# Pubertal Onset in Apparently Healthy Indian Boys and Impact of Obesity

Vineet Surana, Aashima Dabas, Rajesh Khadgawat, Raman Kumar Marwaha[1], V. Sreenivas[2], M. Ashraf Ganie, Nandita Gupta, Neena Mehan[3]

Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, [1]International Life Sciences Institute, [2]Department of Biostatistics, All India Institute of Medical Sciences, [3]Sur Homeopathic Medical College, Hospital and Research Centre, New Delhi, India

## Abstract

**Objective:** Primary - to determine the age of pubertal onset in Indian boys. Secondary - (a) to assess the impact of obesity on pubertal timing, (b) to assess the relationship between gonadotropins and puberty. **Design:** Cross-sectional. **Setting:** General community-seven schools across New Delhi. **Participants:** Random sample of 1306 school boys, aged 6–17 years. **Materials and Methods:** Anthropometric measurement for weight and height and pubertal staging was performed for all subjects. Body mass index (BMI) was calculated to define overweight/obesity. Serum luteinizing hormone (LH), follicle stimulating hormone, and serum testosterone were measured in every sixth subject. **Main Outcome Measure:** Age at pubertal onset-testicular volume ≥4 mL (gonadarche) and pubic hair Stage II. **Results:** Median age of attaining gonadarche and pubarche was 10.41 years (95% confidence interval [CI]: 10.2–10.6 years) and 13.60 (95% CI: 13.3–14.0 years), respectively. No significant difference in the age of attainment of gonadarche was observed in boys with normal or raised BMI, though pubarche occurred 8 months earlier in the latter group. Serum gonadotropins and testosterone increased with increasing stages of puberty but were unaffected by BMI. Serum LH level of 1.02 mIU/mL and testosterone level of >0.14 ng/mL showed the best prediction for pubertal onset. **Conclusion:** The study establishes a secular trend of the age of onset of puberty in Indian boys. Pubarche occurred earlier in overweight/obese boys. The cutoff levels of serum LH and testosterone for prediction of pubertal onset have been established.

**Keywords:** Body mass index, gonadarche, luteinizing hormone, puberty, testosterone

## INTRODUCTION

Puberty is characterized by changes in body structure, sexual maturation, and rapid skeletal growth. The hormonal changes act differently in both genders, resulting in differential patterns of maturation. While sexual maturation considerably precedes linear growth velocity in boys, thelarche is soon accompanied by an increase in height in girls.

The pubertal onset in boys is heralded by an increase in genital size and testicular volume (TV), which is followed by increase in pubic hair (PH).[1] Unlike thelarche and menarche which can clearly define puberty in girls, onset of puberty in boys has not been defined uniformly in literature. The factors which influence timing and tempo of puberty in boys include genetic, nutrition, stress and environment. Although secular trends in age of pubertal onset have been reported globally,[2] the last Indian data available is of the early 1990s.[3] Nutrition and environmental factors play an important role in determining growth and pubertal development. Recent reports show an alarming increase in the prevalence of overweight and obesity worldwide[4] including India.[5,6] Most recent reports confirm an association of early puberty with obesity in girls,[7] though reports with regard to boys are conflicting.[1,8,9]

Thus, we undertook to evaluate the pubertal patterns of apparently healthy Indian boys and study its correlation with neuroendocrine regulatory hormones. We also attempted to study the effect of raised body mass index (BMI) in affecting puberty.

## MATERIALS AND METHODS

This cross-sectional study was carried out in seven schools of Delhi selected from all geographical regions (north, south, east,

**Address for correspondence:** Raman Kumar Marwaha, Flat No. 17, Gautam Apartments, Gautam Nagar, New Delhi - 110 049, India. E-mail: marwaha_ramank@hotmail.com

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west, and central regions). A discussion of the study plan was held with the school authorities for administrative approval. Those schools who wished to participate were provided with a written plan to be circulated to the parents. A written informed consent from parents/guardians was obtained after the personal interaction, while verbal assent was taken from the children who participated in the study. The study was approved by ethics committee, All India Institute of Medical Sciences.

Apparently, healthy boys in the age range of 6–17 years of age were recruited as study subjects (duration of study from January 2013 to December 2013). Children with a history of any systemic illness or taking any treatment for more than 1 month in last 3 months were excluded from the study.

### Methodology
Children underwent a tailored history and clinical examination including anthropometry followed by blood sample collection. All anthropometric measurements were made with subjects dressed in minimal light clothing but without footwear. Height was measured with portable Holtain stadiometer (Holtain Inc., Crymych, Pembs. UK). Weight was measured with the digital weighing machine. BMI was calculated as weight/height$^2$ and status of obesity and overweight was determined as per International Obesity Task Force criteria.[10]

### Pubertal staging
Pubertal stage assessment was carried out by trained professionals of same-sex using Tanner's method.[11] TV was assessed by comparative palpation with the Prader's orchidometer.[12] Testes <1 mL were recorded as 1 mL. Based on volume, testicular assessment was divided into four stages.[13] Stage I (prepubertal stage) included subjects with volume <4 mL, Stage II (early pubertal stage) – volume >4 mL but ≤8 mL, Stage III (mid-pubertal stage) – volume >10 mL but ≤15 mL, and Stage IV (fully matured stage) – volume >15 mL. We defined pubertal onset as TV of 4 mL or greater, consistent to an earlier publication which reported strong correlation between the onset of puberty assessed as per Tanner's method and TV.[12] If there was a discrepancy in two TVs, the larger one was taken into consideration. Precocious puberty was defined as Tanner Stage II before 9 years of age.[11]

### Blood analysis
Every sixth boy was chosen for sampling due to logistic issues. The fasting samples were collected by trained phlebotomist from the antecubital vein. They were centrifuged within 2 h of collection and serum was stored at − 20°C for hormonal assessment. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were measured. All samples were analyzed in one batch to avoid inter-assay variation. Samples were measured by electrochemiluminometric immunoassay in an automated analyzer (Cobas E411 Roche Diagnostics, Mannheim, Germany). For serum LH, the range of detection was 0.1 mIU/mL–200 mIU/mL with intra-assay coefficient of variation (CV) of 0.8%–1.8% and inter-assay CV of 2%–5.5% while for serum FSH, measuring range

was 0.1–200 mIU/mL with intra-assay CV of 1.5%–1.8% and inter-assay CV of 3.8%–5.3%. Serum testosterone had measuring a range of 0.025–15 ng/mL with intra-assay CV of 1.2%–4.7% and inter-assay CV of 2.8%–8.4%.

### Statistical analysis
Descriptive statistics such as mean, median, standard deviation (SD), and range for baseline as well as outcome variables were used. This enabled for identification of any outliers and the type of distribution (normal or otherwise). Median and 95% confidence interval [CI] for pubertal age was calculated by probit analysis by logistic regression model by SPSS software version 20 (IBM corporation, Armonk, NY). For correlation of hormonal data with TV, one-way ANOVA was used to calculate mean and SD. Bonferroni correction was applied to find out the level of significance between various pubertal stages and gonadotropins and sex steroids. Log transformation of data was done to make data normally distributed. Data were adjusted for age and BMI status to look for their effect on the gonadotropins and sex steroids level with relation to pubertal status. Receiver operative characteristic tables and graph were obtained to find out threshold value of the gonadotropins and sex steroids level in relations to the onset of puberty, i.e., progression from Stage I to Stage II. All tests were done keeping a probability of Type 1 error at 5%.

### Sample size calculation
Assuming an SD of age at onset for G2 as 2 years, with an absolute error of margin ±0.25 year (3 months), it was calculated that in a two-sided 95% CI, 246 boys would be needed. For other stages from G3 to G5, similar numbers were adopted. Hence considering all genital stages, a total of 1250 children would be needed.

## RESULTS
Out of the 1315 boys recruited for the study, nine boys were excluded (7 did not consent for pubertal examination, and 2 were older than 18 years of age), leaving 1306 boys for final analysis. Blood samples were collected from 212 (16%) boys. Details of study subjects in different age groups along with their height, weight, and BMI are shown in WebTable 1. The prevalence of overweight or obesity was 19.2%. Table 1 shows the distribution of study subjects according to testicular and PH staging in those with normal or raised BMI. The disparity in the prevalence of different stages of puberty was evident assuming the above-mentioned criteria.

The median age of gonadarche was 10.41 years (95% CI: 10.2–10.6 years), with a difference of 2.5 years between those with Stage II and III and 1.5 years between those with Stage III and IV [Table 2]. In contrast, the median age of pubarche was 13.60 (95% CI: 13.3–14.0 years), suggesting the earlier onset of gonadarche by 3 years. The ages of later pubertal stages as per testicular and PH staging were similar showing good correlation between both methods in later puberty. There was no statistically significant difference in onset of gonadarche between boys with normal BMI and overweight/obese, except

**WebTable 1: Number of subjects in different age groups with height, weight, and body mass index**

| Age at the time of examination | Age group | n | Mean (SD) | | |
|---|---|---|---|---|---|
| | | | Weight (kg) | Height (m) | BMI (kg/m²) |
| 6.5-7.49 | 7 | 13 | 23.63 (3.26) | 1.24 (0.06) | 15.08 (1.70) |
| 7.5-8.49 | 8 | 76 | 25.57 (6.21) | 1.25 (0.07) | 15.99 (2.88) |
| 8.5-9.49 | 9 | 120 | 29.04 (7.78) | 1.31 (0.07) | 16.69 (3.31) |
| 9.5-10.49 | 10 | 190 | 32.50 (8.12) | 1.37 (0.07) | 17.13 (3.35) |
| 10.5-11.49 | 11 | 215 | 35.27 (9.10) | 1.42 (0.08) | 17.44 (3.43) |
| 11.5-12.49 | 12 | 177 | 38.38 (9.09) | 1.46 (0.07) | 17.89 (3.29) |
| 12.5-13.49 | 13 | 151 | 43.14 (10.44) | 1.54 (0.09) | 18.17 (3.41) |
| 13.5-14.49 | 14 | 126 | 49.20 (12.28) | 1.59 (0.09) | 19.27 (3.97) |
| 14.5-15.49 | 15 | 124 | 52.44 (12.35) | 1.65 (0.07) | 19.33 (4.15) |
| 15.5-16.49 | 16 | 79 | 57.41 (13.94) | 1.67 (0.06) | 20.61 (4.60) |
| 16.5-17.49 | 17 | 35 | 62.03 (13.86) | 1.69 (0.08) | 21.66 (4.61) |
| Total | | 1306 | | | |

SD: Standard deviation, BMI: Body mass index

**Table 1: Distribution of pubertal status of boys with normal or raised body mass index using testicular volume and pubic hair staging**

| Pubertal stage | Normal BMI | Overweight/ obese | Total, n (%) |
|---|---|---|---|
| TV | | | |
| TV1 (<4 mL) | 316 | 76 | 392 (30) |
| TV2 (4-8 mL) | 332 | 80 | 412 (31.5) |
| TV3 (10-15 mL) | 191 | 51 | 242 (18.5) |
| TV4 (>15 mL) | 207 | 53 | 260 (19.9) |
| PH | | | |
| PH 1 | 636 | 141 | 777 (59.4) |
| PH 2 | 80 | 34 | 114 (8.7) |
| PH 3 | 73 | 17 | 90 (6.9) |
| PH 4 | 96 | 14 | 110 (8.4) |
| PH 5 | 168 | 47 | 215 (16.5) |
| Total | 1046 | 260 | 1306 |

TV: Testicular volume, BMI: Body mass index, PH: Pubic hair

**Table 2: Estimated median age of pubertal stages irrespective of body mass index status**

| Parameter | Age (years) | | |
|---|---|---|---|
| | 3rd centile (95% CI) | 50th centile (95% CI) | 97th centile (95% CI) |
| TV | | | |
| Stage II | 6.28 (5.5-6.9) | 10.41 (10.2-10.6) | 14.55 (13.9-15.3) |
| Stage III | 10.10 (9.7-10.5) | 13.06 (12.9-13.3) | 16.01 (15.5-16.7) |
| Stage IV | 11.29 (10.9-11.6) | 14.59 (14.4-14.8) | 17.90 (17.4-18.5) |
| PH | | | |
| Stage II | 9.89 (9.3-10.3) | 13.60 (13.3-14.0) | 17.31 (16.5-18.5) |
| Stage III | 11.16 (10.7-11.5) | 14.27 (13.9-14.7) | 17.38 (16.7-18.4) |
| Stage IV | 11.83 (11.3-12.2) | 14.65 (14.4-14.9) | 17.47 (16.9-18.2) |
| Stage V | 11.90 (11.5-12.2) | 14.96 (14.8-15.2) | 18.03 (17.6-18.6) |

PH: Pubic hair, TV: Testicular volume, CI: Confidence interval

that overweight/obese boys attained pubarche approximately 8 months earlier [Table 3].

## Hormonal analysis

The distribution of serum LH, FSH, and testosterone among different stages of testicular growth are shown in Table 4. Mean levels of serum LH increased with increasing pubertal stages with significant difference noted between pubertal Stage II and III. Serum FSH levels also increased with increasing pubertal stages though without any significant difference. Serum testosterone levels increased with increasing pubertal stages with significant differences between different gonadal stages with maximum being between Stage II and III. There was no significant difference in levels of serum LH, FSH and testosterone between subjects with normal or raised BMI across different pubertal stages (data not shown).

Evaluation of the relationship between TV and serum LH, FSH, and testosterone showed good correlation between gonadarche and serum LH and testosterone levels. Area under the curve for serum LH and testosterone was 0.5931 and 0.6558 respectively. The cutoff value of serum LH for predicting gonadarche was 1.02 mIU/mL with sensitivity and specificity of 58.76% and 56.25%, respectively. Similarly, the cutoff value for serum testosterone was 0.14 ng/mL with sensitivity of 59.79% and specificity of 59.18%.

## DISCUSSION

The present study shows the age of pubertal onset, impact of BMI on onset of puberty and serum levels of gonadotropins and testosterone in apparently healthy Indian school boys. To the best of our knowledge, this is one of the first few studies in literature evaluating the correlation between different stages of puberty and sex hormones in Indian children.

In the present study, the median age of gonadarche was 10.41 years, which is comparable to the median age of gonadarche of 10.55 years in Chinese boys (95% CI: 10.27–10.79).[14] Data from NHANES[15] showed that the median age for gonadarche among non-Hispanic black boys, non-Hispanic white boys and Mexican American boys as approximately 9.2,

**Table 3: Impact of body mass index on age of attainment of different pubertal stages**

| | Age (years) | | |
|---|---|---|---|
| | 3rd percentile (95% CI) | 50th percentile (95% CI) | 97th percentile (95% CI) |
| TV staging | | | |
| Stage II | | | |
| Normal | 6.26 (5.5-6.9) | 10.41 (10.2-10.6) | 14.56 (13.9-15.3) |
| Overweight/obese | 6.27 (5.4-6.6) | 10.42 (9.9-10.9) | 14.57 (13.9-15.5) |
| Stage III | | | |
| Normal | 10.15 (9.8-10.5) | 13.10 (12.9-13.3) | 16.04 (15.6-16.6) |
| Overweight/obese | 9.94 (9.4-10.4) | 12.88 (12.5-13.3) | 15.83 (15.3-16.5) |
| Stage IV | | | |
| Normal | 11.27 (10.8-11.6) | 14.58 (14.4-14.8) | 17.88 (17.4-18.5) |
| Overweight/obese | 11.36 (10.8-11.9) | 14.67 (14.3-15.1) | 17.97 (17.4-18.7) |
| PH staging | | | |
| Stage II* | | | |
| Normal | 10.07 (9.5-10.5) | 13.76 (13.4-14.2) | 17.45 (16.6-18.7) |
| Overweight/obese | 9.36 (8.6-9.9) | 13.06 (12.6-13.6) | 16.75 (15.9-17.9) |
| Stage III | | | |
| Normal | 11.11 (10.6-11.5) | 14.22 (13.9-14.6) | 17.33 (16.6-18.3) |
| Overweight/obese | 11.32 (10.6-11.9) | 14.43 (13.9-15.2) | 17.54 (16.7-18.7) |
| Stage IV | | | |
| Normal | 11.74 (11.3-12.1) | 14.56 (14.3-14.8) | 17.38 (16.8-18.1) |
| Overweight/obese | 12.32 (11.7-12.9) | 15.14 (14.6-15.8) | 17.96 (17.2-18.9) |
| Stage V | | | |
| Normal | 11.91 (11.5-12.3) | 14.98 (14.8-15.2) | 18.05 (17.6-18.7) |
| Overweight/obese | 11.84 (11.3-12.3) | 14.91 (14.5-15.3) | 17.98 (17.4-18.7) |

*Stage II - $P<0.05$ for age of pubarche between normal and overweight/obese boys. Rest intergroup comparisons-not significant. TV: Testicular volume, CI: Confidence interval, PH: Pubic hair

**Table 4: Hormonal analysis across different stages of puberty as defined by testicular volume**

| TV stage | n | Serum LH (mIU/mL) | Serum FSH (mIU/mL) | Serum testosterone (ng/mL) |
|---|---|---|---|---|
| 1 | 48 | 0.95 (0.81) | 2.35 (1.05) | 0.29 (0.49) |
| 2 | 97 | 1.32 (1.09) | 2.63 (1.64) | 0.79 (1.27)† |
| 3 | 57 | 2.66 (1.68)* | 3.78 (2.21) | 2.79 (1.92)† |
| 4 | 10 | 3.41 (1.22) | 3.60 (1.18) | 4.89 (1.54)† |

Values expressed as mean (SD). *$P<0.05$ between Stage II and III, †$P<0.05$ between consecutive stages. TV: Testicular volume, LH: Luteinizing hormone, FSH: Follicle stimulating hormone, SD: Standard deviation

10, and 10.3 years, respectively. Similar studies from Italy, Denmark, and Hungary, however, reported the median age for gonadarche to be 11.2, 11.8, and 12 years, respectively.[16-18] This delay in pubertal onset in Europeans when compared to American boys may be due to either racial differences or different timeline of the conduct of these studies.

The pubertal onset in our cohort showed a secular trend as compared to an earlier Indian study by Agarwal *et al.* which reported pubertal onset by Tanner's method at 11.2 years.[3] Similar secular trends were observed in the Copenhagen Puberty Study where puberty evaluated in a combined cross-sectional and longitudinal study in 2006–2008, occurred earlier at 11.66 years (11.49–11.82) in boys as compared to 11.92 years in 1991–1993 cohort.[19] In contrast, the age of gonadarche

increased in Chinese boys from 10.55 years in 2003–2005 to 11.02 years in 2012.[1,14]

A major issue in determining pubertal onset in boys remains lack of uniform single parameter, unlike thelarche which defines pubertal onset in girls. Most studies have defined pubertal onset based on gonadarche instead of pubarche, and have reported a time lag between the onset of both.[20-22] We also reported a difference of 3 years in the present study, similar to an earlier report on Indian boys.[3] This time difference reportedly varied from 9 to 15 months in America and European boys.[16,20-23] Few studies have found a comparable age for gonadarche and pubarche.[1,17] There is only one study on Hungarian boys which has reported attainment of pubarche 2 months before gonadarche.[18]

The association of obesity and pubertal timing in boys may be nonlinear with conflicting data at present.[1,8,9] In this study, we did not find any impact of overweight/obesity on the onset of puberty in boys which is consistent with the observation reported by Rosenfield and Bordini.[24] The Copenhagen Puberty Study also showed secular trends between BMI and age of pubertal onset with raised serum LH in the recent subjects than in subjects evaluated 15 years prior.[19] In contrast, data from few other studies,[1,25] report delayed pubertal onset in overweight/obese boys than those with normal BMI. These discordant findings are postulated to differences in methods used to assess puberty, racial differences and lack of longitudinal studies evaluating BMI and puberty.[25]

There is paucity of Indian data on the correlation between pubertal stages and serum gonadotrophins and testosterone levels. The increase in serum testosterone and gonadotropins with increasing pubertal stages as seen in the present study has also been reported by Chada *et al.*[26] The mean serum LH across different pubertal stages was higher in our cohort than in data by Chada *et al.*, unlike serum testosterone which were similar.[26]

The present study has several limitations, (a) limited number of subjects in the age group of 6–8 and 15–17 years; (b) blood samples for hormonal analysis were collected from a subset of total subjects; (c) all children were considered healthy on the basis of history alone; (d) BMI, which truly does not represent percentage body fat, was used for defining obesity; and (e) pubertal data collection was cross-sectional, not longitudinal.

However, this is the first study which shows secular trends in the attainment of puberty in apparently healthy Indian boys. It also reports positive correlation between pubertal stages and serum LH/testosterone levels and establishes cutoff level of serum LH and testosterone to predict gonadarche. Nevertheless, larger longitudinal studies will be needed in future to estimate pubertal progression in Indian children.

## CONCLUSION

The study establishes a secular trend of the age of onset of puberty in Indian boys. Pubarche occurred earlier in overweight/obese boys. The cutoff levels of serum LH and testosterone for prediction of pubertal onset have been established.

### What we already know

Pubertal onset in boys is variable in timing and pattern across different populations. Its relation with obesity is unclear.

### What this study adds

Gonadarche and pubarche occurred at 10.41 years and 13.6 years in Indian boys. Serum LH level of 1.02 mIU/mL and testosterone level of 0.14 ng/mL showed a significant correlation to gonadarche.

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### Conflicts of interest

There are no conflicts of interest.

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