juul A. Minipuberty of human infancy - A window of opportunity to evaluate hypogonadism and differences of sex development? Ann Pediatr Endocrinol Metab. 2020;25:84-91.
7. Kuiri-Hänninen T, Seuri R, Tyrväinen E, et al. Increased activity of the hypothalamic-pituitary-testicular axis in infancy results in increased androgen action in premature boys. J Clin Endocrinol Metab. 2011;96:98-105.
8. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359:61-73.
9. Tripathy M, Baliarsinha AK, Choudhury AK, Das UK. The role of urinary LH and FSH in the diagnosis of pubertal disorders. Indian J Endocrinol Metab. 2021;25:110-20.
10. Greaves RF, Hunt RW, Chiriano AS, Zacharin MR. Luteinizing hormone and follicle-stimulating hormone levels in extreme prematurity: development of reference intervals. Pediatrics. 2008;121:e574-e80.
11. Mondal R, Ray S, Chatterjee K, et al. Penile length and testicular volume in newborns. Indian J Pediatr. 2016; 83: 1398-404
12. Swamy GK, Ostbye T, Skjaerven R. Association of preterm birth with long-term survival, reproduction, and next-generation preterm birth. JAMA. 2008;299:1429-36.
13. Johannsen TH, Main KM, Ljubicic ML, et al. Sex differences in reproductive hormones during mini-puberty in infants with normal and disordered sex development. J Clin Endocrinol Metab. 2018;103:3028-37.
14. Lamminmäki A, Hines M, Kuiri-Hänninen T, Kilpeläinen L, Dunkel L, Sankilampi U. Testosterone measured in infancy predicts subsequent sex-typed behavior in boys and in girls. Horm Behav. 2012;61:611-6.