ORIGINAL ARTICLE

# Basal and FSH-stimulated Inhibin B in Precocious Puberty

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**ABSTRACT**

**Objective:** To evaluate the role of basal and follicle-stimulating hormone (FSH)-stimulated inhibin B to differentiate premature thelarche from gonadotropin-dependent precocious puberty (GDPP).

**Method:** This was a prospective interventional study**.** Basal and FSH-stimulated inhibin B levels were estimated in girls presenting with thelarche < 8 years age ($n$ = 10), healthy girls with normal pubertal development (pubertal control) ($n$ = 8) and healthy prepubertal girls (prepubertal control) ($n$ = 7). Girls with early puberty were classified as premature thelarche or GDPP based on GnRH agonist stimulation test.

**Results:** Median (IQR) basal inhibin B in premature thelarche was 5.42 (2.91, 30.58) pg/mL and FSH-stimulated inhibin B was 236.72 (111.53, 4431.73) pg/mL ($P$ = 0.043). Median (IQR) basal inhibin B in GDPP was 64.11 (24.96, 792.45) pg/mL and FSH-stimulated inhibin B was 833.66 (500.11-1266.18) pg/mL ($P$ = 0.043). Basal inhibin B was discriminatory between GDPP and premature thelarche ($P$ = 0.032). Median (IQR) basal inhibin B in prepubertal and prepubertal controls was 20.36 (9.61, 29.12) and 75.48 (58.55, 165.55) pg/mL, respectively.

**Conclusion:** Basal inhibin B is useful in differentiation of premature thelarche from GDPP while the role of FSH-stimulated inhibin B needs to be further explored in large sample size.

***Keywords*:** Inhibin B, FSH stimulated inhibin B, Precocious puberty, Premature thelarche

## INTRODUCTION

Inhibin B is secreted by the Sertoli cells in males and the antral follicles in females. In females, inhibin B is involved with the regulation of pituitary follicle-stimulating hormone (FSH) secretion by negative feedback, and it serves as a potential marker for ovarian function and follicular content [1]. Basal inhibin B increases during minipuberty (1-6 months of life) under the influence of FSH. After the completion of minipuberty there is a decrease in the levels of basal inhibin B and these low levels are then maintained throughout the prepubertal period [2]. The pubertal onset is characterized by a rise in the basal inhibin B levels in both genders. Further, inhibin B is stimulable by exogenous FSH at the onset of puberty [3].

Precocious puberty (PP) in girls is characterized by the development of secondary sexual characteristics at less than 8 years of age [4]. PP is more common in girls as compared to the boys [5]. Besides, gonadotropin-dependent precocious puberty (GDPP) and gonadotropin independent precocious puberty (GIPP), isolated variants like premature thelarche and premature adrenarche also exist. Premature adrenarche is due to the early production of adrenal steroids, while the exact pathophysiology of premature thelarche is still unknown [6]. GIPP and GDPP can be easily differentiated based on basal gonadotropin level but differentiation between GDPP and premature thelarche in girls is clinically challenging. Various investigations used to differentiate between both include basal gonadotropins, luteinizing hormone (LH)/FSH ratio, GnRH agonist stimulated LH, uterine length, and ovarian volume. Furthermore, basal inhibin B has also been evaluated to differentiate between these two entities [7-9]. The present analysis was done to explore the role of basal and FSH-stimulated inhibin B in the differentiation of premature thelarche from GDPP.

## METHODS

The present study was a prospective interventional study performed in a tertiary care centre (October 2019 – September 2021). The study protocol was reviewed and approved by Institutional Ethics Committee (INT/IEC/2019/001488).

Girls presenting with development of thelarche < 8 years of age were included. Healthy girls < 8 years without any feature of puberty (prepubertal control), and healthy girls with normal puberty ≥ 8 years (pubertal control) were included for comparison. Participants were enrolled into study after a written informed consent/assent. Relevant history pertaining to perinatal history, history of headache, seizures, past surgery/irradiation and family history of precocious puberty was documented. The demographic and anthropological parameters were documented. Tanner stage was documented at the time of presentation [10,11]. Bone age estimation at baseline was done with *X*-ray of the left hand with wrist and interpreted as per Modified Greulich-Pyle hand and wrist radiographic atlas by an experienced endocrinologist (SC) [12]. Thyroid function test, prolactin, cortisol, luteinizing hormone (LH), FSH, testosterone, estradiol (E2), complete hemogram, liver function test, and renal function test were performed in all the patients. Two samples were taken for basal inhibin B, 20 minutes apart and pooled. The MRI of the pituitary-hypothalamic region was done, if clinically indicated. A dedicated radiologist assessed the ovarian volume. The participants were subjected to the GnRH agonist stimulation test and FSH stimulation test. The subjects were classified into GDPP if they showed signs of progressive puberty, had bone age advancement of 2.5 SDS and post-GnRH agonist LH ≥ 8 IU/L [13]. Diagnosis of premature thelarche was made if there were no features of progressive pubertal development during follow up, no bone age advancement, and post GnRH agonist LH < 8 IU/L [13].

FSH stimulation test: Sample was taken for basal inhibin B at Day 1 between 08:00 and 09:00 am. Injection FSH (Urofollitropin) 150 IU was administered for 3 consecutive days and a sample for FSH-stimulated inhibin B was taken at 24 hours after the last injection (Day 4) between 08:00 and 09:00 AM [3]. GnRH analogue (GnRHa) stimulation test was done 5 days after the FSH-stimulation test [3].

*GnRHa (Triptorelin) stimulation test*: The baseline sample were taken for LH, FSH and E2 between 08:00 and 09:00 AM (2 samples taken 20 minutes apart and pooled). Injection Triptorelin 100µg/body surface area (maximum 0.1 mg) was administered subcutaneously between 08:00 and 09:00 AM. Samples were drawn for LH and FSH at 4 hours and, at 24 hours for E2. Samples were stored at -80°C till analyzed.

The LH, FSH, and E2 was measured by electrochemiluminescence immunoassay (ECLIA) (E-801, Roche Diagnostics, Switzerland). The coefficients of variation (CV) for LH were 2.2% (inter-assay) and 1.6% (intra-assay). The CV for FSH were 4.5% (inter-assay) and 2.8% (intra-assay). The lower limit of detection for LH and FSH was 0.1 IU/L. The CV for estradiol were 4.2% (inter-assay) and 2.6 % (intra-assay) with lower limit of detection 5 pg/mL. The inhibin B levels were measured by quantitative three-step sandwich immunoassay (AL-107-i; Ansh Labs, Webster). The CV for inhibin B was 3.05 to 6.32% (inter-assay) and 4.99 % (intra-assay) with lower detection limit of was 1.6 pg/mL.

*Statistical analysis:* SPSS version 25 was used for analysis of data. Kolmogorov-Smirnov test was used for analysis of normality. Normally distributed data were represented as mean (SD) and non-normal distribution as median (interquartile range, IQR). Cut offs were derived by receiver operating characteristic curves analysis. *P* < 0.05 was considered as significant. The sensitivity and specificity were calculated for differentiation of

premature thelarche from GDPP. The gold standard for ROC was clinical diagnosis of GDPP based on progressive pubertal development during follow up, bone age advancement, and post GnRH agonist LH

**RESULTS**

A total of 25 participants were enrolled into the study; five with premature thelarche, five with GDPP, 7 prepubertal controls, and 8 pubertal controls. The mean (SD) age of healthy prepubertal controls ($n$ = 7) was 6.35 (0.93) years. The median (IQR) basal inhibin B of prepubertal controls was 20.36 (9.61, 29.12) pg/mL. The mean (SD) age of pubertal controls ($n$ = 8) was 12.39 (2.97) years. The median (IQR) basal and FSH-stimulated inhibin B of pubertal controls were 75.48 (58.55, 165.55) and 673.69 (288.84, 2356.50) pg/mL, respectively.

The comparison of children with GDPP and premature thelarche is shown in **Table I**. The basal inhibin B in GDPP was significantly higher than that seen in premature thelarche ($P$ = 0.032) as well as prepubertal controls ($P$ = 0.042). Also, there was a statistically significant difference for basal inhibin B between girls with premature thelarche compared to pubertal controls ($P$=0.005), unlike prepubertal controls ($P$ = 0.541). Basal inhibin B at a cut-off value of 27.87 pg/mL had 80% sensitivity and 81.8% specificity for the diagnosis of GDPP.

There was no statistically significant difference for FSH-stimulated inhibin B in GDPP vs premature thelarche ($P$ = 0.310), GDPP vs pubertal controls ($P$ = 0.77) and, premature thelarche vs pubertal controls ($P$ = 0.306).

**DISCUSSION**

The role of basal and FSH-stimulated inhibin B in the differential diagnosis of precocious puberty in female was evaluated in the present study. Basal inhibin B was able to differentiate between GDPP and premature thelarche. There was significant rise in inhibin B in both groups after FSH injection. A significant rise in the levels of inhibin B after FSH injection in premature thelarche possibly suggests it to be a gonadotropin-dependent phenomenon.

Inhibin B is secreted from small antral follicles in female. The pubertal onset is marked by an increase in the number of small antral and pre-antral follicles, with a rise in basal inhibin B. The stimulation of inhibin B by exogenous FSH depicts maturation of ovarian follicles due to the trophic effect of FSH [3]. A few earlier studies have evaluated the role of basal inhibin B to differentiate between GDPP and premature thelarche. A basal inhibin B cut-off of 30.12 pg/mL was able to differentiate between GDPP and premature thelarche with 80% sensitivity and 89.3% specificity [7]. Basal inhibin B of 20 pg/mL with LH > 0.2 IU/L demonstrated 98% sensitivity and specificity to differentiate progressive from non-progressive form of precocious puberty [8]. In the current study, a cut-off value of 27.87 pg/mL for basal inhibin B had high sensitivity and specificity to differentiate the two conditions. However, fewer number of subjects in each group, limited any extrapolation of these results.

The role of FSH-stimulated inhibin B in the evaluation of delayed puberty has recently been evaluated [3], however its role in the evaluation of precocious puberty is still unexplored. Significant increment in inhibin B was observed in both premature thelarche and GDPP in the current study. The increment was higher in

GDPP, though not statistically significant. The small sample size is likely to affect the interpretation and generalization of these results.

Both premature thelarche and GDPP are characterized by development of thelarche, however, there is no further progression of pubertal events in former group. The reason for this is still unknown. A few postulated mechanisms for the development of premature thelarche are an increase in breast tissue sensitivity to estrogen, transient secretion of estradiol from an ovarian cyst, intake of estrogen in diet, and transient activation of the hypothalamus-pituitary gonad (HPG) axis [14]. In the light of current study, premature thelarche is likely a gonadotropin-dependent phenomenon due to the transient activation of the HPG axis with primarily a slow GnRH pulse activity leading to predominant rise in FSH. This is further supported by presence of higher FSH/LH ratio in subjects with premature thelarche as compared to GDPP. Overall, the above findings suggest that HPG axis is not quiescent in premature thelarche and can be considered as a variant of GDPP rather than variation in normal pubertal development. To conclude basal inhibin B is useful for differentiating premature thelarche from GDPP while role of FSH-stimulated inhibin B still needs to be further explored.

*Ethics clearance:* Institutional Ethics Committee, PGIMER Chandigarh No. INT/IEC/2019/001488, dated:05/08/2019.

*Trial Registry*: CTRI/2019/10/021570

*Contributors:* RW, SC, AB, DD: Study concept and design, patient management, data collection and analysis, manuscript editing. NS, TS, SKB: Data collection, laboratory analysis, patient management, data analysis, manuscript editing. All authors approved the final manuscript and agree to be accountable for all aspects of work.

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*Competing interest:* None stated.

**WHAT THIS STUDY ADDS?**

Basal inhibin B is useful to differentiate premature thelarche and gonadotropin-dependent precocious puberty while FSH-stimulated inhibin B needs further exploration.

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**Table I Baseline Characteristics of Girls With Gonadotropin Dependent Precocious Puberty (GDPP) and Premature Thelarche**

| | *GDPP (n=5)* | *Premature thelarche (n=5)* | *P value* |
|---|---|---|---|
| Age (y) [a] | 5.46 (2.91) | 5.34 (2.2) | 0.943 |
| Height (cm) [a] | 118.06 (23.41) | 110.74 (19.27) | 0.540 |
| Height SDS [a] | 2.13 (1.18) | 0.018 (1.10) | 0.019 |
| Bone age (y) [a] | 8 (4.06) | 5.8 (2.58) | 0.342 |
| Bone age advancement (y) [b] | 2.9 (1.18, 4.15) | 1.7 (0.5, 2) | 0.129 |
| Basal LH (IU/L) [b] | 1.07 (0.94,2.50) | 0.10 (0.10,0.20) | 0.007 |
| Post GnRH LH (IU/L) [b] | 28.80 (12.89,33.93) | 3.3524 (2.02,4.41) | 0.009 |
| Basal FSH (IU/L) [b] | 4.02 (2.74,4.34) | 3.15 (1.95,3.79) | 0.175 |
| Post GnRH FSH (IU/L) [b] | 10.30 (6.86,18.12) | 20.9 (11.07,25.14) | 0.347 |
| LH/FSH [b] | 0.19 (0.08, 0.36) | 0.04 (0.03, 0.06) | 0.047 |
| FSH/LH [b] | 5.36 (3.11, 15.65) | 23.10 (17.5, 31) | 0.047 |
| Basal estradiol (pg/mL) [b] | 15.41 (15.41,38.89) | 5.0 (5.0,8.93) | 0.034 |
| Post-GnRH estradiol (pg/mL) [b] | 154.30 (57.80,299.50) | 23.4 (13,55.93) | 0.028 |
| Basal Inhibin B (pg/mL) [b] | 64.11 (24.96, 792.5) | 5.42 (2.91, 30.6) | 0.032 |
| FSH-stimulated Inhibin B (pg/mL) [b] | 833.66 (500.1, 1266.2) | 236.72 (111.5, 4431.7) | 0.310 |

*Data shown as [a]mean (SD) or [b]median (IQR)*
*FSH Follicle stimulating hormone, GnRH Gonadotropin release hormone, LH Luteinising hormone*