

# A practical GnRH analogue (triptorelin) stimulation test to distinguish constitutional delay of puberty from hypogonadotropic hypogonadism in prepubertal boys

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**SUMMARY:** Özkan B, Topaloğlu AK, Bilginturan N. A practical GnRH analogue (triptorelin) stimulation test to distinguish constitutional delay of puberty from hypogonadotropic hypogonadism in prepubertal boys. *Turk J Pediatr* 2001; 43: 114-117.

At present, no established practical and reliable endocrine test exists for differentiating between constitutional delay of puberty (CDP) and hypogonadotropic hypogonadism (HH). The most discriminating results have been reported by measuring LH and testosterone (T) responses to gonadotropin-releasing hormone (GnRH) analogues. In this study, 23 prepubertal boys aged 14 to 16.5 years underwent a modified triptorelin (a GnRH analogue) stimulation test, and they were followed clinically for up to 24 months. Sixteen subjects developed spontaneous puberty during the follow-up period and thus were diagnosed with CDP, and the remaining seven were diagnosed with HH. Retrospective evaluation of their LH, FSH and T responses revealed significant differences without any overlaps in serum LH levels at 4 h (CDP:  $33.2 \pm 9.3$  vs. HH:  $3.3 \pm 2.6$  mIU/ml,  $p < 0.0002$ ) and in serum T levels at 24 h (CDP:  $369.3 \pm 128.1$  vs. HH:  $61.4 \pm 22.6$  ng/dl,  $p < 0.0002$ ). We conclude that CDP can be clearly differentiated from HH by the LH response at 4 h and/or T response at 24 h after a single-dose triptorelin administration.

**Key words:** constitutional delay of puberty, hypogonadotropic hypogonadism, triptorelin.

Differentiation of teen-age boys with constitutional delay of puberty (CDP) from those with hypogonadotropic hypogonadism (HH) remains difficult because of the similarity in their clinical and laboratory characteristics. At present, no established practical and reliable endocrine test exists for differentiating between these two entities<sup>1</sup>. Thus, the diagnosis of HH is often delayed until the exclusion of CDP by spontaneous appearance of pubertal signs. Recent literature focused on gonadotropin and testosterone (T) responses to gonadotropin-releasing hormone analogues (GnRH analogue) to differentiate between these two conditions. A nafarelin stimulation test has been used the

most and reported as a useful tool to distinguish the two conditions by LH and/or T response in the great majority of the cases<sup>2-4</sup>. Leuprolide was reported to have been useful in one study, although it failed to produce distinctive results in another<sup>5, 6</sup>. The most encouraging results were reported with another GnRH agonist, triptorelin<sup>7</sup>. Triptorelin, administered at 4 am, was reported to have differentiated these two conditions in all subjects by their LH but not by their T response four hours later, at 8 am. Our primary aim was to develop a practical triptorelin stimulation test. To this end, we modified the triptorelin stimulation method in that we administered triptorelin at 8 am instead

of 4 am. Our second modification was the extension of the test to 24 h to see whether the T response could be another diagnostic parameter as in the other GnRH agonist stimulation tests. Here we report similarly differentiating results obtained after using a modified triptorelin stimulation test.

### Material and Methods

Twenty-three prepubertal boys, aged 14-16.5 years, were studied at three different pediatric endocrinology clinics. Location of the cases and the time period of the study were as follows: 10 cases at Hacettepe University, İhsan Doğramacı Children's Hospital between June 1997 and February 1998; 10 cases at Atatürk University between July 1998 and November 1999, and three cases at Mersin University between March 1999 and December 1999. The following inclusion criteria were used: 1. no evidence of systemic diseases to cause delayed puberty, 2. a weight for height within normal standards for Turkish children <sup>8</sup>, 3. a bone age equal to or greater than 11.5 years according to Greulich-Pyle method <sup>9</sup>, and 4. a testicular volume less than 4 ml.

All subjects underwent a triptorelin stimulation test at the time of enrollment after the nature of the study had been explained and informed consents were obtained from the subjects or their families. Blood specimens were collected for baseline LH, FSH, and T levels at 8 am. Then, triptorelin (D-trp<sup>6</sup>-luteinizing hormone-releasing hormone) was administered subcutaneously at a dose of 0.1 mg/m<sup>2</sup> (Decapeptyl<sup>®</sup>, Ipsen-Biotech, Paris, France). Following the triptorelin injection, blood samples for LH, FSH and T were obtained at 4, 8 and 24 h. Serum LH, FSH and T concentrations were determined by chemiluminescent enzyme immunoassay (Immulate, Automated Analyzer, Diagnostic Product Corporation, Los Angeles, CA, USA) in duplicate on the same day. The subjects were regularly followed up until determination of the physical evidence of pubertal advancement for up to 24 months. No subject received short-term T treatment. Sixteen subjects who had spontaneous pubertal development were diagnosed with CDP. In this group, evidence of pubertal advancement was determined in 9 ± 5.2 (5-24) months at the age of 15.6 ± 0.83 (14.6-17) years. Their mean testicular volume was 4.5 ± 1.8 (4-8) ml at the time of the last examination. The remaining seven subjects who showed no

pubertal signs during 17.4 ± 5.0 (12-24) months follow-up period were diagnosed with HH. The mean chronological age of the subjects was 16.8 ± 0.7 (16-18) years at the time of the last examination. Their mean testicular volume was 2.7 ± 0.5 (2-3) ml at the end of the follow-up period. Of the subjects with HH, four had anosmia, micropenis and history of bilateral orchidopexy (Kallmann's syndrome), two had panhypopituitarism and had undergone surgical removal of the craniopharyngioma. Except for these two patients with panhypopituitarism, none of the subjects in the entire study population showed clinical or laboratory features of growth hormone deficiency. Clinical and hormonal variables between the groups were compared with Mann-Whitney U test. Z-scores for 4 h LH and 24 h T responses in the CDP group were calculated using SPSS software package.

### Results

The initial clinical characteristics of the patients are summarized in Table I. There were no statistically significant differences between the groups in height, chronological age, bone age, or testicular volume (Table I). Mean basal values of LH, FSH, and T also did not differ significantly between the groups. The patterns of responses to triptorelin in both groups are shown in Table II. After triptorelin administration, the difference in mean serum LH levels between the groups was most pronounced at 4 h (33.2 ± 9.3 vs. 3.3 ± 2.6 mIU/ml, for CDP and HH respectively,  $p < 0.0002$ ) without any overlaps (min: 14.1, max: 44.4 for CDP and min: 0.7, max: 8.1 for HH groups). Mean incremental LH response, Δ LH (LH at 4 h minus LH at time 0) was significantly higher in the CDP group (32.3 ± 9.2 mIU/ml vs. 2.6 ± 2.5 mIU/ml,  $p < 0.0002$ ). Δ LH at 4 h ranged from 13.4 to 43 mIU/ml for the CDP group, and from 0.0 to 7.7 in the HH group. Although the mean LH responses to triptorelin at 8 and 24 h in subjects with CDP were significantly higher, the responses of the two groups were overlapping. No significant differences in mean FSH response was observed at any timepoints. Mean T response to triptorelin in the CDP group was significantly higher than in the HH group (369.3 ± 128.1 ng/dl vs. 61.4 ± 22.6 ng/dl respectively,  $p < 0.0002$ ) at 24 h without

overlapping (range 110 to 571 ng/dl for CDP, and 21 to 90 ng/dl for HH group).  $\Delta T$  at 24 h (T value at 24 h minus T value at time 0) was  $334.3 \pm 124.0$  ng/dl vs.  $12.8 \pm 34.0$  ng/dl for CDP and HH groups, respectively ( $p$ : 0.0002).  $\Delta T$  at 24 h ranged from 90 to 551 ng/dl for the CDP group, and from -40 to 51 ng/dl in the HH group. Although the mean T responses to triptorelin at 4 and 8 h in subjects with CDP were significantly higher, the responses of the two groups were overlapping.

patients. However, one subject with HH had a T response similar to those of CDP at 8-24 h. Ghai et al.<sup>3</sup> reported that LH levels in subjects with CDP were significantly higher than those in subjects with HH at 4 h. One patient with HH, however, was misclassified in the CDP group by the test. Only 55 percent ( $n$ : 11) of the patients with CDP displayed a significant T response 8-24 h after nafarelin injection. In a smaller study (CDP  $n$ : 6, HH  $n$ : 5), Kletter et al.<sup>4</sup> found that LH, FSH and T responses in

Table I. Initial Clinical Characteristics of the Subjects with Constitutional Delay of Puberty (CDP) and Hypogonadotropic Hypogonadism (HH)

	Age (year)	Height (cm)	Height SDS*	Testicular volume (ml)	Bone age (year)
CDP	$14.8 \pm 0.8$ (14-16.5)	$147.6 \pm 3.2$ (142-154)	$-2.4 \pm 0.8$ (-4.0-1.5)	$2.6 \pm 0.4$ (2-3)	$12.0 \pm 0.6$ (11.5-13)
HH	$15.8 \pm 1.2$ (14-16.5)	$153.5 \pm 8.8$ (144-163)	$-2.0 \pm 1.1$ (-4.0-(-0.99))	$2.4 \pm 0.5$ (2-3)	$12.6 \pm 0.9$ (11.5-14)

Data are means  $\pm$  SD (Range), \*SDS: Standard deviation score.

Table II. LH, FSH and Testosterone (T) Responses to Triptorelin in Constitutional Delay of Puberty (CDP) and Hypogonadotropic Hypogonadism (HH)

	Hour	HH (n:7)	CDP (n:16)	p
LH (mIU/ml)	0.	$0.6 \pm 0.2$ (0.4-1.0)	$0.8 \pm 0.2$ (0.4-1.4)	NS*
	4	$3.3 \pm 2.6$ (0.7-8.1)	$33.2 \pm 9.3$ (14.1-44.4)	0.0002
	8.	$2.8 \pm 1.6$ (0.7-5.0)	$12.8 \pm 5.9$ (4.4-26.5)	0.0003
	24.	$1.1 \pm 0.5$ (0.7-2.1)	$6.5 \pm 4.8$ (0.7-15.6)	0.003
FSH (mIU/ml)	0.	$0.6 \pm 0.2$ (0.1-0.9)	$0.8 \pm 0.3$ (0.6-1.7)	NS
	4.	$5.1 \pm 2.3$ (2.0-7.2)	$4.4 \pm 2.3$ (2.7-9.5)	NS
	8.	$3.3 \pm 2.1$ (1.5 $\pm$ 6.1)	$3.9 \pm 2.8$ (2.2-9.6)	NS
	24.	$1.6 \pm 1.2$ (0.2-3.5)	$1.8 \pm 1.1$ (1.0-4.9)	NS
T (ng/dl)	0.	$22.8 \pm 13.8$ (11-54)	$29.3 \pm 18.7$ (10-72)	NS
	4.	$35.7 \pm 17.1$ (22-70)	$61.8 \pm 24.8$ (20-103)	0.02
	8.	$42.8 \pm 21.3$ (21-82)	$120.6 \pm 35.1$ (50-194)	0.0003
	24.	$61.4 \pm 22.6$ (21-90)	$369.3 \pm 128.1$ (110-571)	0.0002

Data are means  $\pm$  SD (Range), \* NS: non-significant.

## Discussion

Distinguishing CDP from HH in boys with delayed puberty remains a diagnostic challenge. Synthetic GnRH analogues have been used extensively to distinguish CDP from HH. However, a simple and reliable diagnostic test is still lacking<sup>1</sup>. We found that when triptorelin is given at 8 am, the increase in serum LH at 4 h or T at 24 h is sufficient to distinguish those two clinical entities. In a pilot study (HH  $n$ : 8, CDP  $n$ : 3), Ehrmann et al.<sup>2</sup> reported that LH responses to nafarelin in subjects with CDP at the 0.5-4 h did not overlap with those in HH

subjects with CDP were higher than in those with HH without overlapping after nafarelin administration. Recently, Zamboni et al.<sup>7</sup> reported that triptorelin administration at 4 am clearly distinguished CDP ( $n$ : 18) from HH ( $n$ : 10) by the LH responses at 4 h without overlapping. However, starting a test at 4 am on an outpatient basis would be almost impossible in practice, as the authors suggested. Therefore, we modified their method and administered triptorelin at 8 am instead of 4 am and extended the test period to 24 h to see whether the T response could be another

diagnostic parameter. In this study, we were able to reproduce the 4h LH results of the original study in a comparable subject population. This reproduction might not have been possible because of the wellknown nocturnal increase in the number and magnitude of LH pulses in early puberty, which suggests enhanced sensitivity to GnRH during the night<sup>10</sup>. Moreover, we found that the 24h T response is also completely discriminatory. Zamboni et al.<sup>7</sup> looked for T responses only at 4 h; however, based on the results obtained in the other GnRH analogue stimulation studies, larger differences in T response between the two conditions should be expected at 16-24 h<sup>2-4</sup>. For that reason, we extended the study period to 24 h, and we found significantly different, non-overlapping results at 24 h. Our data indicate that an LH level below 14.1 mIU/ml (-2 SD) 4 h or a T level below 110 ng/dl (-2SD) 24 h after triptorelin administration could be useful as a cut-off point to discriminate CDP from HH. We conclude that CDP can be clearly differentiated from HH by the LH response at the 4 h and/or T response at 24 h after triptorelin administration. This test, we think, is superior to the others in that: i) Both 4h LH and 24 h T responses are significantly different between the groups without any overlaps ii) It is practical since it is started at 8 am. The patient can return either at noon on the same day or at 8 am on the following day. The latter option can be particularly useful in that absence from school for the test is unnecessary. iii) Measuring T response at 24 h enables assessment of the integrity of not only pituitary but also gonadal function.

In conclusion, patients with CDP, with bone age equal to or greater than 11.5 years, display a distinct increase in LH at 4 h and in T concentrations at 24 h after 8 am triptorelin

administration, thus enabling clear differentiation of subjects with CDP from those with HH.

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