A Cost-Benefit of GnRH Stimulation Test in Diagnosis of Central Precocious Puberty (CPP)

SUTTIPONG WACHARASINDHU, M.D., M.R.C.P.(UK)*, SUMARLEE SRIVUTHANA, M.D.*,
SUPHAB AROONPARKMONGKOL, B.Sc.*, VORASUK SHOTELERSUK, M.D.*

Abstract

The GnRH stimulation test is the gold standard to diagnose central precocious puberty (CPP). Conventionally, we need at least 2 hours to finish the test which seems to be costly and time consuming. In this study, we described the pattern of LH and FSH levels during the GnRH test in 27 girls who presented with various degrees of precocious puberty. We found that the blood samples at 90 and 120 min after GnRH were not necessary. To save the cost of diagnosis, the basal LH/FSH ratio > 0.2, the 30 min LH/FSH ratio after GnRH > 0.9 and the peak LH/FSH ratio > 1.0 can be used to diagnose CPP with positive predictive values (PPV) of 87.3, 89.4 and 93.8 per cent respectively.

Key word : Precocious Puberty

WACHARASINDHU S, et al
J Med Assoc Thai 2000; 83: 1105-1111

BACKGROUND

Normal pubertal development in humans requires the activation of the luteinizing hormone releasing hormone (LHRH) pulse generator at the appropriate time, 9-13 years in girls and 10-14 years in boys. During the prepubertal period, the LHRH pulse generator is in the juvenile pause secreting very low levels of gonadotropin. Any conditions affecting the early activation of LHRH pulse generator may cause central or true precocious puberty. However, not all girls presenting with early breast development may have precocious puberty. They may have the benign condition which is called premature thelarche and treatment is not required. A previous study hypothesized that premature thelarche and central precocious puberty may represent different positions along a continuum of hypothalamic LHRH neuron activation[1]. The diagnosis of central precocious puberty (CPP) requires many factors including age of onset, degree of advancement in sexual and skeletal maturation, tempo of

* Endocrine Unit, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

progression and the standard laboratory confirmation of central precocious puberty which is the maximal serum luteinizing hormone (LH) concentration after gonadotropin-releasing hormone (GnRH) stimulation[2]. Because bone age advancement is usually found in CPP, eventually, resulting in short adult height if treatment does not intervene[3]. The conventional test requires 5 blood samples including the basal sample for LH, FSH and estradiol and subsequently every 30 minutes after 100 microgram of GnRH (Relisorm-L) for LH, FSH at 30, 60, 90 minutes and for LH, FSH and estradiol at 120 minutes. The test seems to be costly and time-consuming.

In this study, we describe the pattern of LHRH pulse generator during GnRH stimulation test in girls presenting with breast development and in those with early breast development and other signs of puberty such as increased height velocity, pubic hair and menstruation. Regarding the cost-benefit of conventional GnRH test, we evaluated the basal LH, FSH levels and LH/FSH ratio to determine whether they could be used instead of the conventional test to confirm CPP.

MATERIAL AND METHOD

All girls who presented with early breast development before 8 years of age were included in this study and divided into 3 groups depending on the severity of precocious puberty. (Table 1)

Group I : Nine girls presented with early breast enlargement and no other signs of puberty. No advancement of bone age and no history of increased height velocity.

Group II : Ten girls presented with early breast enlargement and no other signs of puberty. Bone age advancement at least one year over the chronological age was demonstrated. Some of them also had history of increased height velocity.

Group III : Eight girls presented with early breast development and other signs of puberty such as pubic hair development or menstruation.

GnRH stimulation tests were performed in all girls and FSH, LH were measured at 0, 30, 60, 90, 120 min and estradiol at 0 and 120 min after giving synthetic GnRH (Relisorm 100 ug) intravenously. The bone age was estimated by the Greulich & Pyle method. Pelvic ultrasonography was performed to exclude ovarian tumor or functional ovarian cysts. Tumor markers including hCG and alpha-fetoprotein were also measured.

Serum FSH, LH and estradiol levels were measured by fluoroimmunoassay.

The mean FSH, LH and estradiol levels were compared between the groups and within the group but at different times.

The statistics used in this study were *t* test and ANOVA and $p < 0.5$ was considered significant.

RESULTS

From all 27 GnRH tests, 20/27 (74.1%) had the peak serum LH at 30 min after GnRH, 6/27 (22.2%) at 60 min and 1/27 (3.7%) at 90 min. No one had peak LH at 120 min.

The peak FSH occurred at 30 min in 7 out of 27 (26%), 10/27 (37%) at 60 min, 5/27 (18.5%) at 90 min and 5/27 (18.5%) at 120 min.

In group I, the mean peak LH was 7.1±4.1 IU/L and FSH 13.46 ± 2.7 IU/L. (Table 2)

The basal LH/FSH ratio was 0.07±0.05 and the peak LH/FSH was 0.53 ± 0.34 (Fig. 1, 2).

Table 1. The clinical data of patients in 3 groups.

Group	N	CA (yr)	Breast stage	Pubic hair	Menstruation
I	9	7.4±1.2	2.1±0.3	1	no
II	10	7.8±0.8	2.7±0.5	1	no
III	8	8.8±4.0	3.5±0.8	1.8±0.5	all
Group	**BA (yr)**	**HtSDS**	**HtSDS for BA**	**Wt SDS**	
I	7.4±1.1	0.5±0.9	0.3±0.5	0.5±1.0	
II	10.5±0.7	1.6±0.7	-0.3±0.7	1.5±0.8	
III	11.7±1.5	2.9±1.4	-0.1±1.2	3.7±1.9	

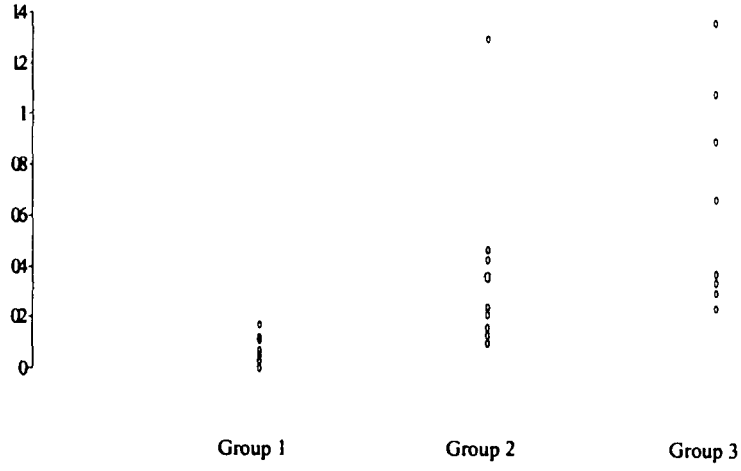

Fig. 1. The basal LH/FSH ratio in 3 groups.

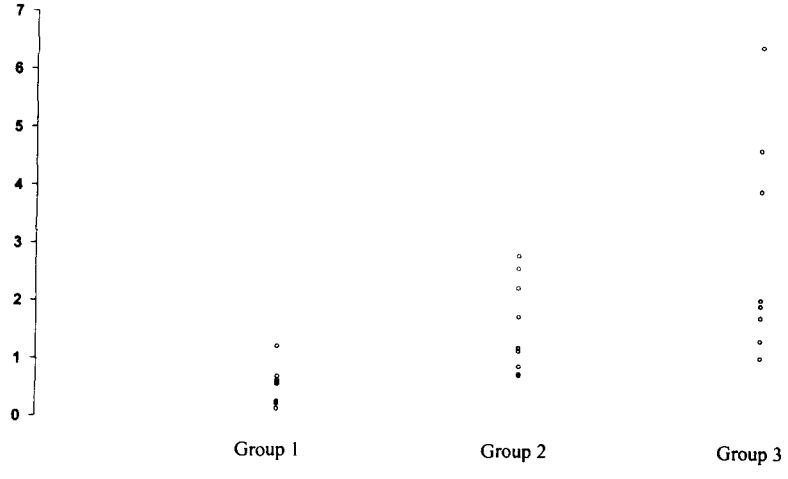

Fig. 2. The peak LH/FSH ratio in 3 groups.

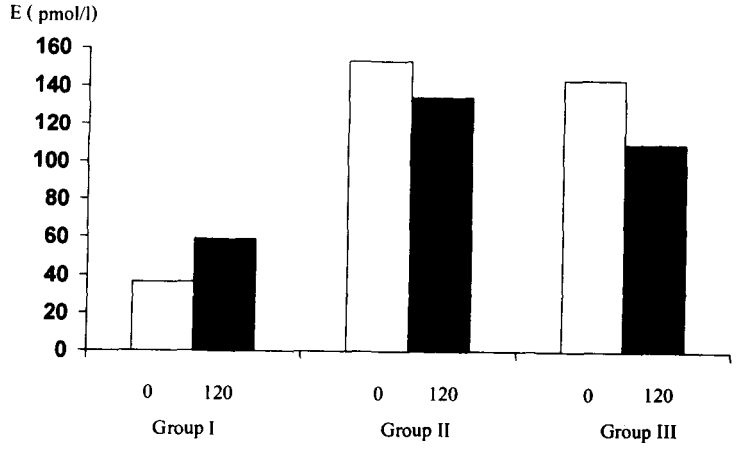

Fig. 3. Estradiol (E) at 0 and 120 min in 3 groups.

Table 2. Serum LH, FSH, LH/FSH, estradiol in 3 groups.

Group	Basal LH (IU/L)	Basal FSH (IU/L)	Peak LH (IU/L)	Peak FSH (IU/L)
I	0.2±0.17	3.98±3.91	7.1±4.1	13.46±2.7
II	1.86±1.45	5.06±1.38	18.75±11.5	12.44±4.76
III	3.65±2.52	5.21±1.85	24.08±13.15	9.31±2.37
Group	Basal LH/FSH	Peak LH/FSH	Basal E2 (pmol/l)	120 min E2 (pmol/l)
I	0.07±0.05	0.53±0.34	36.19±22.05	59.21±73.96
II	0.38±0.35	1.57±0.77	153.5±148.5	134.8±113.6
III	0.66±0.41	2.96±1.92	144.2±116.8	110.0±54.1

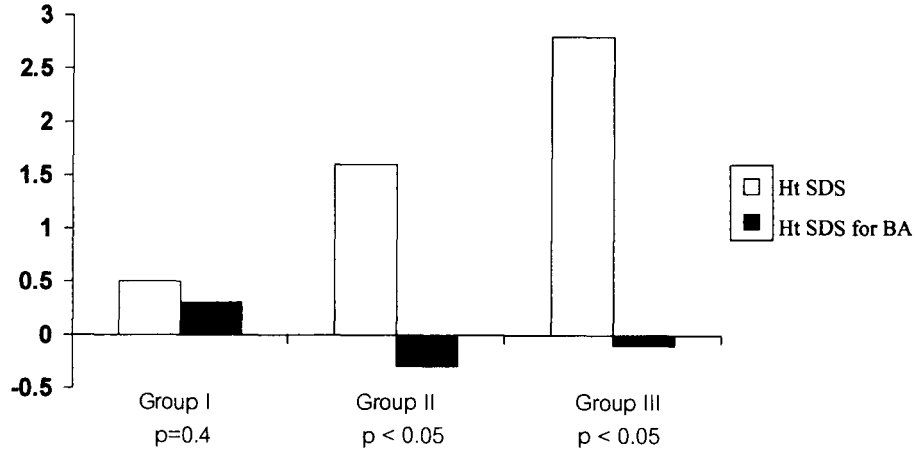

Fig. 4. Ht SDS and Ht SDS for BA in 3 groups.

The means of estradiol at 0 and 120 min were 36.19 ± 22.05 and 59.21 ± 73.96 pmol/l which were not significantly different. (Fig. 3)

In group II, the mean peak LH was 18.75 ± 11.5 IU/L and FSH 12.44 ± 4.76 IU/L (Table 2).

The basal LH/FSH ratio was 0.38 ± 0.35 and the peak LH/FSH 1.57 ± 0.77. (Fig. 1, 2)

The means of estradiol at 0 and 120 min were 153.5 ± 148.5 and 134.8 ± 113.6 pmol/l which were not significantly different. (Fig. 3)

In group III, the mean peak LH was 24.08 ±13.15 IU/L and FSH 9.31 ± 2.37 IU/L. (Table 2)

The basal LH/FSH ratio was 0.66 ± 0.41 and peak LH/FSH 2.96 ± 1.92. (Fig. 1, 2)

The means of estradiol at 0 and 120 min were 144.2 ± 116.8 and 110.0 ± 54.1 pmol/l which were not significantly different. (Fig. 3)

In contrast to the patients in group II and III, the patients in group I had good height prognosis because Ht SDS and Ht SDS for BA were not significantly different. (Fig. 4) If we considered the peak LH > 10 IU/L as the laboratory confirmation of CPP, we found that all patients in group III, 8 of 10 patients in group II and 1 of 9 patients in group I had CPP. Therefore, most of the patients in group I were in the benign group called premature thelarche but most of them in group II and III were in the more serious group (CPP) and treatment should be considered.

The peak LH/FSH ratio of 1.0 may be used to differentiate between premature thelarche and CPP with the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 88.2, 90, 93.8, 81.8 per cent respectively.

Table 3. The cost and benefit of the tests for diagnosis of CPP.

TEST	Sensitivity (%)	Specificity (%)	Positive predictive value (PPV) (%)	Negative predictive value (NPV) (%)	Cost (baht)*
Basal LH/FSH = 0.2	82.5	80	87.5	72.7	700
Peak LH/FSH = 1.0	88.2	90	93.8	81.8	3400
30 min LH/FSH = 0.9	100	80	89.4	100	1500

* 38 baht ≈ 1 US dollar

Additionally, the basal LH/FSH ratio of 0.2 and the 30 min LH/FSH ratio of 0.9 may also be used. The sensitivity, specificity, PPV, NPP and costs of all tests are shown in Table 3.

From this study, no one had ovarian tumor or cyst producing sex hormone.

One of the patients in group I presented with stage II breast development and no advancement of bone age, however, the test showed the peak LH of 16.4 and the peak FSH of 13.2 IU/L, The basal LH/FSH ratio of 0.12 and peak LH/FSH ratio of 1.24 and the 30 min LH/FSH ratio of 1.4. On follow-up, we found that her puberty had progressed and LHRH analogue was started subsequently.

All patients in group III having clinical grounds and laboratory confirmation of CPP met the three cut-off points to diagnose precocious puberty, basal LH/FSH>0.2, peak LH/FSH >1 and 30 min LH/FSH > 0.9.

However, 2 of 10 in group II had peak LH < 10 (8 and 9.5) but clinical grounds supported precocious puberty such as advancement of bone age, increased height velocity and treatment was considered because of the progression of puberty. We found that the first girl had all 3 cut-off levels (basal LH/FSH 0.47, peak LH/FSH 1.9 and 30 min LH/FSH 1.2) and the second girl had 2 out of 3 cut-off levels. (basal LH/FSH 0.37, peak LH/FSH 0.92, 30 min LH/FSH 1)

The weight SDS was higher in group II and III than in group I.

DISCUSSION

The available gold standard used at present to diagnose central precocious puberty (CPP) is the LH-predominant response to GnRH stimulation test.[2,4] Neely et al suggested that the peak LH > 5 IU/L after GnRH stimulation test considered CPP because this figure was above +2 SD for normal prepubertal female subjects[5,7]. However, some studies recommended different figures e.g. > 8 or maximum night time LH > 10 IU/L[6-8]. The peak FSH after GnRH cannot be used to diagnose CPP. From this study, the peak FSH levels in the 3 groups were not significantly different but the changes were seen in the peak LH levels which increased progressively from group I to group III. (Fig. 5) This finding represented the maturation of the LHRH pulse generator of which the LH levels

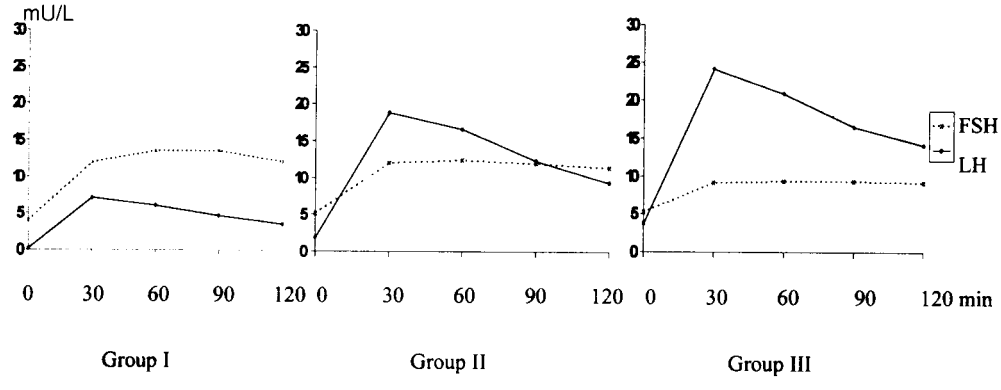

Fig. 5. FSH and LH during GnRH test in 3 groups.

but not FSH had progressively increased from prepubertal to pubertal period. Most of the peak LH levels occurred at 30 min after GnRH and almost 100 per cent occurred at 60 min. The peak FSH occurred at 30, 60, 90, 120 min for 26 per cent, 37 per cent, 18.5 per cent and 18.5 per cent respectively. However, the levels at 4 different times were not significantly different. In addition, the mean E2 at 120 min was not different from the basal E2. To save costs, therefore, we suggested that it was not necessary to take a sample at 90 and 120 min during the GnRH test.

Previous study showed that the spontaneous LH levels correlated strongly with the peak LH after GnRH and it was recommended to use the spontaneous LH to diagnose CPP. The spontaneous level LH > 0.1 mU/L by ICMA detected CPP with 94 per cent sensitivity and 88 per cent specificity (4). Similar results were demonstrated in many studies(8-10). The different immunometric assays with simple multiplication factors were inaccurate(11). Therefore, the peak LH/FSH ratio may be the best predictor for CPP. From our study, if we used the peak LH/FSH, we would reach better sensitivity, specificity and PPV than using the basal LH/FSH. Similar to the study by Oretor et al which suggested that the peak LH/FSH ratio was the best predictor for CPP(7). Angsusingha et al also suggested that the peak LH minus basal LH was the best parameter to diagnose CPP(13). The cost of the standard GnRH test is very expensive and takes at least 2 hours to finish the test. Therefore, we may use the blood sample at 30 minutes after GnRH intravenous which is cheaper, saves time and can be done in out-patient clinics to diagnose CPP and the results are not apparently different. As in a previous study(9), the single sample subcutaneous GnRH test can be used to confirm CPP. Even the basal LH/FSH ratio which is the cheapest way to diagnose CPP can be used in conjunction with clinical ground to diagnose with PPV of 87.5 per cent (Table 3).

The increased adipose tissue was proved to be associated with early puberty in girls(12). In the present study, we supported this because the wt SDS was higher in group II and III than in group I.

The decision to start treatment in girls with early breast development relies not only on the biochemical evidence, but we also have to consider the clinical data of each individual. The biochemical result is a good tool to confirm CPP but it should not be too expensive and should be easy to perform. Furthermore, clinical follow-up is very important to make the decision for treatment in patients with CPP.

(Received for publication February 24, 2000)

REFERENCES

1. Pescovitz OH, Hench KD, Barnes KM, et al. Premature thelarche and central precocious puberty: the relationship between clinical presentation and the gonadotropin response to luteinizing hormone-releasing hormone. J Clin Endocrinol Metab 1988;67:474-9.
2. Lee PA. Laboratory monitoring of children with precocious puberty. Arch Pediatr Adolesc Med 1994; 148:369-76.
3. Kreiter M, Burstein S, Rosenfield RL, et al. Preserving adult height potential in girls with idiopathic true precocious puberty. J Pediatr 1990; 117:364-70.
4. Cutler Jr GB. Precocious puberty. In: Hurst JW, ed. Medicine for the practicing physician. Boston: Butterworth; 1988: 526-30.
5. Neely EK, Wilson DM, Lee PA, et al. Spontaneous serum gonadotropin concentration in the evaluation of precocious puberty. J Pediatr 1995; 127: 47-52.
6. Neely EK, Hintz RL, Parker B, et al. Two-year results of treatment with depot leuprolide acetate for central precocious puberty. J Pediatr 1992; 121: 634-40.
7. Neely EK, Hintz RL, Wilson DM, et al. Normal range for immunochemiluminometric assays. J Pediatr 1995; 127:40-6.
8. Oreter KE, Uriarte MM, Rose SE, et al. Gonadotropin secretory dynamic during puberty in normal girls and boys. J Clin Endocrinol Metab 1990; 71: 1251-8.
9. Eckert KL, Wilson DM, Bachrach LK, et al. A single-sample, subcutaneous gonadotropin-releasing hormone test for central precocious puberty.

Pediatrics 1996;97: 517-9.
10. Garibaldi LR, Picco P, Magier S, et al. Serum luteiniozing hormone concentration, as measured by a sensitive immunoradiometric assay, in children with normal, precocious or delayed pubertal development. J Clin Endocrinol Metab 1991; 72: 888-98.
11. Taylor AE, Khoury RH, Crowley Jr WF. A comparison of 13 different immunometric assay kits for gonodotropin : Implication for clinical investigation. J Clin Endocrinol Metab 1994; 79: 240-7.
12. Genazzani AR, Pintor C, Corda R. Plasma levels of gonadotropins, prolactin, thyroxine and adrenal and gonadal steroids in obese prepubertal girls. J Clin Endocrinol Metab 1978;47:974.
13. Angsusingha K, Wacharasindhu S, Likitmaskul S, et al. LHRH test in normal children and children with pubertal disorders. Siriraj Hosp Gaz 1995: 47: 43-52.

การประเมินการใช้ GnRH stimulation test ในการวินิจฉัยภาวะ Central Precocious Puberty

สุทธิพงศ์ วัชรสินธุ, พ.บ., M.R.C.P.(UK), สุมาลี ศรีวัฒนา, พ.บ.*, สุภาพ อรุณภาคมงคล, วท.บ.*, วรศักดิ์ โชติเลอศักดิ์, พ.บ.**

การวินิจฉัยภาวะ central precocious puberty (CPP) ซึ่งเป็นที่ยอมรับกันโดยทั่วไป จำเป็นต้องทำ GnRH stimulation test การทำ test ดังกล่าวต้องใช้เวลาประมาณ 2 ชั่วโมงซึ่งทำให้เสียเวลาและค่าใช้จ่ายมาก คณะผู้วิจัยได้ทำการศึกษารูปแบบของระดับ LH และ FSH ระหว่างการทำ GnRH test ในเด็กผู้หญิงจำนวน 27 ราย ที่มาพบด้วยเรื่องเป็นสาวก่อนวัยอันควรที่รุนแรงแตกต่างกัน และพบว่าในการวินิจฉัยดังกล่าว ไม่จำเป็นต้องทำการตรวจเลือดที่เวลา 90 และ 120 นาที นอกจากนั้นการใช้ระดับ basal LH/FSH ที่มีค่ามากกว่า 0.2, LH/FSH ที่ 30 นาที หลังให้ GnRH มากกว่า 0.9 และ peak LH/FSH ที่มีค่ามากกว่า 1.0 สามารถทำนายภาวะ CPP ได้ถูกต้องเท่ากับ ร้อยละ 87.3, 89.4 และ 93.8 ตามลำดับ

คำสำคัญ : หนุ่มสาวก่อนวัยอันควร

สุทธิพงศ์ วัชรสินธุ และคณะ
จดหมายเหตุทางแพทย์ ฯ 2543; 83: 1105–1111

* หน่วยต่อมไร้ท่อ, ภาควิชากุมารเวชศาสตร์, คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย, กรุงเทพ ฯ 10330