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Mini-Review

Basal and Stimulated Inhibin B in Pubertal Disorders

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Abstract

Pubertal disorders in the form of delayed puberty (DP) or precocious puberty (PP) can cause considerable anxiety to both children and parents. Since the clinical and biochemical signatures of self-limiting and permanent conditions overlap considerably, it can be hard to determine whether to offer reassurance or intervention. Researchers have thus long been searching for a robust test to indicate whether the process of endogenous puberty is underway and is likely to proceed to completion. Although existing tests are available, such as basal gonadotropins, gonadotropin-releasing hormone (GnRH)-stimulated luteinizing hormone, and basal and human chorionic gonadotropin-stimulated testosterone, their diagnostic specificity is inadequate.

Inhibin B, a glycoprotein hormone, is secreted by Sertoli cells in males and small antral follicles in females. Entry into puberty is characterized by a rise in inhibin B levels in both genders. For the past 2 decades, researchers have been studying the role of inhibin B in the differential diagnosis of DP and PP. Initial studies showed promising results for using inhibin B to distinguish between constitutional (or self-limited) DP and congenital hypogonadotropic hypogonadism. However, diverse population studies have revealed varying cutoffs, limiting the use of basal inhibin B (basal-iB) in routine clinical practice. Recently, the concept of stimulated inhibin B has been introduced, using either follicle-stimulating hormone (FSH) or GnRH-analogs. Both FSH- and GnRH-analog-stimulated inhibin B concentrations were found to be more reliable than basal levels for investigation of pubertal disorders. This review examines the current status of basal-iB in the differential diagnosis of DP and PP, addressing its main advantages and limitations, and shedding light on the role of stimulated inhibin B concentrations.

Key Words: inhibin B, FSH stimulated inhibin B, GnRH agonist stimulated inhibin B, delayed puberty, constitutional delay in growth and puberty, hypogonadotropic hypogonadism, precocious puberty

Abbreviations: basal-iB, basal inhibin B; CHH, congenital hypogonadotropic hypogonadism; DP, delayed puberty; DSL, Diagnostic Systems Laboratories; ELISA, enzyme-linked immunosorbent assay; FSH, follicle-stimulating hormone; GDPP, gonadotropin-dependent precocious puberty; GnRH, gonadotropin hormone releasing hormone; hCG, human chorionic gonadotropin; HH, hypogonadotropic hypogonadism; LH, luteinizing hormone; OBI, Oxford Brooks Innovation; PP, precocious puberty; SLDP, constitutional (self-limited) delayed puberty; TV, testicular volume.

Inhibin B was first recognized in 1932 as a nonsteroidal testicular substance produced by seminiferous tubules that inhibits follicle-stimulating hormone (FSH) secretion (1), although it was only isolated and characterized almost 5 decades later in 1985 (2). Inhibins are glycoprotein hormone members of the transforming growth factor β superfamily; structurally, they are composed of covalently linked alpha and beta subunits (3). When subunit alpha is paired with the βA subunit, it is termed inhibin A and, when paired with the βB subunit, inhibin B (4). In males, the major circulating form is inhibin B, while in females, both inhibin A and B are present (5-7). The rapid decline in inhibin B levels after castration indicates that the testis is a major source of inhibin B in males (8), with the Sertoli cell being the primary site of production (9). Although immature prepubertal Sertoli cells preferentially secrete anti-Mullerian hormone (AMH), in vitro studies have demonstrated they can also secrete inhibin B (10). In females, the production of inhibin B is attributed to granulosa cells of small antral follicles (11), with a significant rise in levels occurring only after the recruitment of pre-antral follicles to small antral follicles (12).

Levels of inhibin B increase shortly after birth due to minipuberty in both males and females, peaking at around 3 months (13), although levels in males are much higher than in females (14, 15). In primates, the achievement of an adult set of Sertoli cells requires 2 phases of proliferation: neonatal and pubertal. Studies in nonhuman primates show that the postnatal activation of the hypothalamic-pituitary-gonadal axis in minipuberty determines adult Sertoli cell number and inhibin B levels (16). In humans, long-term gonadotropin release hormone (GnRH) therapy in adult patients with idiopathic hypogonadotropic hypogonadism normalizes inhibin B levels in subjects with partial spontaneous pubertal development, but not in those with congenital hypogonadotropic hypogonadism (CHH) (17). This implies that an adequate pubertal surge of inhibin B partly depends on the prior postnatal proliferation of Sertoli cells during minipuberty.

Inhibin B is well-known for its physiological function of exerting negative feedback on FSH secretion by pituitary gonadotrophs (18). FSH, in turn, stimulates inhibin B secretion. Therefore, apart from the negative feedback loop of luteinizing hormone (LH) and sex steroids, there is an additional feedback loop involving FSH and gonadal peptides in the hypothalamic-pituitary-gonadal axis. The relationship between FSH and inhibin B changes with age. FSH stimulates

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the secretion of inhibin B during the prepubertal period (19), but once puberty begins, a negative correlation between FSH and inhibin B is observed (20). The negative feedback axis matures during mid-puberty, as indicated by changes in the relationship between FSH and inhibin B during different stages of puberty. During Tanner stage 1 and Tanner stage 2, there is a positive trophic effect on inhibin B, while from stage Tanner stage 3 onwards, inhibin B and FSH have an inverse relationship (21).

After the onset of a negative feedback axis during puberty, there is a transitional shift in the regulation and production of inhibin B. In the fetal testis, inhibin α and βB subunits are found in Sertoli as well as Leydig cells (22). In adults, however, subunit inhibin α is mainly seen in Sertoli cells and subunit inhibin βB in germ cells (23). Therefore, the site of production for prepubertal males appears to be only Sertoli cells, while for adult males it is both Sertoli cells and germ cells. These findings indicate a shift in the sites of production of inhibin B around puberty. However, production still appears to be primarily dependent on Sertoli cells. This is evident from the lack of increase in inhibin B after human chorionic gonadotropin (hCG) injection in men with acquired hypogonadotropic hypogonadism (HH). In contrast, an increase in inhibin B is observed after recombinant FSH or a combination of recombinant FSH and hCG in the same population (24).

Sexual dimorphism is observed in inhibin B levels, with levels being much higher in boys than in girls (14, 15). Post-minipuberty in prepubertal children aged 2 to 6 years, both females and males have low serum levels of inhibin B, but it is often undetectable in girls aged 5 to 9 years, while always measurable in healthy boys (25). From Tanner stage 1 to Tanner stage 2, inhibin B levels increase significantly in both males and females, with a more pronounced increase in boys than girls. In girls, inhibin B levels rise more noticeably from Tanner stage 2 to Tanner stage 3 (26).

Over the years, assays for measuring inhibin B have advanced from nonspecific radioimmunoassay to specific enzyme-linked immunosorbent assays (ELISA) (27, 28). The first-generation inhibin B ELISA assays include the DSL (Diagnostic Systems Laboratories) assay and OBI (Oxford Brooks Innovation) assay. Both assays utilize a capture monoclonal antibody that targets a synthetic peptide from the β subunit. A key requirement for both assays is the oxidation of methionine using hydrogen peroxide to allow for epitope recognition. Additionally, the OBI ELISA requires heating with sodium dodecyl sulfate solution to enhance specificity. There is approximately 0.5% cross-reactivity with inhibin A for both assays (27). The second-generation ELISA (Gen II ELISA) uses a specific monoclonal antibody targeting the β subunit of inhibin B as the capture antibody. This test is simpler, with a shorter turnaround time, and does not require sample pre-treatment or an oxidation step. Importantly, Gen II ELISA shows no cross-reactivity with inhibin A, making it more specific (28). A comparative analysis by Kalra et al demonstrated the performance of the first-generation (DSL and OBI) assays against the second-generation ELISA (29). The slope and intercept values obtained were Gen II = 1.03 OBI-6.77 pg/mL and Gen II = 1.57 DSL + 11.29 pg/mL, respectively. These values are used for standardization of the DSL and OBI assays to the second-generation assay (29).

In males, inhibin B levels reflect Sertoli cell number and function, while in females, they reflect the function and number of small antral follicles (9, 11). FSH acts as a trophic hormone for the proliferation of Sertoli cells at a slower pace in the prepubertal period compared to minipuberty and puberty (19). The rate of proliferation increases just before the onset of puberty, which is marked biochemically by the increase in inhibin B. This forms the basis of using basal inhibin B (basal-iB) to investigate pubertal disorders. In past decade, investigators have tried to explore the role of inhibin B in differential diagnosis of delayed puberty (DP) as well as precocious puberty (PP). The current review discusses the diagnostic performance and clinical utility of basal-iB in present times and also considers the relevance of recently introduced stimulated inhibin B protocols in clinical practice.

Search Strategy

The literature was searched from various databases: PubMed, Science Direct, Web of Science, and Scopus using keywords "*inhibin B* AND *delayed puberty*," "*inhibin B* AND *precocious puberty*," "*stimulated inhibin B* AND *delayed puberty*," and "*stimulated inhibin B* AND *precocious puberty*." A total of 62 full-text articles on delayed puberty and 41 full-text articles on precocious puberty were shortlisted. After removing duplicate and irrelevant articles (eg, inhibin B measured but not used for differential diagnosis and no cutoffs provided for inhibin B), 10 articles for DP and 4 articles for PP were included in the review. (Figure 1)

Inhibin B in the Differential Diagnosis of Delayed Puberty

The lack of appearance of secondary sexual characteristics by 13 years of age in girls and 14 years of age in boys is defined as delayed puberty (DP) (30). This equates to a 2 to 2.5 SDS value above the mean of the population. However, as the commencement of puberty does not guarantee its eventual completion, there is also a secondary definition that should be highlighted, namely the failure to complete pubertal development (Tanner 5) by age 15 to 16 years in girls and 16 to 17 years in boys (27). The various etiologies implicated in DP are CHH, premature ovarian insufficiency (or gonadal dysgenesis), central nervous system tumors, systemic illness, prior treatment of childhood cancer with cranial radiation, gonadotoxic therapies and constitutional (or self-limited) delayed puberty (SLDP).

Among these, distinguishing between SLDP and CHH poses a clinical challenge, especially when evaluating a child at Tanner stage 1 or 2. The distinction between these 2 entities is important, as the management is entirely different. SLDP typically requires monitoring and psychological support, while HH calls for further investigation and appropriate treatment (31). Despite numerous tests, differentiating these conditions is still often difficult. Many clinicians still opt for watchful waiting due to the lack of a definitive test, even when there are clinical red flags for CHH, leading to prolonged stress for both the child and their parents and, potentially, also adverse psychosocial and psychosexual consequences in adult life (32).

Basal Inhibin B

Inhibin B levels begin to rise in late pre-puberty, making it a useful indicator for predicting pubertal onset, and basal-iB has thus been proposed for some years now as part of the workup for differentiating CHH from SLDP. Basal-iB overcomes some of the drawbacks of stimulation tests, such as repeated injections, repeated sampling, and multiple visits.

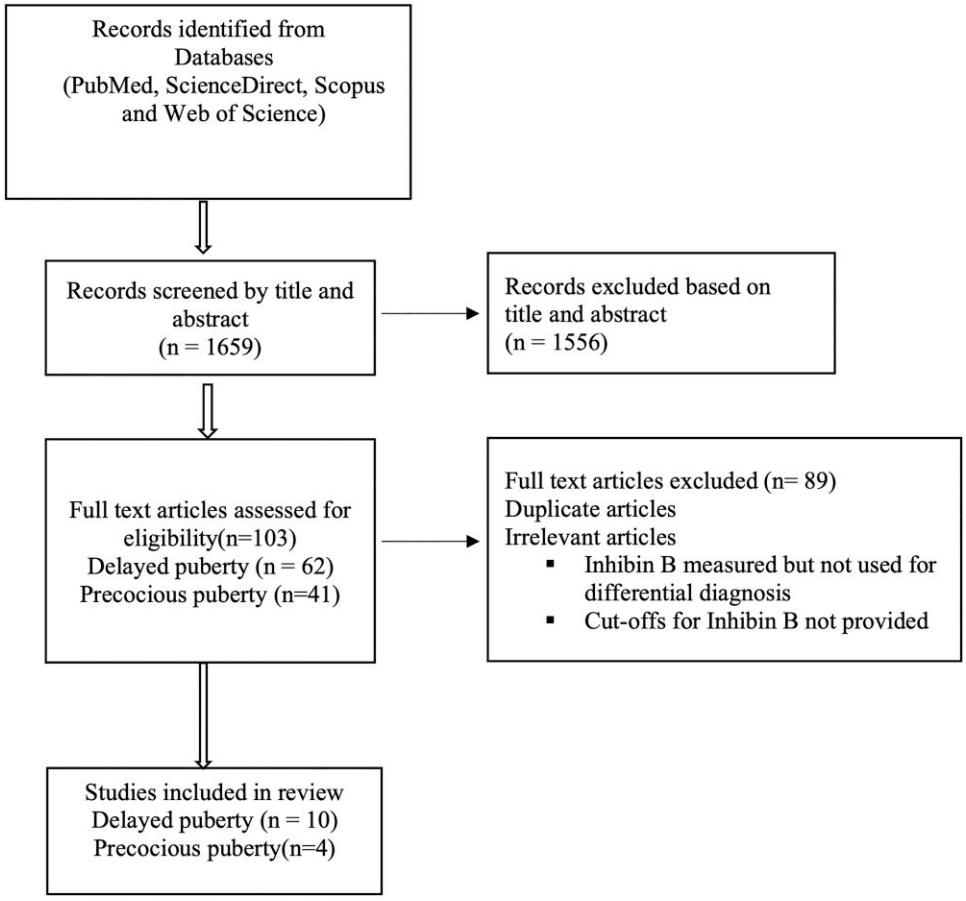

Figure 1. Flow diagram of search and selection of studies.

Several studies have demonstrated that basal-iB has sensitivity and specificity equal to or better than the GnRHa stimulation test (33-37). The diagnostic value of basal-iB in DP differential diagnosis was first explored in boys by Coutant et al in 2010 (38), with a further 7 studies in males and 2 studies in females having been performed since then. Four of these studies were prospective, while the others were retrospective. Coutant et al (38) identified 2 different cutoff values based on testicular volume (TV). A cutoff value of < 35 pg/mL showed 100% sensitivity and specificity for diagnosing CHH, for boys with TV < 3 mL, while a cutoff value of < 65 pg/mL had 86% sensitivity and 92% specificity for diagnosing CHH for boys with TV 3 to 6 mL. The combined sensitivity and specificity for both groups were 93% and 100%, respectively. The major strengths of the study were its prospective design and follow-up until the age of 18 years. However, the assay used was a first-generation DSL assay, which requires standardization to a second-generation assay. Additionally, there was no direct comparison in the same population with the commonly used GnRHa stimulation test. Nevertheless, the study introduced basal-iB as an additional parameter to the armamentarium of investigations used for the differential diagnosis of DP.

Adan et al (2010) (39) found a cutoff of < 100 pg/mL for basal-iB to have 87% sensitivity and 90% specificity for diagnosing CHH vs SLDP in boys with TV < 6 mL. However, it is important to note that the study had a retrospective design, and the assay used was OBI ELISA, which requires standardization to Gen II ELISA. Subsequently, Binder et al (2015) (33) using Gen II ELISA found a cutoff value of basal-iB < 111 pg/mL to have 100% sensitivity and 92% specificity for the same purpose in boys with TV <4 mL. However, the follow-up was only 12 to 18 months for SLDP and 24 months for CHH. Also, the study had a retrospective design and comprised fewer subjects with CHH. Rohayem et al (2015) (40), using a DSL assay, found a cutoff of < 28.5 pg/mL to have 95% sensitivity and 75% specificity for the diagnosis of CHH. It likewise had a retrospective design, and the subjects were aged between 13.9 to 23.2 years, meaning that some were already > 18 years old, which is the conventional age cutoff for presuming CHH anyway. Sukumar et al (2017) (34) found a cutoff value of ≥ 80 pg/mL (Gen II ELISA) to have 80% sensitivity and 96% specificity in boys with TV < 3 mL for the diagnosis of SLDP. Basal-iB was found to have similar sensitivity and higher specificity than GnRHa-stimulated LH, which had a specificity of 93.3%, and hCG-stimulated testosterone, which had a specificity of 87% in the same cohort. Varimo et al (2017) (35) found a cutoff value of 61 ng/L to have 90% sensitivity and 83% specificity to differentiate between SLDP and CHH. The study conducted a retrospective analysis of patients assessed for DP at a single center from 2004 to 2014. In addition to its retrospective design, the study has limitations due to the use of 2 different assays for measuring inhibin B: the OBI ELISA until 2010 and the Gen II ELISA thereafter. While the study notes that inhibin B levels measured by the newer assay were 7% lower, it is unclear whether the OBI assay values were standardized to the Gen II ELISA values when determining the cutoff

Table 1. The characteristics of the studies using basal and stimulated inhibin B for differentiation between self-limited delayed puberty and hypogonadotropic hypogonadism

	Study design	Study subjects	TV (mL)	Age group (years)	Gold standard	Method	Study results
Basal Inhibin B							
Boys							
Coutant et al (2010) (38)	Prospective	16-HH 51-SLDP	< 3 mL 3-6 mL	14-18 years	F/U till 18 years age	DSL ELISA Gen II ELISA corrected	< 35 pg/mL < 66 pg/mL SN-100% SP-100% for diagnosis of HH
						DSL ELISA Gen II ELISA corrected	< 65 pg/mL < 113 pg/mL SN-86% SP-92% for diagnosis of HH
Adan et al (2010) (39)	Retrospective	15-HH 39-SLDP	≤ 6 mL	14-17.4 years	Spontaneous and progressive pubertal development	OBI ELISA Gen II ELISA corrected	< 100 pg/mL < 96 pg/mL SN-87% SP-90% for diagnosis of HH
Binder et al (2015) (33, 41)	Retrospective	9-HH 52-SLDP	≤ 4 mL	13.7-17.5 years	Spontaneous and progressive pubertal development	Gen II ELISA	< 111 pg/mL SN-100% SP-92% for the diagnosis of HH
Rohayem et al (2015) (40)	Retrospective	22-HH 24-SLDP	< 4 mL	13.9-23.2 years	Spontaneous and progressive pubertal development	DSL ELISA Gen II ELISA corrected	< 28.5 pg/mL < 56 pg/mL SN-95% SP-75% for the diagnosis of HH
Sukumar et al (2017) (34)	Prospective	15-HH 15-SLDP	≤ 3 mL	14.1-26.2 years	F/U till 18 years age	Gen II ELISA	≥ 80 pg/mL SN-80% SP-96% for the diagnosis of SLDP
Varimo et al (2017) (35)	Retrospective	10-HH 60-SLDP	≤ 4 mL	14-18 years	Spontaneous and progressive pubertal development	OBI ELISA(till 2010) Gen II ELISA(after 2010)	61 pg/mL SN-90% SP-83% to differentiate between SLDP and HH
Chaudhary et al (2021) (36)	Prospective	Exploratory cohort- 18-Spontaneous puberty 8-HH Validation cohort 9-SLDP 2-HH	≤ 3 mL	Exploratory cohort- 10-26.4 years Validation cohort- 14-18 years	F/U till 18 years age in validation cohort	Gen II ELISA	≤ 50 pg/mL had 100% specificity for the diagnosis of HH ≥ 97.2 pg/mL had 100% specificity for the diagnosis of SLDP Cutoffs when used in validation cohort had 81.8% diagnostic accuracy

(*continued*)

Table 1. Continued

	Study design	Study subjects	TV (mL)	Age group (years)	Gold standard	Method	Study results
Mishra et al (2022) (37)	Prospective	6-HH 23-SLDP	≤ 4 mL	14-16.5 years	F/U till mid-puberty (Tanner stage 3)	ELISA	< 105 pg/mL SN-100% SP-82.6% for diagnosis of HH
Girls							
Binder et al (2015) (33, 41)	Retrospective	9-HH 12-SLDP	NA	13-17.5 years	menarche in SLDP	Gen II ELISA	< 20 pg/mL SN-100% SP-100% for diagnosis of HH
Chaudhary et al (2021) (36)	Prospective	Exploratory cohort- 8-Spontaneous puberty 8-HH Validation cohort 3-SLDP 5-HH	NA	Exploratory cohort- 9.2-34 years Validation cohort- 14-18 years	F/U till 18 years age in validation cohort	Gen II ELISA	≤ 38.4 pg/mL had 100% specificity for the diagnosis of HH ≥ 51.5 pg/mL had 100% specificity for the diagnosis of SLDP Cutoffs when used in validation cohort had 87.5% diagnostic accuracy
Inhibin B after testosterone priming							
Sukumar et al (2017) (34)	Prospective	15-HH 15-SLDP	≤ 3 mL	14.1-26.2 years	F/U till 18 years age	Gen II ELISA	≥ 94.7 pg/mL SN-100% SP-100% for diagnosis of SLDP
FSH stimulated Inhibin B (FSH-iB)							
Chaudhary et al (2021) (36)	Prospective	**Male** Exploratory cohort- 18-Spontaneous puberty 8-HH Validation cohort 9-SLDP 2-HH **Female** Exploratory cohort- 8-Spontaneous puberty 8-HH Validation cohort 3-SLDP 5-HH	≤ 3 mL	Exploratory cohort- 10-26.4 years Validation cohort- 14-18 years Exploratory cohort- 9.2-34 years Validation cohort- 14-18 years	F/U till 18 years age in validation cohort	Gen II ELISA	≥ 116.1 pg/mL SN-100% SP-100% To differentiate spontaneous puberty from HH 100% NPV, PPV, and diagnostic accuracy in validation cohort for diagnosis of SLDP ≥ 116.5 pg/mL SN-100% SP-100% To differentiate spontaneous puberty from HH 100% NPV, PPV, and diagnostic accuracy in validation cohort for diagnosis of SLDP
GnRHa stimulated Inhibin B (GnRH-iB)							
Chaudhary et al (2022) (43)	Prospective	Exploratory cohort- 18-Spontaneous puberty 8-HH Validation cohort 9-SLDP 2-HH	≤ 3 mL	Exploratory cohort- 10-26.4 years Validation cohort- 14-18 years	F/U till 18 years age in validation cohort	Gen II ELISA	≥ 113.5 pg/mL in male and ≥ 72.6 pg/mL in female had 90% sensitivity, 100% specificity and 94% diagnostic accuracy for diagnosis of SLDP

Gen II ELISA = 1.03 OBI – 6.77 pg/mL and Gen II ELISA = 1.57 DSL + 11.29 pg/mL (29).
Abbreviations: DSL ELISA, Diagnostic Systems Laboratories enzyme-linked immunosorbent assay; F/U, follow-up; Gen II ELISA, second-generation enzyme-linked immunosorbent assay; HH, hypogonadotropic hypogonadism; NPV, negative predictive value; OBI ELISA, Oxford Brooks Innovation enzyme-linked immunosorbent assay; PPV, positive predictive value; SLDP, self-limited delayed puberty; SN, sensitivity; SP, specificity; TV, testicular volume.

Table 2. The characteristics of the studies using basal and stimulated inhibin B in combination with other tests for differentiation between self-limited delayed puberty and hypogonadotropic hypogonadism

		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)
Binder et al (2015) (33, 41)						
Basal LH (CLIA) + Basal inhibin B[a]	LH <0.3 IU/L and basal inhibin B < 111 pg/mL for diagnosis of HH	100	98	NA	NA	NA
Chaudhary et al (2022) (43)						
Basal LH (ECLIA)+ Basal inhibin B[a]	LH ≥0.3 IU/L and basal inhibin B ≥ 97.2 pg/mL in male and ≥51.5 pg/mL in female for diagnosis of SLDP	80	66.7	80	66.7	75
Basal inhibin B[a]+ GnRHa (Triptorelin)–LH	Basal inhibin B ≥ 97.2 pg/mL in male and ≥51.5 pg/mL in female and GnRHa-LH ≥14 IU/L for diagnosis of SLDP	80	100	100	75	87.5
Mishra et al (2022) (37)						
Basal LH (CMIA) + Basal inhibin B (ELISA)	Basal LH <0.56 IU/L and basal inhibin B < 105 pg/mL for diagnosis of HH	100	100	NA	NA	NA

Abbreviations: CLIA, chemiluminescent immunoassay; CMIA, chemiluminescent microparticle immunoassay; ECLIA, electro chemiluminescent immunoassay; GnRHa, gonadotropin release hormone agonist; HH, hypogonadotropic hypogonadism; LH, luteinizing hormone; NA, not available; NPV, negative predictive value; PPV, positive predictive value; SLDP, Self-limited delayed puberty.
[a]Basal inhibin B was done by second-generation ELISA.

for analysis. A study (2021) (36) from our center found basal-iB levels of ≤ 50.1 pg/mL to have 100% specificity for the diagnosis of HH and ≥ 97.2 pg/mL to have a specificity of 100% for the diagnosis of SLDP. When these cutoffs were used on a prospective cohort for prediction of pubertal onset (diagnosis of SLDP) in boys with TV ≤ 3 mL, basal-iB had 77.8% sensitivity, 100% specificity, and 81.8% diagnostic accuracy. A recent study done by Mishra et al (2022) (37) found a cutoff level of basal-iB ≤ 105 pg/mL to have 100% sensitivity and 82.6% specificity in boys with TV ≤ 4 mL for the diagnosis of CHH.

Only 2 studies have evaluated the role of basal-iB in differentiating SLDP from CHH in females, in whom the cutoff values were found to be lower. A basal-iB level of < 20 pg/mL had 100% sensitivity and specificity for diagnosing CHH according to Binder et al (41). A study from our center (36) found that a basal-iB level of ≤ 38.4 pg/mL had 100% specificity for the diagnosis of CHH, while a level of ≥ 51.5 pg/mL had 100% specificity for the diagnosis of SLDP. The basal-iB cutoff ≥ 51.5 pg/mL for diagnosing SLDP was then validated in the prospective cohort, showing that it had 66.7% sensitivity, 100% specificity, and 87.5% diagnostic accuracy for diagnosing SLDP in girls (36).

Overall, basal-iB is a simple test, does not require multiple injections, and can be easily done on an outpatient basis, but unfortunately, different studies have provided very different diagnostic cutoffs. Even after correction of DSL assay and OBI ELISA to Gen II ELISA, the cutoff ranges from 56 to 113 pg/mL in boys, which is far too wide to establish a consensus for a single cutoff value for basal-iB for routine use. Moreover, only a cutoff value of 66 pg/mL (standardized to Gen II ELISA) in boys with TV < 3 mL was found to have 100% sensitivity and specificity. All other studies have found good sensitivity (80%-100%) but lower specificity (75%-96%) in males, and only 2 studies have been performed in females and with different cutoffs. Additionally, the cost of basal-iB performance is higher compared to the conventional tests. Hence, although basal-iB would seem to be a reasonable first-line investigation in children presenting with DP, in practice, the ongoing quest for a standard uniform cutoff value limits its applicability.

Inhibin B After Testosterone Priming in Males

A single study has evaluated the role of basal-iB measured after testosterone withdrawal in boys (34). The premise behind the study was that testosterone treatment followed by withdrawal may trigger the activation of the hypothalamic-pituitary-gonadal axis in children with SLDP and hence can increase the sensitivity and specificity of basal and stimulation tests done after withdrawal of testosterone. The protocol used was 3 doses of 100 mg testosterone injected intramuscularly every 4 weeks followed by 8 weeks washout. Evaluations were conducted at baseline and 20 weeks later. It was found that a post-testosterone priming inhibin B level ≥ 94.7 pg/mL had 100% sensitivity and specificity for differentiating SLDP from HH. Inhibin B levels measured after testosterone priming outperformed GnRHa (Triptorelin)- and hCG-stimulation tests in the same population. The study suggests that using inhibin B after testosterone priming might be a good alternative to dynamic tests at the initial presentation. Testosterone administration for 3 months may (i) help alleviate the anxiety associated with DP in SLDP; (ii) have a triggering action in respect of endogenous puberty in SLDP; and (iii) in any case serve as an effective treatment for CHH. Both basal-iB levels and dynamic tests conducted after priming showed improved sensitivity and specificity. However, as there was a significant increase in the TV over 5 months in the SLDP group as a whole over the course of the study, it was not possible to determine whether it was, indeed, the brief exposure to testosterone that led to increased sensitivity and specificity or the natural course of SLDP patients who entered puberty after 5 months.

FSH Stimulated Inhibin B

The concept of stimulability has been used for years for the differential diagnosis of DP. GnRH/GnRHa stimulated LH and hCG-stimulated testosterone are well-established stimulation tests, but stimulation of inhibin B by its trophic factor

Table 3. The characteristics of the studies using basal inhibin B for differentiation between gonadotropin-dependent precocious puberty and premature thelarche

	Study design	Study subjects	Tanner stage for breast development	Age group (years)	Gold standard	Method	Study results
Basal Inhibin B							
Girls							
De Filippo et al (2013) (45)	Prospective	31-progressive form 31-non progressive form	B2	< 8 years	F/U till 9 years age	DSL ELISA Gen II ELISA corrected	> 20 pg/mL > 43 pg/mL SN-60% SP-89% for diagnosis of progressive form
Chen et al (2017) (46)	Prospective	55-progressive central PP 28-slowly progressive central PP 65-premature thelarche	B2	< 8 years	Elevated LH response on GnRHa stimulation test	Gen II ELISA	> 30.12 pg/mL SN-80% SP-89.3% for diagnosis of progressive central precocious puberty
Jeong et al (2020) (47)	Retrospective	48-central PP 35-age-matched controls	B1-B5	<8 years	Bone age advancement more than 1 year and pubertal LH response on Leuprolide stimulation test	Gen II ELISA	> 19.59 pg/mL SN-83.3% SP-82.9% specificity for the diagnosis of central precocious puberty
Chaudhary et al (2024) (48)	Prospective	5-Gonadotropin-dependent PP 5-Premature thelarche 6-Healthy controls	B2	< 8 years	Bone age advancement > 2.5 SDS and elevated LH response on Triptorelin stimulation test	Gen II ELISA	≥ 27.87 pg/mL SN-80% SP-81.8% for the diagnosis of GDPP

Abbreviations: DSL ELISA, Diagnostic Systems Laboratories enzyme-linked immunosorbent assay; GDPP, gonadotropin-dependent precocious puberty; Gen II ELISA, second-generation enzyme-linked immunosorbent assay; GnRHa, gonadotropin release hormone agonist; LH, luteinizing hormone; PP, precocious puberty; SN, sensitivity; SP, specificity.

FSH and its potential role in the prediction of pubertal onset was only recently explored (36). FSH being less tightly regulated than LH causes the proliferation of Sertoli cells during prepubertal period (42), resulting in a priming effect. The study demonstrated that administration of exogenous FSH in an individual with primed testes/ovary results in a significant rise in inhibin B, which can be utilized clinically to differentiate between SLDP and CHH. In boys, the stimulation protocol involved a 300 IU FSH injection given on alternate days for a total of 3 doses, with inhibin B levels measured 24 hours after the final injection (Day-6). In girls, the protocol was a 150 IU FSH injection given daily for 3 days, with inhibin B measured 24 hours after the final injection (Day-4). The study found that a cutoff value of 116.1 pg/mL in males and 116.5 pg/mL in females for FSH-stimulated inhibin B (FSH-iB) had 100% sensitivity, specificity, negative predictive value, positive predictive value, and diagnostic accuracy for differentiating SLDP from HH. The major strength of the study was a derivation of cutoff from the exploratory cohort (children with normal pubertal development and adults with CHH) and its validation in a prospective cohort. Moreover, the performance of FSH-iB was compared with other commonly used tests (basal LH, basal-iB, and GnRHa stimulation test) in the same cohort. However, fewer subjects were included in the validation cohort, which warrants the need for confirmation in a larger cohort. Nevertheless, the study has paved the way for the concept of stimulated inhibin B, and FSH-iB holds the potential to become a promising discriminatory tool in the differentiation of SLDP from CHH in the future.

GnRHa Stimulated Inhibin B

The administration of exogenous GnRHa causes an increase in both LH and FSH levels. FSH, being a natural stimulant for inhibin B, prompted a study to examine the possibility of stimulating inhibin B with exogenous GnRHa and its potential role in the differential diagnosis of DP (43). GnRHa stimulated inhibin B (GnRH-iB) was measured at 24 hours after GnRHa (Triptorelin injection 0.1 mg/m^2). The cutoffs for GnRH-iB were derived from the exploratory cohort and were applied to the prospective cohort for validation. GnRH-iB at a cutoff of 113.5 pg/mL in males and 72.6 pg/mL in females had 90% sensitivity, 100% specificity, and 94% diagnostic accuracy for differentiating SLDP from HH. Although the test is simpler than the FSH stimulation test, sensitivity and diagnostic accuracy were lower. Moreover, the patient population was the same for both tests. So, subjects undergoing GnRHa stimulation had already been subjected to FSH stimulation 5 days earlier and a priming effect by FSH cannot be completely ruled out.

The characteristics of the studies using basal and stimulated inhibin B for differentiation between SLDP and HH are illustrated in Table 1.

Inhibin B in Combination With Other Tests

Inhibin B has been evaluated in combination with other tests to increase the sensitivity and specificity of the diagnosis. Binder et al (33) found that basal LH < 0.3 IU/L in combination with basal-iB < 111 pg/mL had 100% sensitivity and 98% specificity for diagnosing HH. Mishra et al (37) demonstrated that basal LH < 0.5 IU/L in combination with basal-iB < 105 pg/mL had 100% sensitivity and specificity for diagnosing HH. In a study conducted at our center (43), it was found that a combination of basal-iB ≥ 97.2 pg/mL and GnRHa-stimulated LH ≥ 14 IU/L had 80% sensitivity, 100% specificity, and 88% diagnostic accuracy for diagnosing SLDP. However, the combination of basal-iB ≥ 97.2 pg/mL and basal LH > 0.3 IU/L was less effective, with 80% sensitivity, 67% specificity, and 75% diagnostic accuracy. Moreover, a combination of GnRH-iB with basal LH, GnRHa-stimulated LH, or basal-iB was not found to be superior to GnRH-iB alone (Table 2).

Inhibin B in the Differential Diagnosis of Precocious Puberty

The appearance of secondary sexual characteristics in girls younger than 8 years and boys younger than 9 years is known as precocious puberty (PP) (44), which can be gonadotropin-dependent (GDPP) or independent. GDPP is more commonly observed in girls. Differentiating between GDPP and simple premature thelarche can be challenging, especially in Tanner stage 2. Recently, researchers have looked into the role of basal-iB as an indicator of entry into puberty to help distinguish between these 2 conditions.

Basal-iB was first evaluated for differential diagnosis of PP by De Filippo et al (45) and a total of 4 studies have evaluated basal-iB in the differential diagnosis of PP so far, all of them in girls. De Filippo et al followed 62 girls developing thelarche (B2) under 8 years of age and followed until 9 years. There were 31 girls in each group, including both progressive form and nonprogressive forms. It was found that a basal-iB level > 20 pg/mL had 60% sensitivity and 89% specificity in differentiating between progressive and nonprogressive forms of PP. When compared with basal LH levels, an LH level > 0.2 IU/L had a sensitivity of 71% but with a lower specificity of 77%. Combining basal-iB levels > 20 pg/mL and LH levels > 0.2 IU/L resulted in 98% sensitivity and specificity.

Chen et al (46) identified a cutoff value of 30.12 pg/mL to distinguish between slowly progressive and progressive central PP. The study included 55 girls with progressive central PP and 28 girls with slowly progressive central PP. Assessment of basal-iB was found to have 80% sensitivity and 89.3% specificity. However, the study was limited by a short follow-up period of 6 months for the assignment of the 2 groups. Jeong et al (47) compared basal-iB in 48 girls with central PP and 35 age-matched controls. They found that a basal-iB cutoff value of 19.59 pg/mL had 83.3% sensitivity and 82.9% specificity. The study was limited by its retrospective design. A study from our center compared basal-iB levels between GDPP, premature thelarche, and healthy controls. The study found that a basal-iB ≥ 27.87 pg/mL had 80% sensitivity and 81.8% specificity for the diagnosis of GDPP (48). A major limitation of the study was the small number of subjects in each group, which necessitates confirmation of the cutoff value in a larger population. The characteristics of the studies using inhibin B for differentiation between GDPP and isolated premature thelarche are illustrated in Table 3.

Conclusion

Over the years, basal-iB has established itself as a strong candidate marker to differentiate SLDP from CHH, but its use in routine clinical practice remains limited; the major barrier to clinical implementation is the lack of consensus on a single cutoff. Moreover, standardization of assays, uniformity of assays, and use of different TV criteria for the enrollment of

study patients further add to the heterogeneity of the results obtained from different studies. Compared to conventional tests, basal-iB offers better sensitivity and specificity than basal LH. It has comparable sensitivity and specificity to the GnRHa stimulation test, while offering the advantage of ease of performance. The studies combining inhibin B with LH are fewer and have provided different cutoffs, making its routine use challenging. For the selected subset of patients, who are ready to wait for 3 months, testosterone priming is a good initial option. Although the recent concept of stimulated inhibin B (FSH-iB and GnRH-iB) offers the possibility of a more discriminatory marker, these tests are time-consuming and require multiple visits—like other stimulation tests. In this context, the concept of a two-sided cutoff for basal-iB appears promising. Using a two-sided cutoff gives the investigator the privilege of using basal-iB as an initial investigation and proceeding to stimulation tests only when the value falls in the equivocal range. The data for the use of basal-iB in the differential diagnosis of PP remains insufficient. To conclude, both basal- and stimulated inhibin B levels need larger studies with standardized assays in different ethnicities to derive uniform cutoffs for use in routine clinical practice.

Author Contributions

All authors have contributed to the manuscript in a substantive manner and are prepared to take public responsibility for it.

Disclosures

The authors have nothing to disclose

Data Availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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