

Clinical significance of serum AMH and INHB in girls with precocious puberty

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Research Article

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Abstract

Background: Precocious puberty is the second sexual characteristic of girls before 8 years old. The diagnosis of central precocious puberty (CPP) needs to evaluate ovarian function, and ultrasound examination is an auxiliary means for the evaluation of ovary at present. The serum levels of AMH and INHB might be markers of evaluation of ovary according the former research. We investigated the clinical features, serum sex hormones, serum levels of AMH and INHB in 184 girls with precocious puberty, which provided deeper insight into the clinical significance of AMH and INHB in female precocious puberty.

Methods: We evaluated 184 girls with precocious puberty at the Department of Endocrinology of Shanghai Children's Hospital from 2017 to 2021, which was consisted of PT, Tanner stage 2 CPP and Tanner stage 3 CPP. We analysed clinical data from the patients including clinical manifestations, AMH, INHB and other hormone levels, and we analysed AMH and INHB in normal control group either.

Results: The PT group ($P = 0.031$) and Tanner stage 2 CPP group ($P = 0.006$) exhibited significantly higher AMH level than that in normal control group. AMH level showed no significant difference among PT group, Tanner stage 2 CPP group and Tanner stage 3 CPP group. The Tanner stage 2 CPP group exhibited significantly higher level of INHB than that in Tanner stage 3 CPP group ($P = 0.013$) and normal control group ($P = 0.007$). AMH and INHB were positively correlated in the four groups, especially in the PT group ($r = 0.694^{**}$, $P < 0.01$). AMH and basal LH were positively correlated in PT group ($r = 0.296^*$, $P < 0.01$). AMH or INHB showed no correlation with chronological age, bone age, uterine volume, ovarian volume, BMI, E2, SHBG, peak LH, basal FSH, peak FSH, IGF-1 or IGF-BP3. The ROC analysis showed that the AUC of AMH or INHB was relatively low.

Conclusions: In this research, the serum AMH and INHB in 184 girls with precocious puberty were analysed. The PT group and Tanner stage 2 CPP group exhibited significantly higher AMH level than that in normal control group. The Tanner stage 2 CPP group exhibited significantly higher level of INHB than that in Tanner stage 3 CPP group and normal control group. AMH and INHB were positively correlated in the four groups, especially in the PT group. ROC analysis showed that the diagnostic performance of PT or CPP using AMH or INHB was weak.

Background

Precocious puberty is the second sexual characteristic of girls before 8 years old. Central precocious puberty (CPP) refers to the premature activation of hypothalamus pituitary gonad axis, which leads to accelerated bone maturation and short stature of adults [1] [2]. Girls With precocious puberty need evaluation of ovary function in order to determine treatment strategy. In female ovaries, AMH (anti-Mullerian hormone) is produced by growing follicles, and is not affected by day and night node rate change [3], menstrual cycle [4], body mass index [5], blood sample collection time [6]. Therefore, AMH may be suitable as a marker to evaluate ovarian function. Inhibin B (INHB) and AMH are glycoproteins in the transforming growth factor – β family, both of which are produced by ovarian granulosa cells.

Studies have shown that in girls with CPP, the measurement of INHB level is consistent with the severity of clinical manifestation [7]. Therefore, the changes of serum AMH and INHB levels may be used as markers to determine the rate of puberty progression in adolescents. At present, although there are many researches on precocious puberty, there are few researches on AMH and INHB levels of precocious puberty in girls. The role of AMH and INHB in the development of CPP and PT has not been completely evaluated. Our study focused on the relationship of AMH and INHB with clinical characteristics and serum sex hormones in girls with precocious puberty.

Subjects And Methods

Subjects

Methods: 184 cases of precocious puberty in Endocrinology Department of Shanghai Children's Hospital from 2017 to 2021 were selected as the research objects. The clinical characteristics and sex hormone levels of the patients were collected. Informed consent was signed by all patients and their families. According to the clinical manifestations, precocious puberty refers to the development of secondary sexual characteristics in girls before the age of 8 years old. According to GnRH stimulation, precocious puberty is divided into central precocious puberty and simple breast development. There are 65 patients with central precocious puberty in Tanner stage 2, 73 patients with central precocious puberty in Tanner stage 3 and 46 patients with simple breast development. In addition, AMH and INHB were detected in 71 prepubertal girls in Tanner stage 1.

Clinical Manifestations, AMH, INHB And Other Hormones

Tanner stage, bone age, pelvic ultrasound and pituitary MRI excluded CPP secondary to tumour. All patients underwent gonadotropin releasing hormone (GnRH) stimulation to assess hypothalamic-pituitary-gonadal axis function. Sex hormones including anti-Mullerian hormone (AMH), inhibin B (INHB), oestradiol, sex hormone binding globulin (SHBG), basal luteinizing hormone (LH), peak LH, basal follicle-stimulating hormone (FSH) and peak FSH were detected. Serum LH and FSH concentrations were tested with LH and FSH detection kits (Beckman Coulter) and measured with an automatic immunoluminescence analyser (UnicelDxl 800). Serum AMH and INHB were detected in solid-phase sandwich enzyme-linked immunosorbent assays (ELISAs) purchased from Guangzhou Kangrun Biotechnology Co., Ltd. The detection limit of AMH was 0.06ng/ml. The intraassay and interassay coefficients of variation were 5.97% and 7.38%, respectively. The detection limit of INHB was 10pg/ml. The intraassay and interassay coefficients of variation were 4.65% and 6.08%, respectively.

Statistical analysis

SPSS 26.0 software (manufactured by International Business Machines Corporation) was used to analyse these data. The nonparametric data were analysed with the Mann–Whitney U test and are presented as median values. The normally distributed data were analysed with the t-test and are presented as the mean \pm S.D. Spearman correlation analysis was used for correlation analysis. ROC curve was used to evaluate the properties of AMH and INHB in differentiating precocious puberty. A *P*-value <

0.05 was considered to indicate a significant difference, which was indicated as follows; * $P < 0.05$, ** $P < 0.01$.

Results

Clinical and hormonal characteristics

The data analysis showed that the bone age of patients with precocious puberty was significantly progress than the chronical age. The bone age of patients with Tanner stage 2 and Tanner stage 3 CPP were 9.5 years and 10 years, respectively, which were 1.2 years and 1.3 years larger than the chronical age, and showed significant difference ($P < 0.01$). The bone age of Tanner stage 3 CPP was the largest. The ovarian volume ($P < 0.05$) and uterine volume ($P < 0.01$) in CPP group were larger than those in PT group. The GnRH stimulation was consistent with the sexual maturity of the patients, but the oestradiol showed no significant difference in three groups. Both IGF-1 and IGF-BP3 were the highest in Tanner stage 3 CPP group and the lowest in PT group. The PT group ($P = 0.031$) and Tanner stage 2 CPP group ($P = 0.006$) exhibited significantly higher AMH level than that in normal control group. AMH level showed no significant difference among PT group, Tanner stage 2 CPP group and Tanner stage 3 CPP group. The Tanner stage 2 CPP group exhibited significantly higher level of INHB than that in Tanner stage 3 CPP group ($P = 0.013$) and normal control group ($P = 0.007$).

Table 1
Clinical and hormonal characteristics of the patients

	Tanner stage 2 CPP	Tanner stage 3 CPP	PT	Controls
Age(year)	8.3 (6.1–9.6)	8.7 (5.7–9.9) ^{bb}	8.2 (4.8–9.6)	NA
Bone age(year)	9.5 (7–11) ^{cc}	10 (6.5–12) ^{bb}	9.5 (7-11.5)	NA
BMI (kg/m ²)	16.32(12.81–24.84) ^{bb, cc}	17.16 (10.98–24.88)	17.59 (14.31–21.83)	NA
Ovarian volume (mL)	2.62 (1.01–4.39) ^b	2.62 (1.12–5.28) ^b	2.36 (0.44–4.12)	NA
Uterine volume(mL)	2.85 (1.29–9.94) ^{bb}	3.39 (1.05–13.36) ^{bb}	2.26 (0.63–6.16)	NA
Oestradiol (pmol/L)	88 (73–418)	96 (73–445)	104 (73–313)	NA
Peak LH(IU/L)	13.5(6.01–62.3) ^{bb, cc}	22.9(5.5-99.11) ^{bb}	3.83(1.37–4.95)	NA
Basal LH(IU/L)	0.65(0.1–4.59) ^{bb}	1.04(0.13–6.63) ^{bb}	0.33(0.2–1.89)	NA
Peak FSH (IU/L)	12.83 (2.84–40.17) ^{bb}	13.32 (6.44–36.41) ^{bb}	8.78 (2.42–22.38)	NA
Basal FSH (IU/L)	4.46 ± 2.53	5.02 ± 2.40 ^b	3.79 ± 1.65	NA
SHBG(nmol/L)	74.11 ± 23.92 ^{b, cc}	57.53 ± 21.44	63.75 ± 21.3	NA
IGF-1 (ng/mL)	304(192–539) ^{bb, cc}	373(123–672) ^{bb}	258.5(111–498)	NA
IGF-BP3 (ug/mL)	5.59 (3.81–8.28) ^{bb}	5.6 (2.61–7.87) ^{bb}	5.03 (3.12–7.19)	NA
AMH(ng/mL)	3.76 (2.31–6.2) ^{aa}	3.44 (1.93–6.78)	3.45 (2.09–6.03) ^a	3.31 (0.29–8.65)
INHB(pg/mL)	74.06 (15.26–263.8) ^{aa, c}	49.13 (13.3-249.6)	46.23 (15.66–233.1)	37.41 (8.91–319.7)
^a P<0.05, ^{aa} P<0.01: significantly different compared with subjects in control group.				
^b P<0.05, ^{bb} P<0.01:significantly different compared with subjects in PT group.				
^c P<0.05, ^{cc} P<0.01:significant difference between Tanner stage 2 and Tanner stage 3 CPP groups.				

Correlation Analysis

AMH and INHB were positively correlated in the four groups, especially in the PT group ($r = 0.694^{**}$, $P < 0.01$). AMH and basal LH were positively correlated in PT group ($r = 0.296^*$, $P < 0.01$). AMH or INHB showed no correlation with chronological age, bone age, uterine volume, ovarian volume, BMI, E2, SHBG, peak LH, basal FSH, peak FSH, IGF-1 or IGF-BP3.

ROC Curve Analysis

The performance of AMH and INHB were studied by ROC analysis. The AUC of AMH in distinguishing PT from normal control group was 0.68 (SE, 0.0468), and that in distinguishing CPP Tanner stage 2 from normal control group was 0.64 (SE, 0.048). The AUC of INHB in distinguishing CPP Tanner stage 2 from normal control group was 0.63 (SE, 0.048), and that in distinguishing CPP Tanner stage 2 from CPP Tanner stage 3 was 0.62 (SE, 0.047).

Discussion

At present, GnRH stimulation is the gold standard for the diagnosis of CPP, and bone age and ultrasound examination of uterus and ovary are auxiliary means. INHB and AMH are glycoproteins of transforming growth factor – β family, both of which are produced by ovarian granulosa cells. AMH gene is located in the short arm of chromosome 19 (19p13.3) and only expressed in gonad. However, AMH is not considered to be a gonadotropin and produced by preantral follicles in granulosa cells. The cells around the follicles are recruited from the primordial follicle pool, but some of them are not selected as dominant antral follicles [8]. In women, it takes 36 weeks of gestation for fetal ovarian granulosa cells to begin to produce AMH. AMH inhibits the transformation of primordial follicles to primary follicles and maintains primordial follicles at rest [9]. AMH is not expressed in primordial follicles, but can be slightly expressed in granulosa cells of primary follicles. The expression of AMH is the highest in preantral follicles, primary follicles and granulosa cells of antral follicles less than 4 mm in diameter, while the expression of larger antral follicles gradually decreases. About 60% of AMH in human serum is produced and secreted by granulosa cells of follicles with a diameter of 5-8mm [10]. The expression of AMH is limited to primary follicles and preantral follicles, but it is not produced at the higher stage of follicular development [11]. AMH not only inhibits the initial regeneration of follicles, but also inhibits FSH dependent follicular growth [12] [13]. AMH in female ovaries has the functions of regulating initial recruitment and circulating recruitment, inhibiting excessive consumption of primordial follicle pool and formation of dominant follicles [14].

Mini puberty begins in the first few months of a girl's life. The level of AMH in infants increases significantly from birth to 3 months, suggesting that the hypothalamus pituitary ovary axis is temporarily activated [15]. The increase of AMH level in puberty may be the response of ovary to FSH induced follicles. In our study, the elevated AMH levels in patients with PT and Tanner stage 2 CPP may be an

ovarian response to prevent FSH induced follicle growth and premature activation. Former studies with small samples have found that there is a weak negative correlation between the levels of AMH and FSH in normal girls before puberty [16] [17], but no correlation was found in patients with precocious puberty in our study. There was no correlation between AMH and basal LH in healthy women aged 18–24 [18]. AMH and basal LH showed no correlation with each other in CPP group and positively correlated in PT group in our study. Another explanation for AMH variation in healthy female infants may be related to birth weight [19], and there is a negative correlation between BMI and AMH level in adult obese women [20]. There are few studies on the relationship between AMH, INHB and BMI in precocious puberty children. There is no correlation between BMI and AMH or between BMI and INHB in our study, which might be due to the relatively normal weight of patients. The serum AMH levels of healthy girls vary greatly among individuals [21]. Hagen et al. have shown that AMH levels increase by 17% in the first three years before puberty, after the onset of puberty, AMH levels decreased by 30% in the first two years [17]. Previous study showed that compared with the control group before puberty, the level of AMH in the PT group was significantly higher, and the level of AMH in the CPP group was similar to that in the PT group, but the sample was very small, and no statistical difference was found [22]. Part of the former researches selected CPP girls with Tanner stage 2 [23], or CPP patients were composed of Tanner stage 2–4 mixture [24]. Those researches could not explain the impact of Tanner stage on the AMH level of CPP patients. Our study had a larger sample and the Tanner stage of CPP patients was well classified. We demonstrated that the AMH level of PT group was significantly higher than that of prepubertal control group, and the AMH level of CPP group was also higher than that of control group, which was mainly due to Tanner stage 2 CPP patients rather than Tanner stage 3 CPP patients. Because the ovary of girls with Tanner stage 3 CPP was in the transition stage from early puberty to late puberty, which reduces the serum AMH level. The AMH level of patients with Tanner stage 3 CPP in our study is similar to that of fast progressive CPP patients with Tanner stage 2 in former studies, because the AMH level of patients with Tanner stage 2 fast progressive CPP is lower than that of patients with slow progressive CPP. The level of AMH decreased with the maturation of hypothalamic pituitary gonadal axis, which may be a gradual process of precocious puberty. The AMH of patients with Tanner stage 3 CPP was significantly lower than that of patients with Tanner stage 2 CPP, indicating that with the further maturity of puberty, the AMH increased from early adolescence, and the peak level of AMH might be in Tanner stage 2, then declined to a relatively stable state. The latest research also showed that AMH in CPP patients was the lowest in Tanner stage 3 [25], which was consistent with our research, and the change of AMH concentration was similar to a mathematical parabola.

INHB secretes from early antral follicles and dominant follicles by granules cells. The increase of serum INHB level in adolescence seems to be induced by FSH in early adolescence due to active follicular development. During puberty, the level of INHB increased from Tanner stage 1 to Tanner stage 3, increased sharply in Tanner stage 2, reached the peak level in the Tanner stage 3, and then decreased in the Tanner stage 4 of puberty [26]. In our study, INHB was at the highest level in Tanner stage 2 CPP, and decreased significantly in Tanner stage 3 CPP, but it was still elevated compared with PT group and normal group. This suggested that the Tanner stage of peak level of INHB in CPP patients was earlier

than that in girls with normal puberty. Previous studies chose CPP girls in Tanner stage 2, without CPP patients in Tanner stage 3 [23] [27], which easily ignored the influence of CPP in different Tanner stages on INHB level. INHB level might be particularly useful in early puberty. Due to elevated gonadotropin levels, INHB secretion appeared earlier and might be the first step in puberty before growth spurts and bone age. The INHB level of Tanner stage 2 CPP (74.06 pg/mL) was significantly higher than that of Tanner stage 3 CPP (49.13 pg/mL) and normal control group (37.41 pg/mL). Although there was a rise in the precocious girls, INHB is not suitable to distinguish PT group from CPP group, especially between PT group and Tanner stage 3 CPP group. Previous research also showed that INHB was inferior to basal LH in predicting the peak level of LH [28]. Oestradiol and INHB are secreted by antral follicles stimulated by FSH, and the secretion of FSH is inhibited by the negative feedback of gonadal axis. Therefore, the consumption of follicular pool leads to the increase of FSH, the decrease of oestradiol and the decrease of INHB. These three indicators are interdependent rather than independent, and the changes occur relatively late. This suggested that the secretion of INHB was related to the activation of hypothalamus pituitary ovary axis, which might help to determine the early onset of CPP and observe the therapeutic effect of GnRHa. Previous studies showed that INHB level decreased significantly after GnRHa treatment which also reduced AMH level, but returned to the level before treatment after stopping treatment, while changes in AMH levels were not associated with changes in gonadotropin during or after treatment [29] [30]. AMH and INHB in patients with precocious puberty were positively correlated in our study, which was consistent with previous study [31]. But AMH or INHB showed no correlation with other indicators of ovarian function, including uterine volume, ovarian volume, E2 and FSH. The clinical significance of AMH and INHB in precocious puberty needs further verification.

Conclusions

Precocious puberty needs to evaluate ovarian function. In this research, the serum AMH and INHB in 184 girls with precocious puberty were analysed. We demonstrated that the PT group and Tanner stage 2 CPP group exhibited significantly higher AMH level than that in normal control group. The Tanner stage 2 CPP group exhibited significantly higher level of INHB than that in Tanner stage 3 CPP group and normal control group. AMH and INHB were positively correlated in the four groups, especially in the PT group. ROC analysis showed that the diagnostic performance of PT or CPP using AMH or INHB was weak. These findings provide deeper insight into precocious puberty diagnosis and will contribute to its clinical assessment.

Abbreviations

AMH: Anti-Mullerian hormone

INHB: Inhibin B

SHBG: Sex hormone-binding globulin

LH: Luteinizing hormone

FSH: Follicle-stimulating hormone

GnRH: Gonadotropin-releasing hormone

Declarations

Ethics approval and consent to participate

The research was performed in accordance with the principles of the Declaration of Helsinki. The study was approved by the ethics committee of Shanghai Children's Hospital Affiliated with Shanghai Jiao Tong University (2020R153-E01), Shanghai, China). The patients consented to participate in this study.

Consent for publication

The authors and participants agree to publication.

Availability of data and materials

All data generated or analysed during this study are included in this published article. The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competitive interests.

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Authors' contributions

Liu Q designed the experiments and analysed the data. Li P contributed reagents/materials/analysis tools. Yin X helped with the data analysis and presentation. All authors read and approved the final manuscript.

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Figures

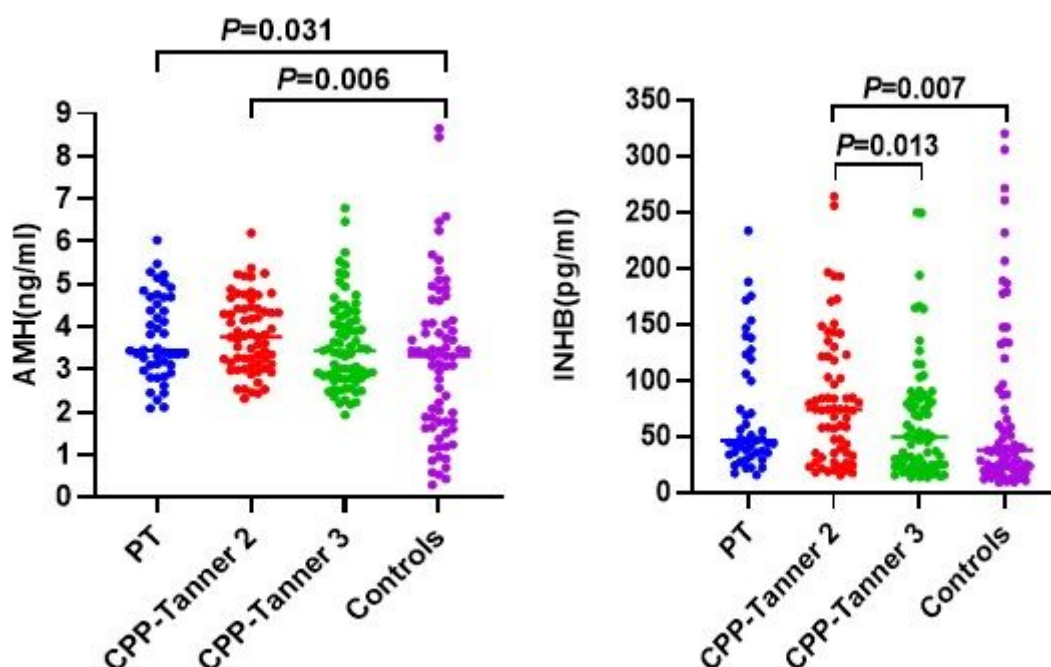


Figure 1

Differences of AMH and INHB in girls with different Tanner stage.

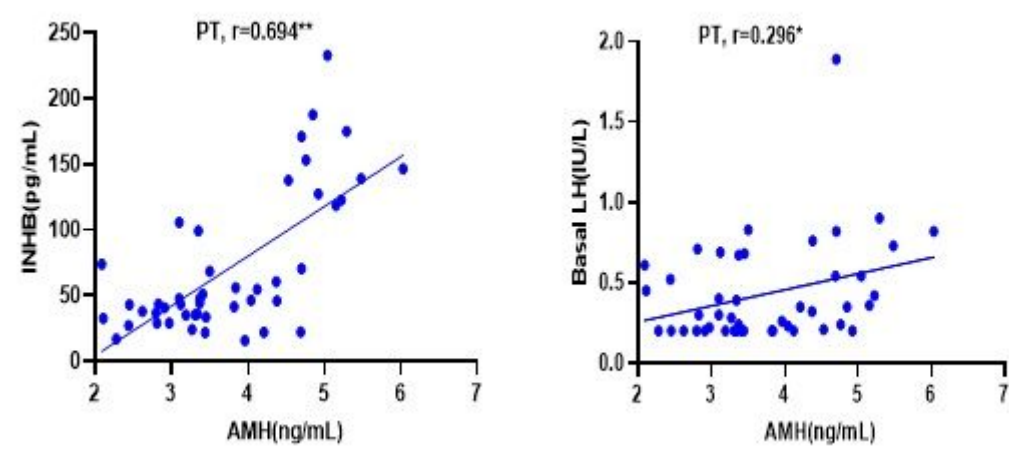


Figure 2

Correlation analysis of AMH and INHB in girls with precocious puberty.

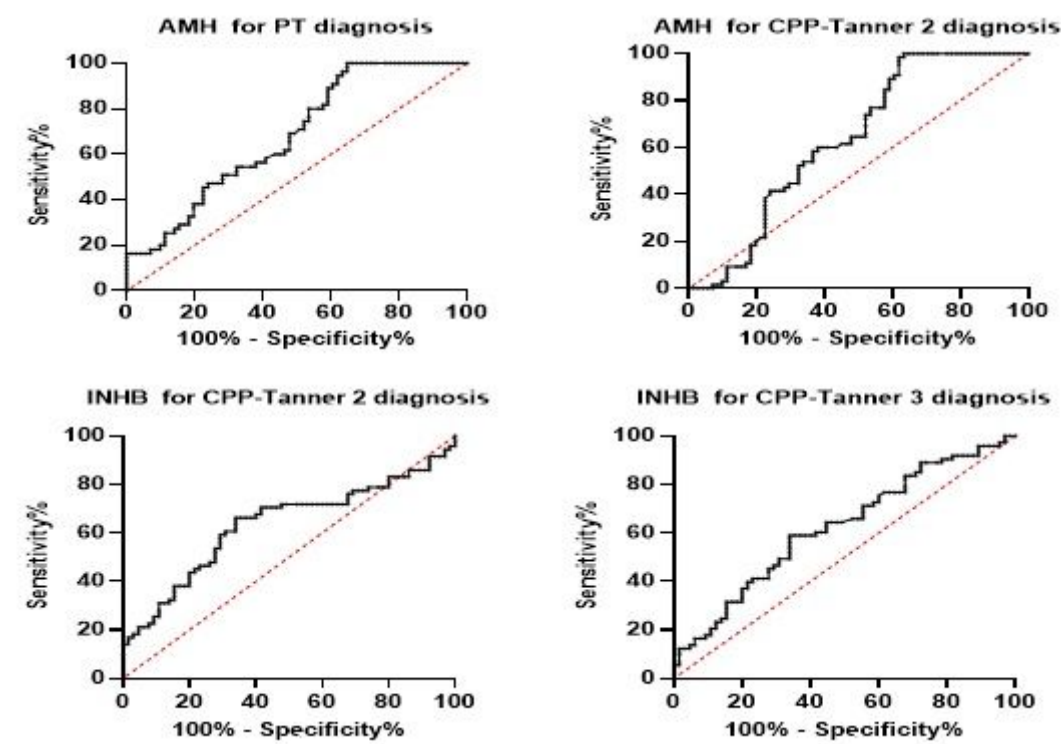


Figure 3

ROC curve analysis of AMH and INHB in girls with precocious puberty.