

## **Age of Onset of Puberty in Apparently Healthy School Girls from Northern India**

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**Objective:** To determine the age of pubertal onset and menarche in school-going girls, and to assess the impact of obesity on pubertal timing.

**Design:** Cross-sectional

**Setting:** Seven schools across Delhi, India.

**Participants:** 2010 school girls, aged 6-17 years

**Methods:** Anthropometric measurement and pubertal staging was performed for all subjects. Menarche was recorded by 'status quo' method. Body mass index was used to define overweight/obesity. Serum gonadotropins and serum estradiol were measured in every sixth participant.

**Main outcome measure:** Age at thelarche and menarche—

analyzed for entire cohort and stratified based on body mass index.

**Results:** Median (95% CI) ages of thelarche, pubarche and menarche were 10.8 (10.7-10.9) y, 11.0. y (10.8-11.2) y and 12.4 y (12.2-12.5) y. Overweight/obese girls showed six months earlier onset of thelarche and menarche than those with normal BMI ( $P<0.05$ ). Serum gonadotropins did not vary significantly in overweight/obese subjects.

**Conclusion:** The study provides the normative data for pubertal growth in Indian girls. Pubertal onset occurs earlier in overweight and obese girls.

**Keywords:** Adolescent, Menarche, Obesity, Pubarche, Thelarche.

The onset of puberty results from activation of complex neuroendocrine regulatory mechanisms resulting in release of pulsatile GnRH secretion but the exact mechanism of this activation is still unclear [1]. The secular trends of decrease in average menarcheal age are documented in literature between the mid-19th and the mid-20th century [1]. However, recent studies have reported a relatively slow decline, no change or modest increase in the average menarcheal age [2,3].

A recently published review on impact of body fat and timing of puberty concluded earlier onset of puberty in girls with increased body mass index (BMI) [4]. However, a study on Indian Bengali girls showed no correlation between menarcheal age and BMI [5]. Thus, there is paucity of data on impact of increasing obesity on pubertal development and progression in Indian children.

We therefore conducted this study to assess age of menarche, and impact of obesity on pubertal development in apparently healthy Indian school children, aged 6-17 years.

### **METHODS**

This cross-sectional study was carried out in seven schools selected from seven out of 11 districts of Delhi representative of all geographical regions (North, South, East, West and Central region) after obtaining permission from school management. All these schools were private schools catering to middle or upper socio-economic status, and were selected as per convenience. All seven schools which were approached agreed to participate.

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They were given detailed written information sheet regarding the study, to be sent to parents of all eligible children. Interaction with parents was undertaken to clear their queries about the study. Written informed consent from parents / guardians and verbal assent was taken from the children who participated in the study. The study was approved by Institutional ethics committee, All India Institute of Medical Sciences (AIIMS), New Delhi.

Apparently healthy girls aged between 6 and 17 years

were recruited from January 2013 to December 2013. Children with history of any systemic illness or taking any treatment for more than one month in last three months were excluded from the study. Girls underwent tailored history and clinical examination, including anthropometry, followed by blood sample collection by trained phlebotomists. All measurements were made with participants dressed in minimal light clothing without footwear. Height was measured with portable Holtain's stadiometer (Holtain Inc., Crymych, Pembs. UK). Weight was measured with the digital weighing machine. BMI was calculated and status of obesity and overweight was determined as per International Obesity Task Force (IOTF) criteria [6]. Pubertal stage assessment was using Tanner's method carried out by trained professional women [7]. *Status quo* method was used to determine the median age of menarche. In *status quo* method, data for menarcheal age is obtained by asking subject of her 'current menstrual status' whether she has achieved menarche or not, and her date of birth [8].

Fasting blood samples were collected in a subset of participants (every sixth student was selected for sampling). All samples were collected and centrifuged within two hours of collection at the study site and serum was stored at -20°C for hormonal assessment later. Serum follicle stimulating hormone (FSH), leutinizing hormone (LH), and estradiol (E2) were measured. All samples were analyzed in one batch to avoid inter-assay variation. Samples were measured by electrochemiluminometric immunoassay (ECLIA) in an automated analyzer (Cobas E411 Roche Diagnostics, Mannheim, Germany). For serum LH, the range of detection was 0.1 IU/L – 200 IU/L with intra assay CV of 0.8-1.8% and inter assay coefficient of variation (CV) of 2-5.5% while for FSH, measuring range was 0.1 IU/L-200 IU/L with intra assay CV of 1.5-1.8% and inter assay CV of 3.8-5.3%. Serum E2 had measuring range of 5-4300 pg/mL with intra assay CV of 2.4-4.3% and inter assay CV of 4.3-9.9%.

Assuming a standard deviation of age at onset for B2 and G2 as 2 years, with absolute error of margin  $\pm$  0.25 year (3 months), it was calculated that in a two-sided 95% confidence interval, 246 girls would be needed. For other stages from B3 - B5 and G3 - G5 similar number were adopted. So considering all genital stages, a total of 2000 was calculated to be sufficient.

**Statistical Analysis:** Median and 95 % CI for pubertal age was calculated by probit analysis using SPSS Software version 20. For correlation of hormone data with breast stages, one-way ANOVA was used. Bonferroni correction was applied to find out 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 to look for their effect on the gonadotropins and sex steroids level with relation to pubertal status. ROC tables and graph were obtained to find out threshold value of the gonadotropins and sex steroids level in relation to onset of puberty *i.e.* progression from stage 1 to stage 2. All tests were done keeping a probability of type 1 error at 5%.

## RESULTS

A total of 2015 girls were recruited for the study. Exclusion of five girls for various reasons (non-availability of date of birth, and age >18 years) resulted in a final tally of 2010 girls. Details of all subjects in different age groups along with their height, weight and BMI are shown in **Table I**. Almost one-fourth (23.8%) girls were either overweight or obese.

Distribution of girls with different stages of breast and pubic hair development according to their BMI is shown in **Table II**. **Table III** shows the median age of onset of various breast and pubic hair stages. The median age of onset of thelarche (B2) was 10.8 years (95% CI-10.7-10.9 years), and for pubarche it was 11.0 years (95% CI 10.8-11.2 y).

A significant shift towards early onset of puberty by six months ( $P<0.05$ ) and early progression from stage 2 to 5 was observed in overweight/ obese girls (**Table IV**). Similarly, appearance as well as progression of pubic hair was approximately 6-9 months earlier in overweight/ obese girls ( $P<0.05$ ).

Out of 2010 girls, 927 had achieved menarche at the time of evaluation (overweight/ obese 266/927; 28.7%; normal BMI-661/927, 71.3%). The median age of menarche was 12.4 years (95% CI - 12.2-12.5 y). The 3<sup>rd</sup> and 97<sup>th</sup> centile for menarche was 10.3 (95% CI 10.1-10.6) years and 14.4 (95% CI 14.1-14.7) years, respectively. The duration of progression from thelarche to menarche was about 1.5 to 2 years.

Overweight/obese girls attained menarche approximately four months earlier than girls with normal BMI ( $P<0.05$ ). The time duration of approximately one and half years was seen between onset of thelarche and menarche, and was not different in overweight/ obese and normal BMI subjects (**Table IV**).

Blood samples were collected from 335 subjects with mean (SD) age 11.7 (0.9) years. Of these, 64 (19.1%) had BMI in overweight/ obese range. The mean (SD) levels of serum LH, FSH and estradiol were 4.21 (6.75) IU/L, 5.15 (2.48) IU/L and 64.5 (67.6) pg/mL, respectively. The distribution of LH, FSH and estradiol in different stages of

**TABLE I** AGE AND ANTHROPOMETRIC CHARACTERISTICS OF THE STUDY PARTICIPANTS ( $N=2010$ )

| Age at the time of examination (y) | Age group (y) | Number | Weight (kg)<br>Mean (SD) | Height (m)<br>Mean (SD) | BMI ( $kg/m^2$ )<br>Mean (SD) |
|------------------------------------|---------------|--------|--------------------------|-------------------------|-------------------------------|
| 6.5-7.49                           | 7             | 9      | 24.6 (4.49)              | 1.23 (0.08)             | 16.18 (1.89)                  |
| 7.5-8.49                           | 8             | 167    | 25.5 (5.12)              | 1.26 (0.06)             | 15.93 (2.31)                  |
| 8.5-9.49                           | 9             | 195    | 28.8 (6.95)              | 1.31 (0.07)             | 16.71 (3.02)                  |
| 9.5-10.49                          | 10            | 226    | 32.9 (7.55)              | 1.37 (0.07)             | 17.37 (3.18)                  |
| 10.5-11.49                         | 11            | 270    | 38.6 (9.52)              | 1.43 (0.07)             | 18.67 (3.63)                  |
| 11.5-12.49                         | 12            | 259    | 42.6 (10.45)             | 1.48 (0.08)             | 19.23 (3.80)                  |
| 12.5-13.49                         | 13            | 215    | 48.8 (11.27)             | 1.53 (0.07)             | 20.71 (4.10)                  |
| 13.5-14.49                         | 14            | 205    | 51.8 (10.88)             | 1.56 (0.06)             | 21.36 (4.28)                  |
| 14.5-15.49                         | 15            | 227    | 52.8 (11.46)             | 1.56 (0.06)             | 21.58 (4.27)                  |
| 15.5-16.49                         | 16            | 181    | 53.0 (10.78)             | 1.57 (0.06)             | 21.58 (4.28)                  |
| 16.5-17.49                         | 17            | 56     | 51.7 (9.37)              | 1.55 (0.05)             | 21.39 (3.83)                  |

**TABLE II** DISTRIBUTION OF BREAST AND PUBIC HAIR STAGES ACCORDING TO BMI OF GIRLS

| Stage              | Normal BMI, n | Overweight/Obese, n | Total |
|--------------------|---------------|---------------------|-------|
| <i>Breast</i>      |               |                     |       |
| B1                 | 552           | 108                 | 660   |
| B2                 | 290           | 81                  | 371   |
| B3                 | 186           | 55                  | 241   |
| B4                 | 409           | 235                 | 644   |
| B5                 | 80            | 10                  | 90    |
| Total              | 1517          | 489                 | 2006* |
| <i>Public hair</i> |               |                     |       |
| P1                 | 565           | 124                 | 689   |
| P2                 | 256           | 76                  | 332   |
| P3                 | 160           | 48                  | 208   |
| P4                 | 475           | 212                 | 687   |
| P5                 | 62            | 27                  | 89    |
| Total              | 1518          | 487                 | 2005* |

\*Details of breast staging in 4 girls and pubic hair staging in 5 girls were excluded as written staging on paper were non-readable and could not be confirmed.

breast development is shown in *Web-Table I*. Though serum LH progressively increased from B1 to B5, significant difference was observed between B1 and B2 only. Similarly, significant increase in serum FSH was seen between stage 1 and 2 only but the peak serum FSH levels were seen in stage 3, followed by a small fall in the consequent stages. Serum estradiol progressively increased from stage 1 to stage 5 with significant difference being noted between each stage till stage 4. Serum LH level of 0.88 IU/L showed sensitivity and specificity of 68.9% and 69.6% respectively for prediction

of thelarche (B2). Similarly, sensitivity and specificity values for E2  $>34.5$  pg/mL were 69.9% and 69%, respectively (*Web Table II*). No impact of overweight/obese was observed on serum levels of LH, FSH and estradiol levels.

## DISCUSSION

The present study provides the normative data regarding age of pubertal onset in healthy Indian schoolgirls. We documented an early onset of puberty in obese and overweight girls. We also showed that serum levels of LH, FSH and estradiol correlate well with stage of puberty.

The present study has several limitations. The main limitation is use of *status quo* method for assessment of age of menarche. Prospective method by follow-up every 3-6 months would be more accurate [9]. Other limitations are: (a) limited number of subjects in the age group of 6-8 and 15-17 years; (b) blood samples for hormonal analysis were not collected from all girls; (c) all children were considered healthy on the basis of history alone; and (d) BMI, which may not truly represent percentage body fat, was used for defining obesity.

The present study reports the median age of thelarche to be 10.8 years which is comparable to the earlier Indian report of 1992 [4]. Among published studies in last 10 years, pubertal onset occurred earliest among Chinese girls (9.2 years) [10], followed by American girls [11,12], and then the Danish girls [13], which highlights that racial differences affect pubertal timing. The median age of onset of pubarche in our study was not different from other races, except black American population, where it is documented to occur earlier [11,12,14].

**TABLE III** ESTIMATED MEDIAN AGE (y) OF ONSET OF BREAST AND PUBIC HAIR STAGES IRRESPECTIVE OF BMI STATUS

| Parameter          | 3 <sup>rd</sup> centile (95% CI) | 50 <sup>th</sup> centile (95% CI) | 97 <sup>th</sup> centile (95% CI) |
|--------------------|----------------------------------|-----------------------------------|-----------------------------------|
| Breast Stage 2     | 8.4 (8.1-8.7)                    | 10.8 (10.7-10.9)                  | 13.3 (12.9-13.7)                  |
| Breast Stage 3     | 10.3 (10.1-10.6)                 | 12.6 (12.5-12.8)                  | 14.9 (14.5-15.3)                  |
| Breast Stage 4-5   | 10.0 (9.5-10.5)                  | 13.25 (13.0-13.5)                 | 16.5 (15.9-17.1)                  |
| Pubic hair Stage 2 | 8.6 (8.1-8.9)                    | 10.9 (10.8-11.2)                  | 13.4 (12.9-14.0)                  |
| Pubic hair Stage 3 | 10.3 (9.8-10.7)                  | 12.5 (12.3-12.8)                  | 14.7 (14.1-15.5)                  |
| Pubic hair Stage 4 | 10.4 (9.9-10.9)                  | 13.1 (12.8-13.3)                  | 15.7 (15.2-16.4)                  |

**TABLE IV** AGE (y) OF DEVELOPMENT OF BREAST AND MENARCHE IN RELATION TO BODY MASS INDEX STATUS

| Parameter               | 3 <sup>rd</sup> percentile (95% CI) | 50 <sup>th</sup> percentile (95% CI) | 97 <sup>th</sup> percentile (95% CI) | P value |
|-------------------------|-------------------------------------|--------------------------------------|--------------------------------------|---------|
| <i>Breast Stage 2</i>   |                                     |                                      |                                      |         |
| Normal BMI              | 8.58 (8.2-8.8)                      | 10.94 (10.8-11.1)                    | 13.30 (13.0-13.7)                    | P<0.05* |
| Overweight/obese        | 8.03 (7.6-8.4)                      | 10.40 (10.2-10.7)                    | 12.77 (12.4-13.2)                    |         |
| <i>Breast Stage 3</i>   |                                     |                                      |                                      |         |
| Normal BMI              | 10.66 (10.3-10.9)                   | 12.74 (12.6-12.9)                    | 14.82 (14.4-15.4)                    | P<0.05* |
| Overweight/obese        | 9.86 (9.4-10.2)                     | 11.94 (11.7-12.0)                    | 14.02 (13.6-14.6)                    |         |
| <i>Breast Stage 4-5</i> |                                     |                                      |                                      |         |
| Normal BMI              | 10.50 (9.9-10.9)                    | 13.57 (13.3-13.8)                    | 16.65 (16.2-17.3)                    | P<0.05* |
| Overweight/obese        | 9.30 (8.6-9.9)                      | 12.37 (11.9-12.8)                    | 15.45 (14.9-16.1)                    |         |
| <i>Menarche</i>         |                                     |                                      |                                      |         |
| Normal BMI              | 10.48 (10.1-10.8)                   | 12.46 (12.3-12.6)                    | 14.45 (14.1-14.9)                    | P<0.05* |
| Overweight/obese        | 10.08* (9.6-10.4)                   | 12.06* (11.9-12.3)                   | 14.04* (13.7-14.6)                   |         |

\* P value between normal BMI girls and girls with BMI in overweight/obese range for each centile.

The mean/median age of menarche in studies from different parts of the world, including India, ranged from 12-13.4 years [10-15], similar to our findings. Menarche is also reported to occur earlier in black population than whites [11,14]. The duration of progression from thelarche to menarche in our study was shorter in contrast to 2.4-3 years as observed in some other studies [10,13,14]. A secular trend of decline in average menarcheal age which was reported earlier [1] has gradually either slowed down or stopped in last few decades. A recent report has even showed a modest increase in the average age of menarche [16].

The early onset of puberty among obese girls as documented in our study, has been previously reported [6, 16-19]. In our cohort, the gonadotropins and estradiol progressively increased with pubertal stages with FSH peak at stage 3 of puberty as documented earlier [20]. The best sensitivity and specificity cutoffs of LH and E2 are still prone to misclassify true thelarche if interpreted in absence of clinical examination. It would therefore be

appropriate to combine clinical examination along with LH levels for prediction of stage B2, especially in overweight/obese girls where clinical examination alone at times, may mislead thelarche. Though, the clinical observation in the present study suggest early onset of thelarche and menarche in overweight/obese girls, serum gonadotropin levels were lower than girls with normal BMI, as reported earlier [21]. This paradoxical observation has been suggested to result from excess adiposity, which in the absence of substantial sex steroid excess, may subtly suppress hypothalamic-pituitary-gonadal function in early pubertal girls [21].

The present study provides normative data for age of onset of puberty for Indian girls, and documents an earlier onset of puberty in overweight/obese girls. These data should be validated by longitudinal studies in different settings within the country.

*Contributors:* RK, RKM: conceived and designed the study, and supervised manuscript writing; VS, AD: designed the research plan, analyzed the data, and drafted the manuscript; NM: data

**WHAT IS ALREADY KNOWN?**

- Pubertal onset has shown secular trends worldwide with earlier onset in obese girls.

**WHAT THIS STUDY ADDS?**

- Pubertal onset in Indian girls occur at 10.3 years with average interval of 1.5 to 2 years till menarche. Puberty occurs earlier in overweight/ obese Indian girls.

collection; VS: analyzed the data and performed the statistical analysis; MGA: manuscript preparation; NG: supervised laboratory work. All authors read and approved the final manuscript.

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