CLINICAL STUDY

The human chorionic gonadotropin test is more powerful than the gonadotropin-releasing hormone agonist test to discriminate male isolated hypogonadotropic hypogonadism from constitutional delayed puberty

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Abstract

Objective: The effectiveness of biological investigations aiming at discriminating isolated hypogonadotropic hypogonadism (IHH) from constitutional delayed puberty (CDP) in male patients is still controversial. We revisited the diagnostic power of the basal serum testosterone level, the Triptorelin test and the human chorionic gonadotropin (hCG) test in a cohort of 33 boys with delayed puberty.

Design: Boys were aged 14.2 to 26.2 years at referral. A 5-year-long clinical follow-up after the initial study allowed confirmation of the diagnosis. At the end of the follow-up period, IHH was found in 13 patients while the other 20 had normal spontaneous pubertal development (CDP).

Results: At referral, a basal morning testosterone level >1.7 nmol/l was observed in 55% of patients with CDP exclusively (predictive positive value (PPV) = 100%; predictive negative value (PNV) = 59%). For CDP, the PPV of the LH peak 3 h after Triptorelin was 100% by setting the upper threshold at 14 IU/l and the PNV was 72%. However, no lower threshold could discriminate IHH from CDP in the remaining patients with an LH peak 3 h after Triptorelin <14 IU/l. In CDP patients, the PPV of the serum testosterone increment after hCG stimulation ($\Delta T/hCG$) was 100% for values >9 nmol/l (PNV = 72%). In IHH patients, the PPV of $\Delta T/hCG$ was 100% for values <3 nmol/l (PNV = 82%). Only 29% of the studied population had a $\Delta T/hCG$ between these lower and upper thresholds and therefore could not have been classified initially.

Conclusions: (i) Dynamic testing for the diagnosis of delayed puberty is useful only when the basal testosterone level is lower than 1.7 nmol/l; (ii) in that case, the hCG test has better discriminating power than the Triptorelin test and appears as the best cost-effective investigation. It prevents useless and expensive investigations in about one-half of CDP patients with a basal morning testosterone level lower than 1.7 nmol/l.

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Introduction

Delayed puberty (DP) is observed in as many as 3% of boys in the general population (1). In most cases, it is due to constitutional delayed puberty (CDP), a benign condition which recovers spontaneously. In a few cases, however, DP reveals a more threatening hypothalamic or pituitary lesion or is due to a sporadic or a familial genetic hypogonadotropic hypogonadism (HH). The diagnosis of HH is easy in the case of Kallmann's syndrome, or other complex syndromes such as Prader–Willi (2), Laurence–Moon or Bardet–Biedl (3) syndromes, and mutation in the Dax-1 (4) or leptin (5) genes. However, most HH cases are isolated

(IHH), without other endocrine, neurologic or malformation disturbances. Sporadic IHH can be due to either genetic or acquired abnormality. So far, genetic investigations have provided few explanations for IHH. No mutation in the gonadotropin-releasing hormone (GnRH) gene has been found in men (6) in contrast to mice (7). Mutations in the luteinizing hormone (LH) β subunit (8), follicle-stimulating hormone (FSH) β subunit (9) and GnRH receptor (10–13) genes have been previously reported in only a few patients with sporadic IHH. Conversely, IHH can reveal a pituitary or supra-pituitary tumor where it is necessary to perform magnetic resonance imaging (MRI) for a precise diagnosis and early adequate treatment.

At the time of referral, it is often difficult to distinguish boys with CDP from those with IHH. Both conditions present effectively with similar clinical and hormonal features. Only the demonstration of a complete and spontaneous recovery can distinguish CDP from IHH. It is therefore most important to design a cost-effective strategy for male DP. Many testing protocols have been investigated to address this issue, such as nocturnal sampling for LH (1, 14), urinary gonadotropin measurement (15, 16), plasma LH measurement by a highly sensitive immunofluorometric assay (17), GnRH agonist (GnRHa) stimulation test (18–21) or a human chorionic gonadotropin (hCG) test (22–26). So far no consensus has emerged about a single, reliable and easy test with acceptable sensitivity and specificity. In many studies reporting on these tests, CDP and other causes of DP, mainly IHH, were clearly distinct when data were expressed as means. However, a significant overlap was frequently observed between the two situations, thus limiting the diagnostic power of the test for a given individual. Moreover, the certainty as to whether partial IHH could be excluded in every patient with CDP was not established in most of the previously published series. This requires a long follow-up of patients, which was not fulfilled in most of the studies.

In this study, we took advantage of our ability to follow-up a cohort of patients with CDP or IHH for more than 5 years. In all patients, initial testing included a GnRH analog test and an hCG test. We have chosen to compare these two tests because they do not require the patients to stay overnight or for several days in hospital. Also, they are the ones most studied in the literature, but few authors have compared these tests with each other (27) or with others (18).

Materials and methods

Patients and initial work-up

Thirty-three male Caucasian patients with DP, aged 14.2 to 26.2 years at the time of referral, were included in this study before androgen replacement therapy. All subjects were admitted to the Department of Endocrinology and Diabetology at the University of Lille. Patients having a pituitary deficiency other than hypogonadotropism were excluded from the study. A search for anosmia, measurement of testis volume, weight, height, pubertal development assessment and bone age determination (according to the Greulich and Pyle atlas (28)) were performed in all subjects.

An i.v. catheter was inserted, and samples were obtained for determination of basal morning (0800 h) testosterone, LH and FSH serum levels.

The GnRHa was administered as a single s.c. injection of 0.1 mg Triptorelin (Ipsen-Biotech Laboratories, Paris, France). This GnRH analog has a longer

plasma half-life than native GnRH with an elimination half-life more than 80 min, which is about fivefold that of natural GnRH. Its metabolic clearance rate is about threefold less than that of natural GnRH (29). Blood samples for LH, FSH, testosterone and estradiol (E_2) determinations were then obtained 3, 6, 12 and 24 h after Triptorelin injection.

Five days later, hCG was given as a single 5000 IU i.m. injection and the serum testosterone level was assayed on a venous blood sample drawn 72 h after the hCG injection.

This study was approved by the ethical committee of Lille University Hospital.

Patient follow-up

A 5-year-long clinical follow-up enabled confirmation of the diagnosis for all the patients. Normal pubertal development occurred in 20 patients, with testis volume and plasma testosterone levels spontaneously reaching adult values. They were then classified as having CDP. Conversely, the other 13 patients whose pubertal development did not progress or remained incomplete during the follow-up were classified as having IHH. During the follow-up of treated patients, serum testosterone was evaluated 2 or 4 weeks after the last hCG or testosterone i.m. injection respectively.

Hormone assays

Serum testosterone was determined by radioimmunoassay (Coat-A-Count Testosterone; Diagnostic Products Corporation, Los Angeles, CA, USA). The sensitivity was 0.17 nmol/l. The intra-assay coefficients of variation were 10.8% and 4.7% when plasma testosterone levels were 0.7 and 3.5 nmol/l respectively. The inter-assay coefficients of variation were 11% and 6.4% when plasma testosterone levels were 2.6 and 9.2 nmol/l respectively.

The LH and FSH assays were carried out by a micro-particle immunoassay (AxSYM; Abbott Laboratories, Abbott Park, IL, USA). The sensitivity of the LH and FSH assays were 0.5 and 0.4 IU/l respectively. The LH intra- and interassay coefficients of variation were 4.5% and 4.3% respectively for a 4.8 IU/l level. The FSH intra- and interassay coefficients of variation were 3.7% and 3.1% respectively for a 5.3 IU/l level.

Statistical analyses

Variables were compared between groups by the Mann–Whitney test. Results are expressed as means $\pm$ s.d. The difference was considered significant when $P < 0.05$.

Predictive positive values (PPV) and predictive negative values (PNV) of the serum LH threshold 3 h after Triptorelin injection and the difference between the serum testosterone level before and 72 h after the hCG

injection ($\Delta T/hCG$) for CDP diagnosis were determined by the ratios: number of patients with CDP above the threshold/number of patients above the threshold and number of patients with IHH below the threshold/number of patients below the threshold respectively.

PPV and PNV of $\Delta T/hCG$ for IHH diagnosis were determined by the ratios: number of patients with IHH below the threshold/number of patients below the threshold and number of patients with CDP above the threshold/number of patients above the threshold respectively.

Results

Clinical data

Table 1 shows that patients with IHH were older at the time of referral, with a smaller growth delay, an older bone age, a higher body mass index (BMI) and a lower testis volume than patients with CDP. However, this last parameter overlapped with the CDP group greatly (Fig. 1A). Two patients with IHH had a testis volume >4 ml while it was <4 ml in seven patients with CDP.

Table 1 Clinical data in both groups (CDP and IHH). Values are expressed as means (S.D.).

	CDP	IHH	P
Age (years)	15.31 (0.99)	19.9 (3.32)	<0.001
Height (m)	1.51 (0.11)	1.69 (0.14)	<0.01
BMI (kg/m^2)	19 (4.46)	23.14 (3.92)	<0.01
Bone age (years)	12.6 (1.8)	14.3 (0.5)	<0.01
Testis volume (ml)	4.75 (1.83)	2.71 (1.58)	<0.01
Cryptorchidia (n)	2 (1 bilateral)	8 (4 bilateral)	NA
Anosmia (n)	0	4	NA
Ichthyosis (n)	0	1	NA

NA, not applicable.

Figure 1 (A) Individual values and centiles of testis volume in both groups. (B) Individual values of basal testosterone in both groups.

Anosmia was found in four unrelated patients, who were therefore considered as having sporadic Kallmann's syndrome.

Baseline hormonal data

LH and FSH serum levels were significantly higher in CDP than in IHH (Table 2). However, the range of individual values overlapped each other (Table 2). The mean basal morning serum testosterone level was significantly higher in CDP than in IHH (Table 2). Figure 1B shows that no individual value from the IHH group was >1.7 nmol/l. However, 50% of patients with CDP overlapped with the range of those with IHH.

Triptorelin test

As shown in Fig. 2, no difference was observed between CDP and IHH for serum levels of E_2 at any time during the test. The mean FSH serum level peaked between 3 and 6 h after Triptorelin injection in both groups. A significant difference in the mean levels was observed only 24 h after injection (CDP vs IHH: 3.52 ± 2.12 and 1.98 ± 2.25 IU/l respectively, $P < 0.05$), but no clear-cut threshold could separate the two groups.

The serum LH level peaked 3 h after injection in both groups and was significantly higher in CDP than in IHH (18.4 ± 9.4 vs 3.4 ± 4.1 IU/l respectively, $P < 0.001$). It remained significantly higher at the later times of testing but the difference between the two groups was less. As shown in Fig. 3, a discriminating threshold could be set at 14 U/l, since all patients with IHH were below this level while 14 out of the 19 patients with CDP were above. Therefore, the test yielded a 100% PPV and a 72% PNV for CDP. However, it left 56% of the patients from the total population unclassified.

The serum testosterone level increased slowly up to 24 h after injection in the CDP group while no significant increase was observed in patients with IHH. However, the individual 24 h serum testosterone level varied widely in the CDP group. No clear-cut threshold could therefore properly separate the two groups. The same conclusion was drawn when the difference between the 24-h and 0-h testosterone levels was used instead of the absolute 24-h testosterone level.

Table 2 Baseline hormone levels in both groups (CDP and IHH). Values are expressed as means (S.D.).

	CDP	IHH	P
Testosterone (nmol/l)	2.61 (1.89)	0.86 (0.37)	<0.05
DHAS ($\mu\text{mol}/\text{l}$)	3.77 (1.68)	4.71 (2.05)	NS
SHBG (nmol/l)	69.98 (37)	60.77 (32.4)	NS
FSH (IU/l)	2.16 (1.05)	1.03 (0.69)	<0.01
LH (IU/l)	1.67 (1.87)	0.53 (0.25)	<0.01

DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin; NS, not significant.

Figure 2 Responses after Triptorelin injection (0, 3, 6, 12 and 24 h) of (A) LH, (B) FSH, (C) estradiol (E2) and (D) testosterone (T). Values are expressed as means $\pm$ s.d. in both groups.

hCG test

The serum testosterone increment after hCG stimulation ($\Delta T/hCG$) was significantly higher in CDP than in IHH patients (14.5 ± 8.7 nmol/l vs 3.1 ± 2.4 nmol/l respectively, $P < 0.0001$). Figure 4 shows the individual values for $\Delta T/hCG$. All patients with values > 9 and < 3 nmol/l had CDP and IHH respectively. They represented 72% and 69% of the CDP and IHH groups respectively. The PPV and PNV for CDP diagnosis in the case of $\Delta T/hCG > 9$ nmol/l were 100% and 72% respectively. The PPV and PNV for IHH diagnosis in the case of $\Delta T/hCG < 3$ nmol/l were 100% and 82% respectively. Between 3 and 9 nmol/l, four and five patients had CDP and IHH respectively (Fig. 4). These

thresholds therefore left 29% of the patients from the total population unclassified. In this subset of patients, the 3-h LH level after Triptorelin did not discriminate since it was always < 14 U/l.

Discussion

Some of our patients ($n = 6$) had overt HH at the time of referral (no pubertal signs at 20 years of age or more and/or evidence of Kallmann's syndrome). No difference in initial hormonal results was observed between these patients and those in whom the diagnosis of IHH could not be established at once (data not shown). They were therefore gathered up into a

Figure 3 Individual values of LH 3 h after Triptorelin injection in both groups.

Figure 4 Individual values of $\Delta T/hCG$ (difference between serum testosterone at 0 and 72 h after the hCG injection) in both groups.

single group of patients with IHH in order to consolidate the comparison with patients with CDP, within the design of this study. We agree however that, in practice, such patients should not undergo any testing to distinguish them from CDP.

Our data confirm previous reports (1, 26, 30) concluding that a basal testosterone level >1.7 nmol/l has a good predictive value as a first screening test to discriminate idiopathic IHH from CDP. In patients with CDP, it means that puberty has spontaneously started, despite the absence of clinical evidence. Using this threshold would have prevented useless investigations in 55% of the CDP group and 33% of the total number of patients.

In patients with a basal testosterone level <1.7 nmol/l, further investigations are required. Priority should be given to those tests which yield optimal specificity and avoid missing the diagnosis of IHH. However, the tests should also have enough sensitivity to prevent the maximum number of patients with CDP from having useless investigations. In others studies, regardless of whether basal testosterone assay or tests were used, CDP and IHH were always clearly distinct when the data were expressed as means. However, the individual values from the two populations overlap almost constantly, except in a few studies. Morel *et al.* (15) found no overlap by using a urinary FSH test.

However, the two groups of patients were small (six with CDP and eight with IHH). In another study, Brown *et al.* (31) compared 42 prepubertal normal boys with 11 patients with idiopathic IHH or Kallmann's syndrome. No overlap was observed between these two groups when using the mean nocturnal LH plasma level (ultrasensitive assay). However, others have not confirmed the results of this study. In addition, this procedure is quite complicated and expensive, since it requires the patient to stay in hospital overnight.

In our hands, the Triptorelin test yielded unsatisfactory results. This confirms earlier studies with a different GnRHa (20, 32). It disagrees, however, with the studies of Zamboni *et al.* (21) and Kauschansky *et al.* (27). These authors were able to classify all their patients properly by using a cut-off threshold set at 8 U/l (27) or at about 10 U/l (21) for the LH serum level 4 h after a 0.1 mg/m^2 Triptorelin s.c. injection. In these studies, CDP was ascertained by the spontaneous occurrence of pubertal signs only 1 year after the initial investigation. These discrepancies with regard to the diagnostic power of the GnRHa test can thus be explained by: (i) varying etiologies of IHH among the different series, (ii) selection of more or less severe cases of CDP, (iii) differences in the sensitivity of the LH assays and (iv) differences between the nature and route of administration of the GnRHa.

In our study, the superiority of the hCG test over the Triptorelin test relied upon the ability to define a lower and an upper threshold for the level of testosterone, below and above which patients with IHH and CDP were properly assigned (PPV = 100%) respectively. The upper threshold for $\Delta T/hCG$ at 9 nmol/l is close to the one set by others at 7 nmol/l, with a different protocol of hCG injections (26).

In a previous study (27), no overlap in the testosterone response to hCG was observed between the two groups using a cut-off level at 8 nmol/l. However, this study used a different protocol (1500 U injected i.m. on 3 alternate days and blood samples for testosterone determination drawn on day 7). In addition, the pubertal stage was reassessed only 1 year after the initial referral. One cannot exclude, therefore, that in this series some cases of CDP were mixed up with patients having IHH.

The hCG test in the management of male DP is reliable, cheap, easy and comfortable for the patients (no hospitalization needed). According to our diagnosis strategy illustrated in Fig. 5, patients with basal testosterone < 1.7 nmol/l and $\Delta T/hCG$ < 9 nmol/l must have an MRI investigation because it can reveal a pituitary or supra-pituitary tumor in patients with IHH. When there is evidence for Kallmann's syndrome (anosmia and/or family history), MRI is of no use since finding of olfactory bulb aplasia (33) would not change the management of the hypogonadism. For the moment, the majority of cases of IHH with normal MRI corresponds with idiopathic IHH for which hormonal investigations are still mandatory. Whether genetic studies will modify this strategy in the future remains so far speculative.

In conclusion, dynamic testing for the diagnosis of DP is useful only when the basal testosterone level is lower than 1.7 nmol/l. In that case, the hCG test offers the best cost-effective investigation, with a better discriminating power than the Triptorelin test. It saves useless and expensive investigations in about

half of the CDP patients in whom the basal testosterone level is lower than 1.7 nmol/l. This has a non-negligible medical and psychological impact on the patient and his family.

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Figure 5 Diagnostic strategy for the management of DP in males.

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