ORIGINAL PAPER

The Usefulness of GnRH and hCG Testing for the Differential Diagnosis of Delayed Puberty and Hypogonadotropic Hypogonadism in Prepubertal Boys

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Abstract. [Objective] Discrimination between hypogonadotropic hypogonadism (HH) and constitutional delayed puberty (DP) is difficult in prepubertal patients. We retrospectively analyzed the usefulness of the gonadotropin-releasing hormone (GnRH) test using time-resolved fluoroimmunoassay (TR-FIA) for the measurement of gonadotropins and the human chorionic gonadotropin (hCG) test to discriminate between HH and DP during the prepubertal period.
[Method] Gonadal function was evaluated by the hCG test and the GnRH test using TR-FIA in 16 prepubertal boys who were suspected of having HH, of whom after long-term observation 11 boys were finally diagnosed with HH and 5 boys with DP.
[Results] In the GnRH test, peak LH levels were below the normal range in all HH patients and within the normal range in 4 of the 5 DP patients. Peak FSH levels were within the normal range in 4 of the 5 DP patients and below the normal range in 9 of the 11 HH patients. In the hCG test, testosterone levels after 3-day administration of hCG were below 50ng/ml in all HH patients except one 6-month-old infant and above 50ng/dl in all DP patients.
[Conclusion] GnRH testing using the TR-FIA assay is useful for diagnosis of HH during the prepubertal period. The combination of GnRH and hCG tests might improve diagnostic accuracy.

Key words: Hypogonadotropic hypogonadism, GnRH test, hCG test, Delayed pubert, TR-FIA

Introduction

Differentiation between hypogonadotropic hypogonadism (HH) and constitutional delayed puberty (DP) in the prepubertal period remains difficult despite advances in endocrinological techniques. Accurate diagnosis makes it possible to induce puberty with appropriate timing and to improve quality of life in HH patients. We previously reported the usefulness of the LHRH test relying on a standard RIA assay [1] for the diagnosis of HH, but the sensitivity and specificity of plasma LH measurement in the GnRH test are unsatisfactory. Dunkel et al. [2] and Job [3] reported that the hCG test was more reliable than the GnRH test for diagnosis of gonadotropin deficiency. Although sensitivity differed significantly between these two reports, both suggested that the GnRH and hCG tests were of limited value in the diagnosis of HH during the prepubertal period. In more recent reports, more sensitive immunoradiometric assays (IRMA) [4, 5], instead of the standard RIA assay and TR-FIA, (Delfia FSH, LH, Pharmacia-Upjohn) have been recently used as an ultra-sensitive non-RIA assay for LH and FSH [6]. To clarify the usefulness of TR-FIA and the discriminative power of GnRH and hCG testing, we evaluated gonadal function by GnRH testing using TR-FIA and hCG testing in 16 prepubertal boys with suspected HH.

Subjects

Sixteen Japanese prepubertal boys suspected during the prepubertal period of having HH were studied. After long-term observation 11 boys were diagnosed with HH and 5 boys with DP. Those diagnosed with HH included seven with Kallmann syndrome (KS), one with DAX-1 gene mutation, one with idiopathic isolated HH, one with

Accepted: August 4, 2003
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Table 1. Clinical features in hypogonadotropic hypogonadism and delayed puberty

Subject	Diagnosis	Age at examination	Cryptorchidism	Migrating testes	Micropenis	Small testis	Anosmia
1	KS	2	+		+	+	+
2	KS	16	+	+	+	+	+
3	KS	12	+		+	+	
4	KS	10		+	+	+	+
5	KS	12	+		+	+	±
6	KS	12	+	+		+	+
7	KS	13	+	+	+	+	
8	IsoGnD	10		+	+		
9	DAX-1	3					
10	MPHD	17			+	+	
11	CHARGE	8	+				
12	CDP	10		+		+	
13	CDP	11	+			+	
14	CDP	12					
15	CDP	13		+		+	
16	CDP	8			+	+	

KS: Kallmann syndrome, IsoGnD: isolated gonadotropin deficiency, DAX: isolated gonadotropin deficiency with DAX-1 gene mutation, MPHD: multiple panhypopituitarism, CHARGE: CHARGE association patient, and CDP: constitutional delayed puberty.

panhypopituitarism, and one with CHARGE association. Table 1 shows the final diagnosis, clinical features, and age at examination.

Methods

All patients underwent both hCG and GnRH testing. The GnRH stimulation test was performed by intravenous injection of gonadorelin acetate (Tanabe), at a dose of 100 μg/m^2 followed by blood sample collections for LH and FSH measurements at 0, 30, 60, 90 and 120 min after injection. The hCG (Teikokuzouki) was injected intramuscularly at a dose of 3,000IU/m^2 once a day for three days, and blood sampling for testosterone was done daily from day 1 to day 4. Serum LH and FSH levels were measured by TR-FIA (Pharmacia). The intra-assay coefficient of variation ranged from 4.21% to 6.85% and the inter-assay coefficient of variation from 1.99% to 7.00%. The limit of sensitivity of the assay was 0.08mIU/ml for LH and 0.05mIU/ml for FSH. Testosterone was measured by RIA (Diagnostic Products Co., USA).

Results

1) Clinical features

All KS patients had cryptorchidism or migrating testes. All KS patients except Cases 3 and 7 had hyposmia or anosmia. Cases 2 and 3 were KS brothers with an identical R631X nonsense mutation of the KAL1 gene, but they had significantly different phenotypes of olfactory sensitivity and renal abnormalities. Case 2 (the elder brother) was anosmic and had two normal kidneys, while Case 3 (the younger brother) had normal olfactory sensitivity and right renal agenesis. Case 7 had a normal sense of smell, although he carried a splice mutation in intron 4 of the KAL1 gene. Gene analysis was performed because he had right renal aplasia, which suggested X-linked KS. Case 9 showed high general hyperpigmentation at initial observa-

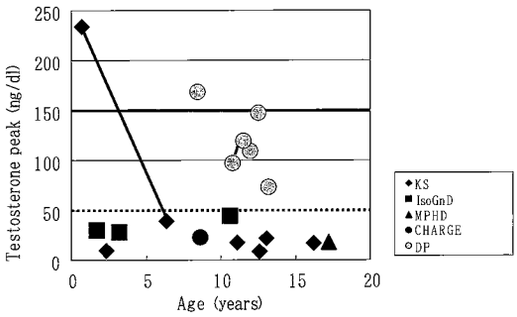

Fig. 1. Peak testosterone responses to the hCG test (■—■, ○—○ the hCG test was repeated at intervals). Wide solid line shows the lower limit of normal response. Dotted line shows the lower limit of subnormal response.

tion at 6 years and was subsequently found to have a DAX-1 mutation. Case 10 had a history of complicated delivery with breech presentation and asphyxia; he was diagnosed at age 11 years with growth hormone deficiency characterized by short stature and was later found to have HH. Case 11 was diagnosed as CHARGE syndrome by association with deafness, corneal dysplasia, and deviation of aortic valve on initial consultation; HH was detected during the observation period.

Five patients with delayed puberty were also suspected of having HH at an earlier diagnosis because of the presence of cryptorchidism, migrating testes, micropenis or small testis, but they were eventually diagnosed with delayed puberty due to development of spontaneous puberty. Case 14, who lacked clinical features of HH, was diagnosed at initial observation with HH because of his low peak LH response to GnRH, but he later developed spontaneous puberty.

2) hCG test

The results of the hCG stimulation test are shown in Fig.1 The normal range of peak testosterone response to hCG stimulation is more than 150ng/dl [1]. Peak testosterone levels were less than 50ng/dl in all HH patients (KS, IHH, MPHD and CHARGE association) except Case 6. The peak testosterone level in Case 6 was 237ng/dl at initial examination at age 6 months, but it had decreased to below 50ng/ml at the second examination at 7 years. The peak testosterone levels ranged from 50 to 150ng/dl in four DP patients. Only one patient with DP (Case 16) responded with a level greater than 150ng/dl. Case 12 (DP) was tested three times by stimulation with hCG, to which the testosterone response gradually improved from low (101ng/dl at 10 years and 123ng/dl at 11.3 years) to the normal range (423ng/dl at 12.4 years).

3) GnRH test

Fig.2(a) shows the peak LH response to GnRH. Peak LH levels were below the normal range in all HH patients, but within the normal range in all DP patients except Case 14. In Case 14, peak LH was below the normal range at 12.3 years but rose to 8.2mU/ml (normal pubertal response) at age 13.5 years. His testis volume increased and growth spurt occurred after administration of anabolic steroid at 13.6 years. We conclude that administration of anabolic steroid induced puberty in Case 14.

Fig.2(b) shows the distribution of peak FSH levels in the GnRH test. Peak FSH levels were within the normal range in two HH patients but below the normal range in the remaining nine HH patients. Only one of the DP patients showed a low FSH response, but his peak LH level was within the normal range at age 10 years.

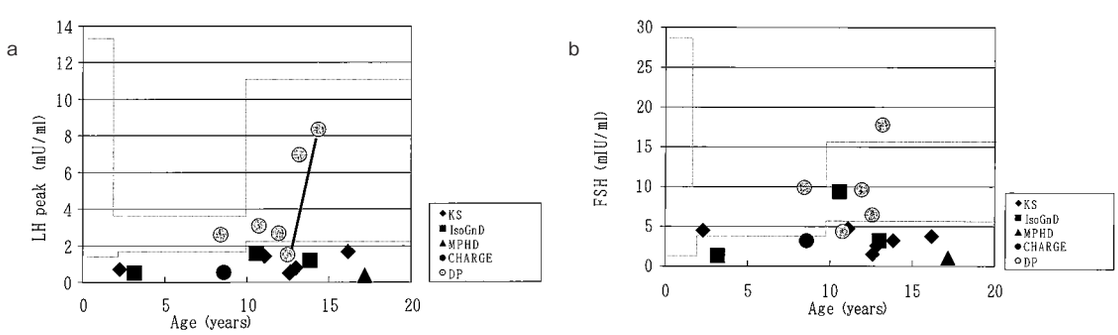

Fig. 2 (a). Peak LH levels by the GnRH test. Dotted lines show the upper and the lower limits of normal response for age group.
(b). Peak FSH levels by the GnRH test. Dotted lines show the upper and the lower limits of normal response for age group.

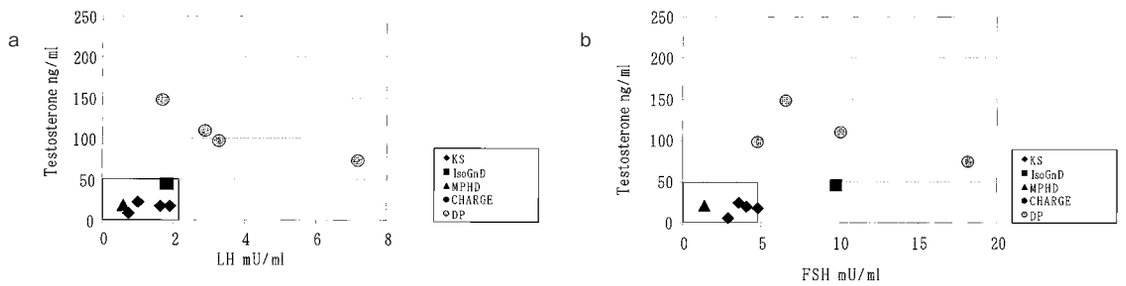

Fig. 3 (a). Distribution of peak LH levels by the GnRH test and peak testosterone levels by the hCG test in subjects aged 10 years and older. The rectangular open box indicates subnormal levels.
(b). Distribution of peak FSH level by the GnRH test and peak testosterone by the hCG test in subjects aged 10 years and older. The rectangular open box indicates subnormal levels.

4) Combination of GnRH and hCG testing in subjects aged 10 years and older

Fig.3(a) shows the distribution of peak LH levels in the GnRH test and the testosterone peak levels in the hCG test in patients at age 10 years and older. The rectangular box indicates peak LH levels less than 2.0mIU/ml and peak testosterone levels less than 50ng/dl. All HH patients' levels were within the rectangular box, while all DP patients' levels were outside. The rectangular box in Fig.3(b) indicates peak FSH levels below the normal range (FSH<4.38mIU/ml) coupled with peak testosterone levels less than 50ng/dl. Most HH patients and all DP patients showed the same pattern as the LH/hCG peak combination. Only one HH (isolated GnD) patient had a normal FSH with low testosterone response. Two HH patients under 10 years old exhibited low peak LH and low peak FSH levels, while one DP patient under 10 years showed normal peak levels (not shown).

Discussion

The hCG test has been reported to diagnose HH before puberty [1]. However, the GnRH test using RIA assay was noted to be of limited value in predicting sexual maturation in boys with HH. False low peak LH in the GnRH test was found in 18% of patients with isolated GHD who completed sexual maturation later. A normal peak LH response was found in 16 of 39 GHD patients (41%) in whom sexual maturation did not occur spontaneously [1]. Dunkel et al. reported that peak LH levels in GnRH tests were subnormal in only 65% (15/23) of patients with HH [2]. These reports suggested that a single GnRH test using RIA assay was insufficient to discriminate between HH and DP and that the GnRH test should be repeated to diagnose HH.

Immunoradiometric assay (IRMA) provides improved diagnostic potential and sensitivity for LH in the diagnosis of puberty and delayed pubertal development [4, 5]. TR-FIA, which is a non-RIA assay, has higher sensitivity than IRMA [7]. Fujiwara et al. [7] showed a strong correlation between IRMA and TR-FIA in LH and FSH measurements (LH: r=0.953, n=371 and FSH: r=0.991, n=399). The limit of sensitivity of this TR-FIA assay was 0.08mIU/ml for LH and 0.05mIU/ml for FSH; that of IRMA was 0.15mIU/ml for both LH and FSH [8].

In the present study, the false normal peak LH response rate was 0% in the patients with HH (0/11); the false low response rate was 16.7% in DP patients (1/6). Therefore, the sensitivity and the specificity of peak LH levels in the diagnosis of HH were 100% and 83.3%, respectively. The peak LH level in a repeated GnRH test was normal in one DP patient with a false low LH peak; in this patient, the peak testosterone level in the hCG test was within the normal range. The sensitivity and the specificity of peak FSH levels in the diagnosis of HH were 81.8% and 83.3%, respectively. Dunkel et al. [9] suggested that peak LH levels were more reliable than peak FSH levels in the diagnosis of HH. The present study produced comparable results.

The hCG test results can also compensate for erroneous results of a GnRH stimulation test. According to Tanaka et al. [1], the normal peak testosterone level after an hCG stimulation test is greater than 150ng/dl. The sensitivity and the specificity of the hCG test in the diagnosis of

HH were 91.7% and 100%, respectively, when the cut-off value was 50ng/dl. However, a diagnosis of HH is difficult in an infant less than 6 months old. Case 6 had an initial testosterone level by the hCG test of 237.5ng/dl at age 6 months, but his peak testosterone fell to below 50ng/dl at 7 years. Forest [10] also reported age-related changes in post-hCG testosterone levels in the first six months of life and suggested that peak testosterone levels by the hCG test are useful in the diagnosis of hypogonadism in boys older than 6 months old. A testosterone value of 50ng/dl in response to hCG stimulation could be used as a cut-off value in the diagnosis of HH and serve to distinguish between HH and DP. When both GnRH and hCG testing are performed in suspected patients aged 10 years and older, both the peak LH level by the GnRH test and the testosterone level by the hCG test have excellent diagnostic value.

Androgen deficiency causes the absence of sexual development, regression of sexual function and spermatogenesis, anemia, muscle atrophy, decrease in bone mineral density, increase in fat mass, and behavioral and mood alternations. In particular, males who do not produce sufficient testosterone at the appropriate adolescent age may experience behavioral and cognitive impairments. Testosterone deficiency may negatively affect personal and workplace relationships [11]. Correct diagnosis is required for appropriate timing of sex steroid supplement and improvement of quality of life in HH patients.

In conclusion, the diagnostic value of GnRH for diagnosis of HH is improved by using TR-FIA to measure LH and FSH. When used in combination with the hCG test, the GnRH test using TR-FIA could reduce HH diagnostic errors in boys.

Acknowledgements

We thank Mitsuko Sakai for assaying LH, FSH, and testosterone and Seiko Honjyo for a great deal of help. We are also grateful to Dr. John Parks and Milton Brown (Emory University) for their special advice. This study was supported by the grant from Ministry of Health, Labor and Welfare.

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