

Accuracy of various tests alone and in combination to differentiate IHH from CDGP

Pratap Kumar Mishra, Ipsita Mishra¹, Arun Kumar Choudhury¹, Anoj Kumar Baliarsinha¹, Swayam Sidha Mangaraj², Sweekruti Jena¹, Binoy Kumar Mohanty³

Department of Endocrinology, Capital Hospital, Bhubaneswar, Odisha, ¹Department of Endocrinology, SCB Medical College and Hospital, Cuttack, Odisha, ²Department of Endocrinology, IMS and SUM Hospital, Bhubaneswar, Odisha, ³Department of Endocrinology, MKCG Medical College and Hospital, Berhampur, Odisha, India

Abstract

Context: Constitutional delay in growth and puberty (CDGP) is a normal physiological variant of delayed puberty in both sexes and is the most common cause of delayed puberty. Idiopathic hypogonadotropic hypogonadism (IHH) is due to deficiency in or insensitivity to gonadotropin-releasing hormone (GnRH) with normal structure and function of the anterior pituitary after exclusion of secondary causes of hypogonadotropic hypogonadism. To differentiate CDGP from IHH is crucial because it not only helps in decision making in management but also lessen anxiety of the parents. **Aim:** In this study we aimed to find out the accuracy of hormonal tests used individually as well as in various combinations to distinguish cases of IHH from CDGP. **Methods:** A cohort of 34 boys with delayed puberty were recruited in this study. Detailed history, clinical examination, hormonal analysis including basal serum testosterone, inhibin-B, LH, FSH as well as GnRH analogue stimulated gonadotrophins and testosterone along with hCG stimulated testosterone was done. At 6 months follow-up, detailed clinical examination was repeated and the cohort was followed until 2 years. **Results:** Out of the 29 boys taken for final analysis, CDGP was diagnosed in 23 boys and IHH in 6 boys. Basal LH, basal inhibin-B, 3 hours post leuprolide LH and 72 hours post hCG testosterone were significantly higher in CDGP than IHH. However, no statistically significant difference was observed between basal FSH, basal testosterone and 3 hours post leuprolide FSH between these two groups. When basal LH (cut-off <0.565 IU/L) and basal inhibin-B (cut-off <105 pg/ml) were taken together the sensitivity and specificity were increased to 100% as was for the combination of basal LH (cutoff <0.565 IU/L) and 3 hours post leuprolide LH (cutoff <6.16 IU/L) for diagnosis of IHH. Both combinations have PPV of 100% and NPV of 100%. A combination of 3 hours post leuprolide LH with 72 hours post hCG testosterone also has good sensitivity (100%), specificity (96%), PPV (90%) and NPV (100%). **Conclusion:** Differentiating IHH from CDGP is a challenging task due to considerable overlap in their clinical as well as hormonal profiles. Therefore we suggest that a combination of basal LH and basal inhibin-B may be considered as a useful screening tool to differentiate IHH from CDGP rather than the cumbersome and invasive stimulation tests.

Keywords: Basal inhibin B, basal LH, CDGP, IHH

INTRODUCTION

Puberty is a complex physiological process that marks the transition from childhood to adulthood, tightly regulated and modulated by an interplay of genetic, hormonal and environmental factors. Reactivation of the gonadotropin-releasing hormone (GnRH) neurons results in increased pulsatile secretion of GnRH, which leads to the pulsatile secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), maturation of the gonads and increased gonadal steroid output. The mean age at onset of puberty in boys is 11 years, with the normal limits being 9 to 14 years.^[1] The normal age of puberty in white girls ranges

from 7 to 13 years and that of African American girls ranges from 6 to 13 years.^[1]

Puberty is said to be delayed when the secondary sexual characters do not develop by the age of 13 years in females

Address for correspondence: Dr. Ipsita Mishra,

c/o Dr H N Mishra, 344 Manavilas Lane, Ice Factory Road, College Square, Cuttack - 753003, Odisha, India.
E-mail: ipsita.mishra1981@gmail.com

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and 14 years in males.^[2] This creates significant anxiety and psychological stress both in parents as well as affected children. CDGP is a physiological variant and is the most common cause of delayed puberty in both sexes. IHH, is a cause of hypogonadotropic hypogonadism (HH) due to deficiency in or insensitivity to GnRH, where the function and anatomy of the anterior pituitary are otherwise normal and secondary causes of HH are absent. During the initial evaluation, it is very difficult to make a clear distinction between these conditions because of the considerable overlap in clinical as well as hormonal profiles. Under both conditions, gonadotropin levels are low due to, functional immaturity of hypothalamo-pituitary axis in CDGP and decreased GnRH secretion or action in IHH respectively. Certain clinical characteristics like family history of CDGP, short stature, delayed bone age (appropriate for height age) and absence of adrenarche with delayed gonadal development favours the diagnosis of CDGP. Features like tall stature, anosmia, cryptorchidism, small testicular volume (1-2 cc), pubarche in absence of gonadarche are evident in IHH. However, these clinical findings are often not diagnostic.

A clinical diagnosis is required at outset to predict the clinical course and prognosis. It also helps in formulating a protocol for better patient management. Clinical outcome at the age of 18 years represents the gold standard to differentiate these two entities. We studied the role of various hormonal parameters alone and in combination as adjuncts to the clinical parameters to help differentiate CDGP from IHH, in treatment naïve individuals.

MATERIALS AND METHODS

Study design and population

This prospective cohort study was conducted in the Department of Endocrinology, SCB Medical College and Hospital, Cuttack. Boys presenting with non-development of secondary sexual characters with a testicular volume ≤ 4 ml by 14 years of age and having no apparent cause of delayed puberty other than CDGP or IHH were enrolled. The upper age limit was taken as 16.5 yrs. A total of 34 patients after meeting the exclusion criteria were included. All study participants underwent a detailed history enquiry and clinical examination using a preformed proforma. The parents were clearly explained about the study protocol and written informed consent was taken from all study participants. The ethical clearance for the study was obtained from our Institutional ethical committee.

Laboratory tests

The enrolled cohort underwent a baseline hormonal analysis as per study protocol. A blood sample for the measurement of basal serum concentration of testosterone, inhibin-B, LH, and FSH was collected between 8-9 am with overnight fast. Subsequently injection leuprolide acetate at a dose of 10 $\mu\text{g}/\text{kg}$ body weight was given subcutaneously and repeat blood samples were drawn at 3 hours and 24 hours for measurements of gonadotropins and serum testosterone respectively. Following the next 24 hours, human chorionic

gonadotropin (hCG) 1500 IU was given deep IM in the gluteal region for 3 consecutive days and the blood sample for measurement of serum testosterone was obtained 24 hrs after the last dose of hCG. Measurement of FSH, LH and testosterone was done by Chemiluminiscent Microparticle Immunoassay (CMIA) methods (Abbott Architect Plus i 2000 SR)). Inhibin B was measured by the enzyme-linked immunosorbent assay.

After enrolling the patients and doing necessary hormone estimation as required in the study protocol, trial testosterone therapy was given to patients who were more apprehensive about puberty induction. In them, intramuscular injection of 50 mg testosterone enanthate was given for 3 consecutive months initially followed by 3 month break. During the next 6 monthly follow up, testosterone was given in escalated doses (for an initial 3 month period; maximum dose of 100 mg for those with a strong suspicion of CDGP) to all those in whom, testicular volume failed to reach >4 ml to stimulate puberty. The patients were followed up at 6 months intervals and clinical examinations including auxological, pubertal staging and measurements of testicular volumes were made and recorded. In view of the stipulated study period, the participants were followed up till mid puberty (until Tanner stage 3).

Diagnosis of IHH or CDGP

A diagnosis of CDGP was made when the testicular volume reached ≥ 8 ml, any point during follow up and IHH was assumed if the testicular volume was ≤ 5 ml during 24 months of follow up. It is evident from the literature that healthy boys reach a testicular volume ≥ 8 ml during midpuberty,^[3,4] where as a testicular volume ≥ 5 ml is rarely reached in adolescence with proven IHH.^[5,6] Patients who had testicular volumes between >5 ml and <8 ml were excluded from the final analysis as we could not categorize them in either category (CDGP/IHH) at the end point of our study.

Statistical analysis

Data were expressed as the mean and standard deviation and analysed using the IBM SPSS 20 statistical software (IBM Corp., Armonk, NY, USA). Nonparametric tests (Mann-Whitney U test) were performed to compare between means. Receiver operating characteristic (ROC) analysis was performed for the different diagnostic tests, and ROC curves were plotted to examine the trade-off between sensitivity and 1-specificity. To compare the diagnostic accuracy of the tests, the area under the curve (AUC) was calculated with 95% confidence limits. Cut-off points were determined by maximizing the difference between the number of true-positive test results (sensitivity) and the number of false positive results (1-specificity). Based on the established cut-off points, the diagnostic sensitivity and specificity were reported for each test in isolation and in different combinations. Positive predictive value (PPV) and negative predictive value (NPV) were calculated as the pre standard formula. For all tests a probability (p) < 0.05 was considered significant.

RESULTS

Thirty four boys aged 14-16.5 years were enrolled in the study. All had testicular volumes ≤ 4 ml. Two boys had a testicular volume between 5 and 8 ml at 24 months of follow-up and therefore did not fulfill the definition of either IHH or CDGP. Three boys were lost to follow up. Thus 29 boys were taken for final analysis.

At the end of the study, CDGP was diagnosed in 23 boys and IHH in 6 boys. The mean age at presentation was 15.33 ± 0.68 years in IHH and 14.64 ± 0.6 years in the CDGP group [Table 1]. CDGP patients were comparatively short with a mean height of 152.6 ± 4.25 cm compared to 165.6 ± 2.56 cm in IHH group ($P=0.001$) [Table 1]. IHH group had significantly ($P=0.001$) more weight and BMI than CDGP group [Table 1]. The mean testicular volume was comparable between groups at baseline ($P=0.26$) [Table 1]. Though the IHH group had more delayed bone age in comparison CDGP group it was statistically not significant ($p=0.581$) [Table 1].

Basal LH, basal Inhibin-B, 3 hours post leuprolide LH and 72 hours post hCG testosterone were significantly higher in CDGP than IHH [Table 2]. However, no statistically significant difference was observed between basal FSH, basal testosterone and 3 hours post leuprolide FSH between these two groups [Table 2].

On analysis of the ROC plot [Table 3], the area under curve (AUC) for 3 hours post leuprolide LH was highest, followed by 72 hours post hCG testosterone, basal inhibin-B and basal LH [Figures 1 and 2]. The AUC for basal testosterone and

that of basal FSH was lower in comparison to others [Table 3]. Basal inhibin-B with a ROC generated cut-off of <105 pg/ml was 100% sensitive and 82.6% specific to pick up IHH [Table 3, Figure 3a]. Basal LH with a cut-off of <0.565 IU/L also had sensitivity and specificity similar to that of basal inhibin B for identifying IHH [Table 3, Figure 3b]. The discriminatory value of post 3 hours leuprolide LH was excellent when a cutoff of 6.61 IU/L was considered [Table 3, Figure 3c]. Seventy-two hours post hCG testosterone is also a good discriminator after stimulated LH [Table 3, Figure 3d]. Others were poor discriminators of IHH [Table 3]. When basal LH and basal inhibin-B were taken together the specificity was increased to 100% [Table 4 and Figure 4] as for the combination of basal LH and 3 hours post leuprolide LH for diagnosis of IHH. A combination of 3 hour post leuprolide LH with 72 hours post hCG testosterone also has good sensitivity and specificity. Other combinations have good discriminatory capacity but are slightly below the above-mentioned combinations.

Thus in the basal state -inhibin B, LH; stimulated state -3 hours post leuprolide LH, 72 hours post hCG testosterone and combinations- basal LH +basal inhibin-B, basal LH +3 hours post leuprolide LH have higher diagnostic power.

Table 1: Baseline clinical characteristic in CDGP and IHH groups

Clinical parameters	CDGP (mean \pm SD)	IHH (mean \pm SD)	P
Age (yrs)	14.64 \pm 0.60	15.33 \pm 0.68	0.04
Height (cms)	152.6 \pm 4.25	165.6 \pm 2.56	0.001
Height SDS	-1.19 \pm 0.82	0.32 \pm 0.29	0.001
Weight (kg)	49.91 \pm 6.2	67.83 \pm 6.14	0.001
BMI (kg/m ²)	21.34 \pm 1.98	24.3 \pm 1.3	0.002
Bone age (yrs)	13.44 \pm 0.49	13.5 \pm 0.54	0.581
Baseline mean testicular volume (ml)	2.58 \pm 0.81	2.17 \pm 0.75	0.26

Table 2: Hormonal parameters in CDGP and IHH groups

Hormonal Parameters	CDGP (mean \pm SD)	IHH (mean \pm SD)	P
Basal FSH (U/L)	1.82 \pm 1.21	0.96 \pm 0.41	0.09
Basal LH (IU/L)	1.44 \pm 1.01	0.36 \pm 0.09	0.004
Basal Testosterone (ng/dl)	22.81 \pm 3.97	20.46 \pm 4.61	0.254
Basal Inhibin-B (pg/ml)	176.95 \pm 63.36	62.78 \pm 24	<0.001
3 hours post leuprolide FSH (U/L)	9.91 \pm 5.43	5.38 \pm 2.57	0.080
3 hours post leuprolide LH (IU/L)	19.28 \pm 9.69	3.47 \pm 1.37	<0.001
24 hours post leuprolide Testosterone (ng/dl)	34.39 \pm 6.26	27.23 \pm 4.98	0.019
72 hours post hCG Testosterone (ng/dl)	251.22 \pm 90.59	82.33 \pm 13.38	<0.001

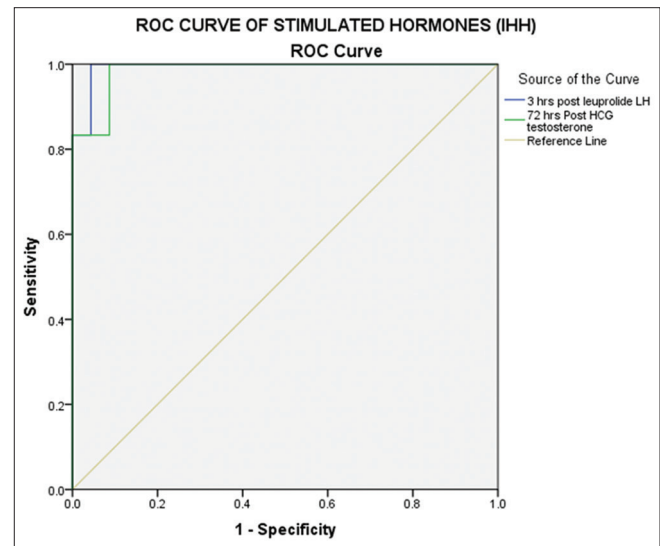


Figure 1: ROC curve of stimulated hormones (IHH)

Table 3: Accuracy of individual hormonal parameters, AUC and ROC generated cut-off of IHH groups

Hormonal Parameters	Cutoff (<)	AUC (95% Confidence Interval)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Basal FSH (IU/L)	1.155	0.732 (0.563-0.928)	0.83 (0.36-0.99)	0.69 (0.47-0.86)	0.41 (0.16-0.71)	0.94 (0.69-.99)
Basal LH (IU/L)	0.565	0.870 (0.737-1.0)	1 (0.51-1)	0.83 (0.61-0.94)	0.6 (0.27-0.86)	1 (0.79-1)
Basal Testosterone (ng/dl)	19.71	0.656 (0.362-0.950)	0.67 (0.24-0.94)	0.78 (0.56-0.92)	0.44 (0.15-0.77)	0.9 (0.66-0.98)
Basal Inhibin-B (pg/ml)	105	0.964 (0.901-1.0)	1 (0.51-1)	0.83 (0.61-0.94)	0.6 (0.27-0.86)	1 (0.79-1)
3 hours post leuprolide FSH (IU/L)	6.41	0.739 (0.533-0.925)	0.83 (0.36-0.99)	0.65 (0.43-0.82)	0.38 (0.15-0.67)	0.92 (0.67-0.99)
3 hours post leuprolide LH (IU/L)	6.16	0.993 (0.970-1.0)	1 (0.51-1)	0.95 (0.76-0.99)	0.86 (0.42-0.99)	1 (0.81-1)
24 hours post leuprolide Testosterone (ng/dl)	29.67	0.812 (0.638-.985)	0.67 (0.24-0.94)	0.78 (0.56-0.91)	0.44 (0.15-0.77)	0.9 (0.66-0.98)
72 hours post hCG Testosterone (ng/dl)	110	0.986 (0.949-1.0)	1 (0.51-1)	0.91 (0.70-0.98)	0.75 (0.35-0.95)	1 (0.80-1)

DISCUSSION

Delayed puberty is distressing in the sense that, it imposes undue physical, psychological and emotional stress on affected children and their parents. At presentation, the clinical as well hormonal profiles of these conditions are so overlapping that, these conditions are not readily differentiated.

In this prospective study, we aimed to differentiate IHH from CDGP by studying the pattern of basal and stimulated hormones along with the role of combinations of various basal and stimulated hormone levels.

We found basal LH with a cut-off of <0.565 U/L has the highest sensitivity (SN) and specificity (SP) in identifying IHH. Sequera *et al.*^[7] demonstrated that a basal LH of more than 0.65 IU/L excluded a diagnosis of complete IHH which is in agreement with our findings. Recently, Binder *et al.*,^[8] in their study involving 53 CDGP and 9 IHH patients demonstrated that a basal LH of ≤ 0.3 IU/L has a similar sensitivity to our finding, to diagnose IHH. The cut-off obtained in their study was lower than that of our cut-off though the same assay methodology has been used in both. The basis of this difference seems unknown. The discriminating potential of basal FSH found in our study is low and is comparable to other studies.^[9,10] In a recent small study ($n = 7$), Grinspon *et al.*^[11] reported basal FSH level below 1.2 IU/L in boys presenting with delayed puberty had the highest PPV in those with a confirmed diagnosis of IHH.

Inhibin B, a heterodimeric glycoprotein product of the Sertoli cells of testis, is now being used as a biomarker of the testicular function. The mean concentration of serum inhibin B, in males increases between pre-puberty and the first stage of puberty.^[12] From genital stage 2 onwards, it remains relatively constant, despite a rise in mean concentration of serum FSH. Coutant *et al.*^[13] demonstrated that a single inhibin B level of 35 pg/ml or less had a good PPV to identify patients with IHH from those with CDGP. Binder *et al.*^[8] taking a still higher cut-off found that inhibin B < 111 pg/ml had the highest sensitivity for the detection of IHH. We found that basal inhibin-B with a ROC generated cut-off of < 105 pg/ml has very good sensitivity in concordance with other studies. Recently, Chaudhary *et al.*^[14] demonstrated that FSH stimulated inhibin B at a cut off of 116.14 pg/ml in males has very good sensitivity and specificity for labeling entry into puberty.

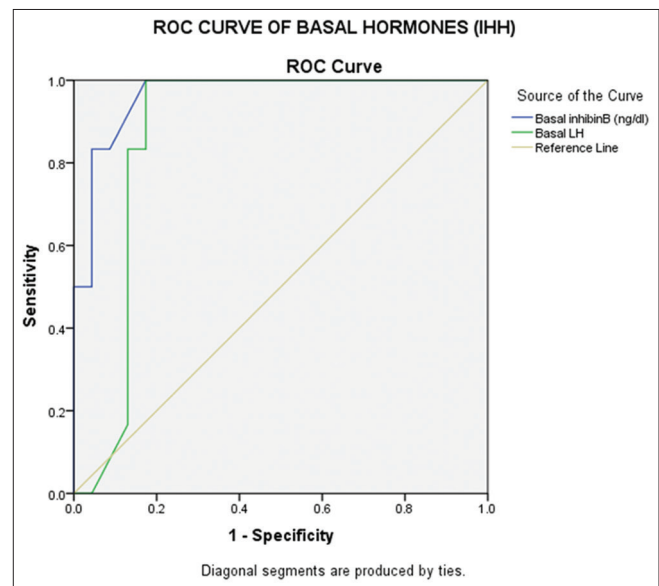


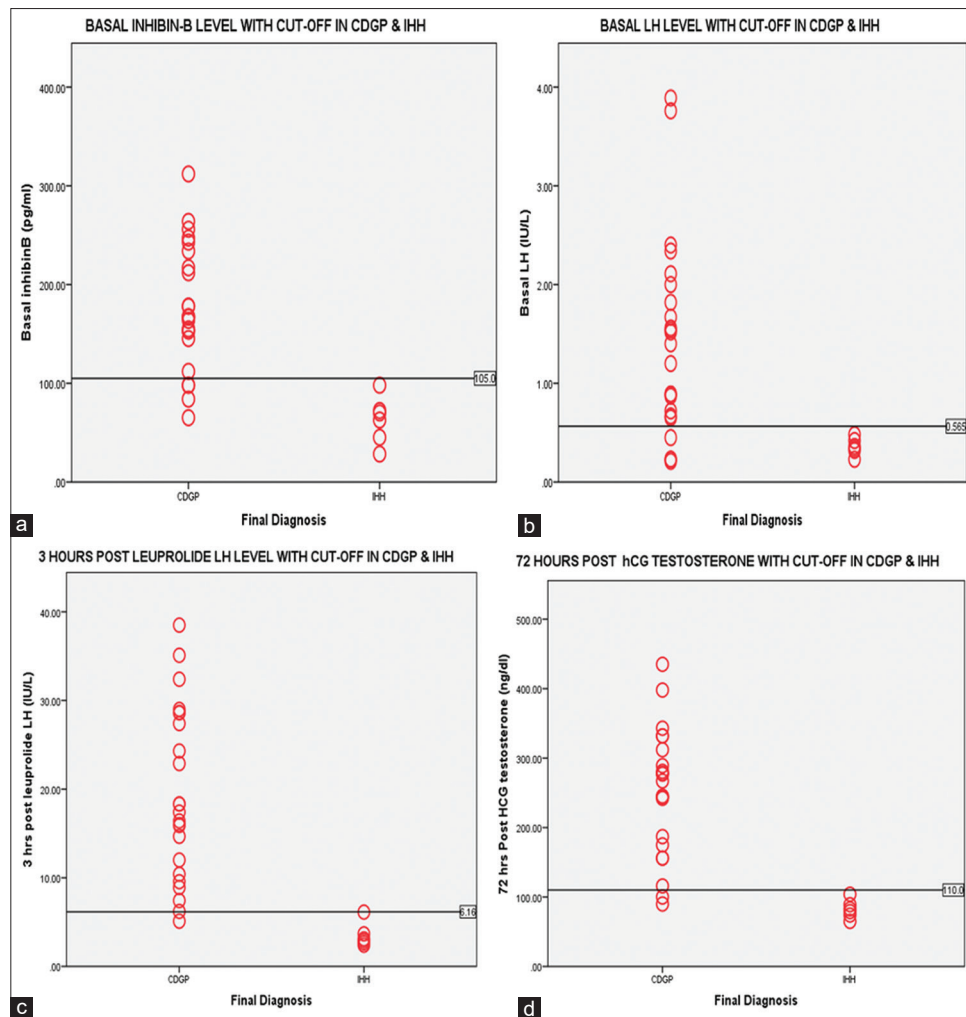
Figure 2: ROC curve of basal hormones(IHH)

We demonstrated that LH responses (3 hours) to subcutaneous leuprolide acetate (10 mcg/kg) stimulation clearly discriminated between IHH and CDGP groups, with no overlap in the hormonal levels between IHH and CDGP group. With a ROC generated cut-off of <6.16 IU/L, it is the most discriminating single hormone with the highest sensitivity and specificity to identify IHH. In agreement with our findings, Street *et al.*^[15] demonstrated stimulated LH of 2.8 IU/L or less has high discriminatory power to diagnose IHH. However, Lanes *et al.*^[16] did not demonstrate the same diagnostic utility of leuprolide stimulation testing, with significant overlap between the two groups. Similar to our finding, Binder *et al.*^[8] also demonstrated that a 4 hours post triptorelin LH value of less than 0.53 IU/L high sensitivity and specificity to diagnose IHH. A recent study found that Kisspeptin stimulated LH is a good predictor to determine pubertal outcomes in children with delayed puberty. The study found that those who demonstrated a rise in LH of 0.8 miu/ml or greater progressed through puberty, whereas those who had LH response to ≤ 0.4 miu/ml did not progress through puberty even till 18 years of age.^[17]

We found a good discriminating potential of 72 hours post hCG testosterone also. A previous study, using 3 days and 19 days

Table 4: Accuracy of combined hormonal parameters for IHH

Hormonal Parameters in combinations	Cut-off (<)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Basal inhibin-B (pg/ml) + Basal LH (IU/L)	Inhibin-B- 105 LH-0.565	1 (0.51-1)	1 (0.82-1)	1 (0.51-1)	1 (0.82-1)
Basal inhibin-B (pg/ml) +3 hours post leuprolide LH (IU/L)	Inhibin-B- 105 3 hrs LH-6.16	1 (0.51-1)	0.95 (0.76-0.99)	0.85 (.42-0.99)	1 (0.81-1)
Basal inhibin-B (pg/ml) +24 hours post leuprolide Testosterone (ng/dl)	Inhibin-B- 105 24 hrs testo-29.67	0.67 (0.24-0.94)	0.95 (0.76-0.99)	0.8 (0.29-0.88)	0.91 (0.71-0.985)
Basal inhibin-B (pg/ml) +72 hours post hCG Testosterone (ng/dl)	Inhibin-B- 105 72 hrs testo-110	1 (0.51-1)	0.95 (0.76-0.99)	0.85 (.42-0.99)	1 (0.81-1)
Basal LH (IU/L) +3 hours post leuprolide LH (IU/L)	LH-0.565 3 hrs LH-6.16	1 (0.51-1)	1 (0.82-1)	1 (0.51-1)	1 (0.82-1)
3 hours post leuprolide LH (IU/L) +72 hours post hCG Testosterone (ng/dl)	3 hrs LH-6.16 72 hrs testo-110	1 (0.51-1)	0.96 (0.76-0.99)	0.90 (.62-0.99)	1 (0.81-1)

**Figure 3:** (a,b,c,d) Basal Inhibin B, Basal LH, 3 HRS POST Leuprolide LH, 72 HRS POST hCG Testosterone cut offs in CDGP & IHH groups

protocol of hCG stimulation tests, found that peak testosterone concentrations on day 4 of 3.6 nmol/litre (104 ng/dl) offered the best sensitivity and specificity for the diagnosis of IHH,^[9] which was similar to our results.

The combination of basal LH (cut-off <0.565 IU/L) coupled with basal Inhibin-B (cut-off- <105 pg/ml) and

basal LH (cut-off < 0.565 IU/L) coupled with stimulated LH (cut-off- <6.16 IU/L) have outstanding performance when the combined performance of various basal and stimulated hormones were tested. In our study, we found that basal LH and the basal inhibin-B level below the described cut-off have more discriminating power than 3 hours post leuprolide

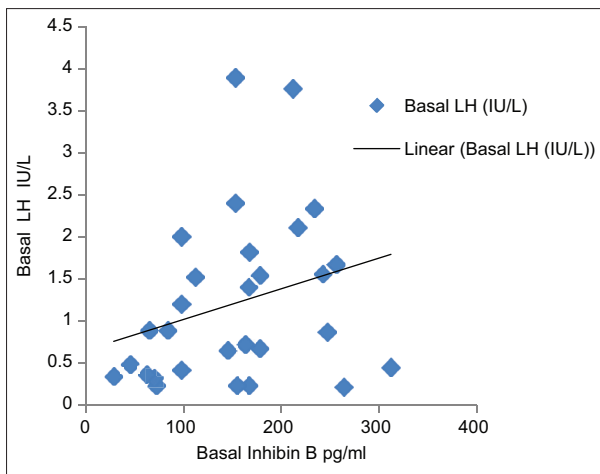


Figure 4: Scatter plot showing positive linear correlation between Basal LH and Basal Inhibin B

LH which is usually considered a gold standard test in differentiating two conditions. For the first time, we have demonstrated the superior efficacy of using a combination of basal LH and basal inhibin-B below the described cut-off in predicting IHH as compared to 3 hours post leuprolide LH, which can be performed as an outdoor diagnostic procedure to discriminate between IHH and CDGP while evaluating for delayed puberty. Other combinations did not add further to the diagnostic power.

Our study has certain limitations which deserve particular mentions. First is the shorter duration of follow up period; in view of the limitation of the study period, we have followed up the cohort till mid puberty, though minimum follow up, up to the age of 18 years is considered the gold standard. The study included a relatively small number of participants. A few cases of partial IHH may have been missed where the testicular volume is high, which might be included in CDGP group. The presence of anosmia was not tested by a standardized protocol which may have missed subtle hyposmic cases. In spite of all the major strength of the study lies in its prospective nature with close follow up schedules.

CONCLUSION

Differentiating IHH from GDGP is a challenging task. The novel finding of our study is that the combination of basal LH (cut-off <0.565 IU/L) and basal inhibin-B (cut-off <105 pg/ml) has a sensitivity and specificity of 100% to diagnose IHH. Apart from that, the combination of basal LH (cut-off <0.565 IU/L) and 3 hours post leuprolide LH (cut-off <6.16 IU/L) has similar sensitivity and specificity. Other combinations have slightly less diagnostic yields. These findings underscore the importance of combining different tests to increase diagnostic accuracy. Therefore we suggest that a combination of basal LH and basal inhibin-B may be considered as a useful screening tool to differentiate IHH from CDGP rather than the cumbersome and invasive stimulation tests. However, large-scale studies are required to validate these

findings to be used as simple and cost-effective outdoor procedures.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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