Accuracy of Endocrine Tests for Detecting Hypogonadotropic Hypogonadism in Girls

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Objective To assess the accuracy of inhibin B and the gonadotropin releasing hormone agonist test for the diagnosis of hypogonadotropic hypogonadism (HH).

Study design We performed a retrospective analysis of data collected 2009-2014 using a strict clinical protocol. All prepubertal nonunderweight girls, aged 13-17.5 years with Tanner breast stage B1/B2 and low estradiol levels, were tested and re-examined at 6-month intervals (n = 21). Constitutional delay of growth and puberty was defined by spontaneous menarche; HH was identified by association with specific causes of HH or no spontaneous progress of puberty during follow-up. Inhibin B was measured using enzyme-linked immunosorbent assay, and follicle-stimulating hormone and luteinizing hormone (basal and stimulated by triptorelin) were measured using a chemiluminescence immunoassay.

Results The cohort comprised 12 girls with constitutional delay of growth and puberty and 9 girls with HH. The causes of HH included hypopituitarism (n = 3), Prader-Willi syndrome, chromosomal aberration, intellectual disability syndrome with ataxia, and idiopathic causes (n = 2). Each measurement, basal inhibin B <20 pg/mL or stimulated follicle-stimulating hormone (4 hours) <11 IU/L, demonstrated a sensitivity and a specificity of 100% for the detection of HH. Stimulated luteinizing hormone (4 hours) <9 IU/L showed 100% sensitivity but only 83% specificity.

Conclusions Inhibin B seems to be the ideal measurement for detecting HH in girls. The gonadotropin releasing hormone agonist test is an alternative diagnostic modality, although this approach is more invasive and laborious. *(J Pediatr 2015;167:674-8)*.

Puberty normally begins in girls before the age of 13 years.[1] Girls older than 13 years without breast development exhibit either constitutional delay of growth and puberty (CDGP) or hypogonadism. Primary hypogonadism is easily diagnosed by elevated basal serum gonadotropin levels. In contrast, the basal gonadotropin levels in hypogonadotropic hypogonadism (HH) are frequently indistinguishable from those in girls with CDGP. Hereditary HH is caused by midline brain malformations, pituitary gland malformations, Kallmann syndrome, and monogenic hypopituitarism.[2] The acquired causes of HH include craniopharyngioma, germinoma, Langerhans cell histiocytosis, cranial irradiation, and severe head injury.[3] Functional HH occurs frequently in girls with anorexia and systemic illnesses and represents a transitory delay of the maturation of the hypothalamic-pituitary-gonadal axis.[4]

For effective counseling and therapy, it is necessary to make a distinction between CDGP and HH; however, this clinical differentiation is occasionally difficult, especially in the absence of hypopituitarism or anosmia. The availability of an accurate and feasible endocrine test for HH would prevent the wait-and-see attitude maintained by many physicians who delay diagnosing girls with HH. In contrast to the significant amount of diagnostic studies on delayed puberty in boys, there is a paucity of studies on girls with delayed puberty.[5] Research on this topic may be challenged by the rarity of CDGP in girls, as this prevalence is approximately 5-fold lower than that in boys.[4] Additional problems encountered during the assessment of girls at the onset of puberty include technical difficulties in obtaining reliable and valid information on the size of the ovaries[6] and inaccuracy and imprecision of the present immunoassays at low estradiol levels.[7]

The only specific study on this topic was reported by Odink et al, who performed frequent 24-hour samplings of gonadotropins in 13 girls, including 11 girls with HH.[8] These authors found higher and more frequent luteinizing hormone (LH) pulses in 2 girls with CDGP than in 11 girls with HH. Because 24-hour blood samplings are invasive and laborious, alternative approaches for routine endocrine diagnostic tests are needed. The gonadotropin releasing hormone agonist (GnRHa) test is well established in the diagnostics of delayed puberty in boys.[5] Measurements of basal inhibin B also represent a meaningful alternative for diagnosing male HH based on recent publications.[9,10] Although pilot studies were performed to study the principal functionality of the GnRHa test in girls with diverse pubertal disorders, the

BMI	Body mass index
CDGP	Constitutional delay of growth and puberty
FSH	Follicle-stimulating hormone
GnRHa	Gonadotropin releasing hormone agonist
HH	Hypogonadotropic hypogonadism
LH	Luteinizing hormone

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practical clinical problem of distinguishing CDGP from HH was not addressed.[11,12] Recently, normative data were reported regarding the response to GnRHa in healthy girls of different levels of pubertal maturity.[13] In this retrospective observational study, the accuracy of inhibin B and of the gonadotropins after GnRHa challenge for the diagnosis of female delayed puberty was analyzed.

Methods

Between 2009 and 2014, girls with delayed puberty were intensively evaluated at the Department of Pediatric Endocrinology of the University Children's Hospital in Tuebingen, Germany. This retrospective study included all patients who were assessed during this time period and who had met the inclusion criteria. The study fulfilled the requirements defined by the Ethical Committee of the Medical Faculty of the University of Tuebingen for retrospective studies. Data collection and analysis were performed after the patients were clinically evaluated according to a strict clinical protocol for girls with delayed puberty (Figure 1). When a disorder frequently associated with HH was observed, the pediatric endocrinologist made the diagnosis of HH before the 30-month follow-up period.

All females, who ranged in age between 13 and 17.5 years and demonstrated Tanner breast stage B1 or B2 and low estradiol serum levels (≤10 pg/mL), were tested and evaluated every 6 months. Girls with Tanner breast stage B2 were included only when the breast development had remained static for a longer time period (>6 months), and the uterine volume was found to be infantile. In total, 38 girls were tested. However, 17 girls had to be excluded from this analysis for different reasons: advanced puberty with breast stage 3 or more (n = 11), age <13 years (n = 2), Turner syndrome (n = 2), Noonan syndrome (n = 1), and underweight (body mass index [BMI] SDS_{LMS} <P3) (n = 1). In the remaining cohort, there were no girls with eating disorders, severe chronic organic diseases, elevated gonadotropin levels, or competitive exercise activities >10 hours per week.

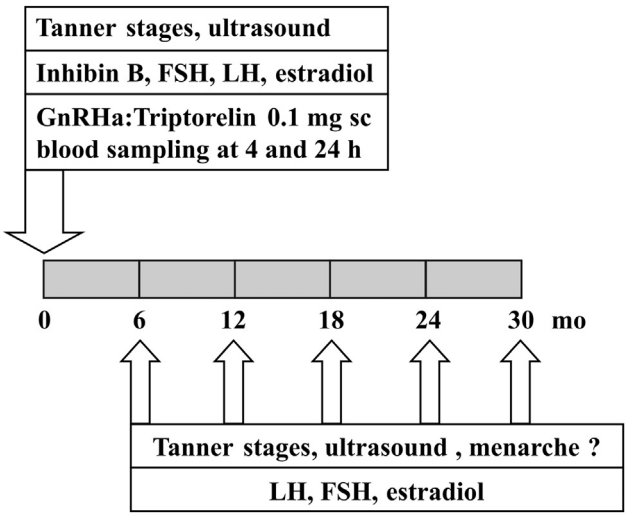

Figure 1. Schematic diagram of the routine diagnostics used in girls with delayed puberty.

The initial clinical examination performed by a pediatric endocrinologist included auxology (height, sitting height, arm span, and weight), pubertal staging according to Marshall and Tanner,[14] and an intensive clinical examination. Syndromic features, clinical signs of hypopituitarism, and other disorders associated with hypogonadism were documented. Olfactory function was quantified using Sniffin' Sticks (Burghart Messtechnik GmbH, Wedel, Germany) in girls suspected to have HH.[15] Left hand radiograms were performed to determine bone age. The rating was conducted by 3 experienced pediatric endocrinologists using the Greulich and Pyle radiographic atlas.[16] Uterine and ovarian volumes were measured using transabdominal ultrasound with the GE Healthcare ultrasound LOGIQ E9 and a curved array 2.8- to 5-MHz transducer (GE Healthcare, Munich, Germany) as previously described.[6] The initial blood analysis included measurements of inhibin B, follicle-stimulating hormone (FSH), LH, prolactin, and estradiol. Subcutaneous injection of 100 μg triptorelin acetate (Decapeptyl IVF 0.1 mg/mL; Ferring Arzneimittel, Kiel, Germany) was followed by blood samplings after 4 and 24 hours for determination of the FSH, LH, and estradiol concentrations. The test commenced between 10 a.m. and 3 p.m.

Diagnosis of CDGP or HH

CDGP was diagnosed according to the presence of spontaneous menarche during the follow-up period. HH was assumed in the presence of septo-optic dysplasia, hypopituitarism, Prader-Willi syndrome, hyposmia, or after 30 months in the absence of spontaneous progress of puberty, which was defined as no significant increase of basal gonadotropins and no increase of the ovarian volume after a short break of estradiol substitution during follow-up.

The induction of puberty by the administration of 0.25 mg estradiol valerate orally was offered to all girls with an LH peak <8 IU/L[17] and to those who reported to suffer from delayed puberty and who had asked for treatment. One-third of the CDGP cohort received 0.25 mg estradiol valerate daily for 3 months; these patients repeated the medication schedule once after a 3-month break. All 9 girls with HH were continuously treated with estradiol valerate at a dose that was increased stepwise at 6-month intervals.

Magnetic resonance imaging scans of the brain were performed in all of the girls with HH to exclude acquired defects of the hypothalamic-pituitary axis or to detect congenital pituitary gland malformations. The only exception was the girl with Prader-Willi syndrome.

Hormone Assays

Inhibin B was measured using the inhibin B Gen II enzyme-linked immunosorbent assay (Beckman Coulter Inc, Brea, California). This enzyme-linked immunosorbent assay is a