

EVALUATION OF THE COMBINATION OF GnRH AND HCG TESTS IN DIFFERENTIATING CONSTITUTIONAL DELAY OF GROWTH AND PUBERTY FROM HYPOGONADOTROPIC HYPOGONADISM IN MALES

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Delayed puberty in males is defined by the absence of signs of puberty by the age of 14 years. Pubertal delay has either a central origin, which can be permanent (hypogonadotropic hypogonadism, HH) or temporary (constitutional delay of growth and puberty, CDGP), or has a peripheral origin, hypergonadotropic hypogonadism.¹

CDGP is a benign condition affecting 3% of normal boys at age 14 years² and accounting for 95% of cases of delayed puberty in males.³ About 0.1% of boys remain prepubertal by the age of 18 years⁴ and, despite major advances in understanding normal and disordered puberty,⁵ the differentiation of CDGP from hypothalamic or pituitary HH before the age of 18 remains difficult. The presence of inherited somatic abnormalities, such as anosmia, midline deformities, unilateral renal agenesis or micropenis, favor a diagnosis of HH, but it is not common to have these associated features in clinical situations.

Several tests have been studied to distinguish these entities: acute i.v. GnRH (LHRH)^{6,7} and short-term intramuscular human chorionic gonadotropin administration (HCG),⁸ or a combination of the two;⁹ GnRH administered as an infusion,¹⁰ or in a pulsatile fashion,^{11,12} morning plasma testosterone levels;¹³ and prolactin response to thyrotropin-releasing hormone¹⁴ or metoclopramide.¹⁵ Unfortunately, most of these tests have failed to differentiate the two groups. Frequent nocturnal sampling to detect the spontaneous sleep-related LH secretion does yield good discrimination between the two conditions but is cumbersome and expensive. Urinary gonadotropin excretion may be helpful but this method may require years of observation before the difference becomes clear.¹⁶

The use of GnRH agonists has been explored.^{17,18} A single dose of a GnRH agonist (nafarelin or triptorelin) has been shown to distinguish CDGP from HH during the teenage years.^{19,20} Another promising method is the measurement of free alpha subunit in the circulation after i.v. GnRH.²¹ The method used at the London Centre for Paediatric Endocrinology (LCPE) to distinguish CDGP

Accepted for publication 22 May 2001. Received 23 January 2001. from HH in males with central delayed or arrested puberty is the combination of GnRH test and a three-day HCG test. This combination has been used because it has been suggested that it maximizes sensitivity and specificity.⁹

The aim of this study was to compare investigation results for boys with delayed puberty who had both GnRH and HCG tests with the actual pubertal outcome in the following years in order to evaluate the effectiveness of the method in differentiating the two conditions.

Materials and Methods

The discharge summaries of all patients admitted to the Middlesex Hospital from 1990 to 1995 and the admission books of Great Ormond Street Hospital (GOSH) for Children from 1994-95 were reviewed. (The Endocrine Departments of these two hospitals have operated as the London Centre for Paediatric Endocrinology since 1994.) Forty-seven male patients referred for delayed or arrested puberty or short stature, and who had both GnRH and HCG tests, were identified. Fourteen children were excluded from the analysis: 10 did not attend the follow-up clinic and the case notes or growth cards of 4 patients could not be found.

Pubertal delay was defined as absence of signs of puberty by 14 years of age. Pubertal arrest was defined as failure to progress in puberty for a period more than twelve months in those who had already shown some signs of puberty. Patients excluded from the study were those with an iatrogenic cause for delayed puberty, e.g., brain tumors treated with radio- and/or chemotherapy (n=61) and children with a peripheral cause, hypergonadotropic hypogonadism (n=5).

The GnRH (*Gonadorelin*) was given at a dose of 2.5 mg/kg by i.v. bolus up to a maximum of 100 mg. Blood was collected from an indwelling cannula for basal (pre-stimulation) and serial serum LH and FSH concentrations at 20 and 60 minutes post-stimulation. Results were compared with those of a historical group (unpublished observations at GOSH).

Post-stimulation LH and FSH concentrations below peak levels were considered as abnormal response. LH was assayed by an automated two-step immunometric assay

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(AxSYM, Abbott Laboratories, Maidenhead, UK). The assay was standardized against International Reference

TABLE 1. Clinical characteristics of children in the HH and CDGP groups, at the time of the endocrine investigations.

	CDGP		P-value*	HH	
	Pre-pubertal (Group A) median (range)	Arrested puberty (Group B) median (range)		Pre-pubertal (Group C) median (range)	P-value**
Genital size (cm)	1.0 (1-3)	2 (1-4)	0.03	1.0 (1-2)	0.41
Left testicular volume (mL)	2 (1-3)	4.5 (4-8)	0.0002	1 (0-2)	0.02
Right testicular volume (mL)	2.0 (1-3)	4.0 (0-8)	0.01	1 (0-2)	0.03
Height SD score	-1.5(-4.2-2.6)	-0.99 (-2.6-0.2)	0.32	-1.1 (-2.8-1.4)	0.25
Age (years)	13.5 (10.6-15.2)	15.0 (12.1-17.5)	0.05	14.2 (10.9-16.1)	0.50

*Comparison between groups A&B; **comparison between groups A&C.

TABLE 2. Endocrine parameters for children in the HH and CDGP groups.

	CDGP		P-value*	HH	
	Pre-pubertal (Group A) mean (SEM)	Arrested puberty (Group B) mean (SEM)		Pre-pubertal (Group C) mean (range)	P-value**
Basal LH (IU/L)	0.96±0.0	1.97±0.5	0.02	1.02±0.2	0.64
Basal FSH (IU/L)	2.0±0.3	3.5±0.5	0.01	0.98±0.3	0.03
Basal testosterone (nmol/L)	1.1±0.2	2.6±0.8	0.06	0.84±0.2	0.4
Peak LH (IU/L)	8.5±1.5	22.9±7.3	0.02	2.1±0.6	0.02
Peak FSH (IU/L)	5±0.5	8.4±1.2	0.02	2.0±0.5	0.004
Peak testosterone (nmol/L)	12.8±2.2	17.8±5.8	0.3	6.6±3.5	0.1

*Comparison between groups A&B; **comparison between groups A&C.

Preparation 80/552, and the minimum detection limit of the assay was 0.5 IU/L.

FSH was assayed by an automated two-step immunometric assay (AxSYM, Abbott Laboratories, Maidenhead, UK). The assay was standardized against International Reference Preparation 78/549 and the minimum detection limit of the assay was 0.4 IU/L.

HCG (*Pregnyl*) was given on three consecutive days at a dose of 1000 units/day for children 1-10 years old and 1500 units/day for >10 years old. Serum testosterone concentration was measured pre-stimulation and 24 hours after the third HCG injection. Results were compared to values obtained from a historical group. Testosterone response below 8 nmol/L was taken as poor testicular response. Testosterone was assayed by coated tube radioimmunoassay (Diagnostic Products Corporation, Los Angeles, California, USA). The minimum detection limit of the assay was 0.14 nmol/L.

Growth cards and case notes were reviewed to obtain the height of the patients at presentation and at the end of the observation period. The coefficient of variation for height, measured by a stadiometer, was 0.1% at a height of 100 cm.

The period of observation was the period of time during which the patients were followed up for pubertal development.

Pubertal outcome in the following years was documented by the Tanner staging method. Testicular volume was measured by an orchidometer.

Medication, if any, used to induce puberty and its duration was documented. Patients were diagnosed as HH if they failed to increase their testicular volume to 8 mL or more and were androgen dependent for progression through

and maintenance of other secondary sexual characteristics.

CDGP was diagnosed if puberty was achieved with testicular volumes >8 mL, either spontaneously or with induction therapy.

Statistical Analysis

Statistical analysis was conducted using SPSS 8.0 for Windows. For descriptive statistics the Mann-Whitney U test was used for continuous nonparametric data. For the diagnostic tests, the sensitivity and specificity were calculated by standard methods. Statistical significance was taken as $P < 0.05$.

The study was approved by the Research and Development Office at GOSH and University College London Hospital.

Result

Thirty-three patients with a median age of 14 years (range 10.6 to 17.5) were included in the study. Of these, 25 were prepubertal at presentation and 8 had arrested puberty. By reviewing the final diagnosis, 7 patients (21%) failed to enter or progress in puberty despite a trial of induction therapy (HH), and 26 patients (79%) progressed into puberty (CDGP). Patients were divided into 4 groups according to their pubertal status at presentation: group A, CDGP who were prepubertal (n=20); group B, CDGP with arrested puberty (n=6); group C, HH who were prepubertal (n=5); and group D, HH with arrested puberty (n=2). Three patients with CDGP had growth hormone insufficiency (GHI), one from group A and 2 from group B. GHI was also found in two patients with HH, one from group C and one from group D. Three patients (2 from group A, and one

from group D) received treatment for induction of puberty prior to the endocrine investigations, but this was stopped at

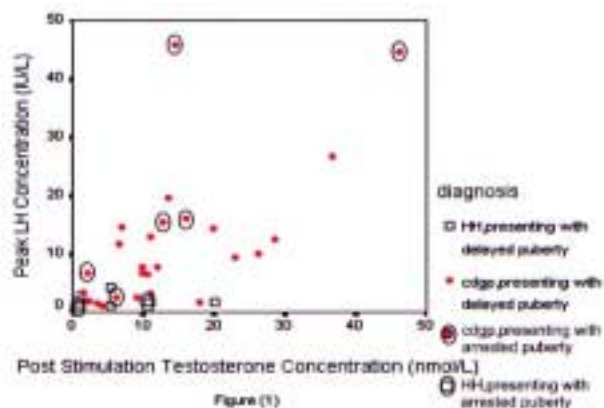


FIGURE 1. Post-HCG testosterone and peak LH concentrations.

least 3 months before the investigations so as not to interfere with the results. Descriptive data at the time of investigation are shown in Table 1. There was no significant difference between prepubertal boys with HH or CDGP at the time of presentation in mean age, height SD score and genital size. Mean testicular volumes were less in the HH, but there was overlap with CDGP.

The follow-up period for the HH group had a median of 3.2 years (range 2.1 to 5.3) and the CDGP group, 2.5 years (range 0.7 to 6.5). All patients with HH received testosterone injections on a regular basis for induction of puberty and had to continue it indefinitely as they failed to achieve a testicular volume >8 mL. Of the CDGP, 5 patients (19%) had no form of induction therapy. Eighteen (69%) had testosterone injections (50-100 mg monthly) for a mean period of 15±13.3 months, 2 (8%) had oxandrolone (2.5 mg daily orally) for a mean period of 7±5.6 months and one (4%) had both: oxandrolone for 6 months followed by testosterone injections for 9 months. On the last follow-up there had been no significant difference between the two groups in the size of the genitalia and height SD score, however, the HH group had significantly smaller testicular volumes, as expected.

Group A had significantly higher peak LH and peak FSH concentrations as compared to group C. Mean basal and peak testosterone concentrations were not significantly different in the two groups. Within the CDGP group, the basal and peak LH and FSH concentrations were significantly higher in group B as compared to group A (Table 2). Although mean peak LH concentration was higher in prepubertal children with CDGP when compared to those with HH, there was significant overlap between values in the two groups, as can be seen in Figure 1. Similarly, there was overlap in the mean peak FSH concentration between the two groups, Figure 2. The GnRH test was abnormal in all the boys with HH, and in 8 out of 26 boys (31%) with CDGP. The HCG test was abnormal in

5 out of 7 boys (71%) with HH and in 8 of those with CDGP (31%), Figure 1.

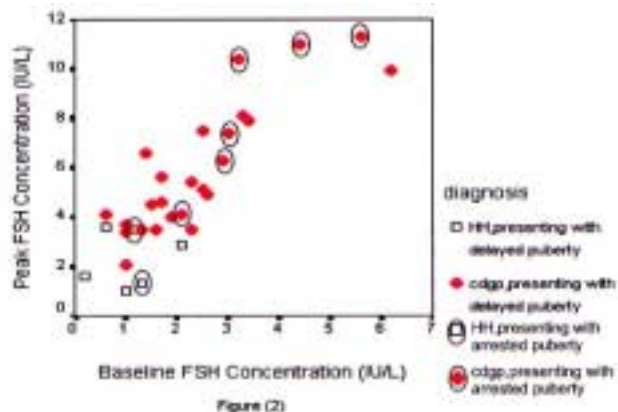


FIGURE 2. Baseline and peak FSH concentrations.

Evaluation of Combination of GnRH & HCG Tests

The GnRH and HCG results were analyzed according to the Sackett et al.²² approach.

1. Both tests are required to be normal to exclude HH.

The first assumption made was the requirement for both tests to be normal to exclude HH. A normal GnRH test was defined as a peak LH value >6 IU/L, while a normal HCG test was defined as a post-HCG testosterone value >8 nmol/L. The sensitivity of this combined test was 100% and specificity was 62%. This means that if both tests are normal the diagnosis of HH is excluded.

2. Both tests are required to be abnormal to diagnose HH.

The second assumption was the requirement for both tests to be abnormal to diagnose HH. This test combination has a sensitivity of 71% and a specificity of 81%. This means that 29% of the males who were ultimately diagnosed as HH were initially missed.

The GnRH test alone had a sensitivity of 100%, and a specificity of 69%.

Discussion

A clear definition of the roles of GnRH and HCG tests for the identification of patients with HH or CDGP has important implications. A clear diagnosis of HH allows detailed counselling to be given to the adolescent and family with respect to the prospects for future fertility. As far as treatment is concerned, in HH one is more likely to induce puberty with testosterone, which needs to be given by a parenteral route, whereas in CDGP, oral therapy with the anabolic agent oxandrolone is the preferred option.²³ Our data show that prepubertal patients with CDGP cannot be distinguished at presentation from those with HH on the basis of their clinical characteristics, such as age, genital size, height and testicular volumes. Previous studies have reported similar findings.^{19,20}

Mean baseline concentrations of LH and testosterone were similar between children with CDGP and HH. In our

series, none of the HH patients had a normal GnRH test. When the test was abnormal, there was overlap between the mean peak LH concentrations of the two groups which prevented these two groups of subjects from being clearly separated, as seen in previously published data.²⁴ The HCG test was abnormal in one-third of the CDGP and two-thirds of the HH patients. Combining the GnRH and HCG tests failed to improve the discriminative power of the former. This appears to be in contrast to the findings of Dunkel et al.,⁹ who found that the combination discriminates better between CDGP and HH. This is probably the result of a different method of analysis. Within the CDGP group, there was a significant difference in the basal and peak LH and FSH concentrations between groups A and B, which confirms previous observations that these values vary with the stage of puberty.⁶

We conclude from our study that in males with delayed puberty, adding the HCG test to the GnRH test does not increase the sensitivity of the latter in detecting cases of hypogonadotropic hypogonadism.

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