Original Study

Gonadotropin and Estradiol Levels after Leuprolide Stimulation Tests in Brazilian Girls with Precocious Puberty

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A B S T R A C T

Study Objective: To determine the best cutoff value on the leuprolide stimulation test for the diagnosis of central precocious puberty (CPP) in a Brazilian population.

Design, Setting, and Participants: This observational study included 60 girls with CPP, as shown on the basis of serum concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) before and 3 hours after subcutaneous administration of 500 μg leuprolide acetate and by measuring serum estradiol concentrations 24 hours later. Six months later, each subject was clinically evaluated to determine whether she had experienced progressive or nonprogressive puberty.

Main Outcome Measures: Analyzing the best cutoff for LH after subcutaneous administration of 500 μg leuprolide acetate.

Results: The best cutoff was a 3-hour LH level of greater than 4.0 mIU/mL, providing the highest sensitivity (73%) and specificity (83.1%), whereas a 3-hour LH level greater than 8.4 mIU/mL had a specificity of 100%. A 24-hour E2 concentration greater than 52.9 pg/mL had a sensitivity of 68% and a specificity of 74%. There was no association between pubertal development and disease progression. Signs such as thelarche and pubarche did not determine the evolution of the disease ($P = .17$). Clinical condition was associated with bone age/chronological age ($P = .01$), basal LH ($P < .01$), 3-hour LH ($P = .02$), baseline LH/FSH indices ($P < .01$) and after 3 hours ($P < .01$), and E2 at 24 hours ($P = .02$).

Conclusion: The optimal parameter indicating hypothalamic–pituitary–gonadal axis activation in our sample was a 3-hour LH level greater than 4.0 mIU/mL. A diagnosis of CPP, however, should be based on a set of criteria and not on an isolated measurement, because typical laboratory findings associated with CPP may not be present in all patients.

Key Words: Precocious puberty, GnRH analog testing, Leuprolide stimulation, Luteinizing hormone, Estrogen

Introduction

Precocious puberty (PP) is defined as the onset of pubertal development before 8 years of age in girls.[1,2] In most individuals, this condition is caused by the activation of the hypothalamic–pituitary–ovarian axis, resulting in the pulsatile secretion of hypothalamic gonadotropin-releasing hormone (GnRH), which promotes an increased secretion of luteinizing hormone (LH) and, to a lesser degree, of follicle-stimulating hormone (FSH).[3] Although central PP (CPP) may be caused by hypothalamic lesions, resulting from tumors, malformations or irradiation, most cases in girls have no central lesion detected on magnetic resonance imaging (MRI).[4] CPP has early effects on bones due to the sensitivity of bone growth plates to estrogen, resulting in premature closure of growth cartilage and short stature.[5,6] In addition, girls with this disorder may have problems with sexuality and may express feelings of loneliness and exemplary behavior.[7]

The strategy usually used to diagnose CPP includes clinical monitoring of pubertal development and growth pattern, assessment of bone age by radiography, pelvic ultrasound, measurement of hormone profiles, and searching for possible factors responsible for the onset of PP.[8,9] Studies have shown that a single basal LH measurement may be adequate to confirm but not to refute the presence of CPP[10] and can be used as a screening test to identify girls with CPP and to determine those who should undergo GnRH stimulation tests.[11] The gold standard for the diagnosis of CPP, however, is the assessment of gonadotropin response, mainly of the secretion of LH in response to GnRH[12] or its analog, aGnRH.[13] The administration of aGnRH initially leads to the hypersecretion of LH and FSH, known as the flare-up effect, followed by pituitary desensitization and the suppression of LH and FSH after approximately 10 days of continued aGnRH use.[14] This desensitization to gonadotropins results from the downregulation of GnRH receptors, leading to a decrease in intracellular signaling.[14] Because the aGnRH test is based on the flare-up effect, activation of the hypothalamic–pituitary–ovarian axis usually results in the increased secretion of gonadotropins. The use of different assays in previous studies, however, has prevented a consensus from being reached on the interpretation of these tests. We therefore sought to determine the optimal

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cutoff values of LH for the diagnosis of CPP in a Brazilian population.

Methods

This prospective observational study enrolled 61 girls who came to the Childhood and Adolescence Gynecology Clinic of the Ribeirão Preto Medical School, University of São Paulo (HCFMRP/USP), from 2009 to 2010 due to the presence of secondary sexual characteristics before age 8 years and in whom clinical examinations showed secondary isosexual characteristics suggesting a diagnosis of PP. PP was clinically evaluated using the Marshall and Tanner criteria (breast development stage M2 or higher and pubic hair development in stage P2 or higher).[15] All subjects underwent computed tomography (CT) of the sella turcica, pelvic ultrasound to evaluate the morphology and volume of the ovaries and uterus, and radiography of the left wrist to assess bone age, which was determined as described previously.[16] The growth curve of each girl was also monitored. If the first signal of pubertal development was pubarche, androgen concentrations were measured to exclude congenital adrenal hyperplasia, evaluating testosterone and dehydroepiandrosterone sulfate basal levels and performing cortrosyn test, in which 17-hydroxyprogesterone and cortisol levels were measured 1 hour after stimulus. All subjects were submitted to aGnRH testing at their first clinic visit, when a fasting venous blood sample was obtained for baseline FSH and LH concentrations, followed by the subcutaneous administration of 500 μg leuprolide acetate (Lupron). Three hours later, when supposedly the peak of LH in response to GnRHa could be observed, a second blood sample was collected to evaluate the FSH and LH concentrations, and 24 hours later, a third blood sample was collected to assess estradiol (E2) levels.

Six months later, all patients were scheduled for a second visit, in order to evaluate the progression of puberty. Tanner stage, growth curve, and bone age on radiography were evaluated in this second clinical visit, after which the subjects were divided into 2 groups: one with PP (n = 30) and the other with nonprogressive puberty (NPP) (n = 31). A legally authorized representative of each subject signed an informed consent form for participation in the study, which was approved by the HCFMRP/USP Ethics Committee.

LH, FSH, and E2 levels were determined by chemiluminescence, using specific antibodies and an amplified enzyme in a DPC Immulite 2000 analyzer (Diagnostic Products Corporation, Los Angeles, CA), a random, automated system. The analytical sensitivities of these assays, established in our laboratory, were 0.05 mIU/mL for LH, 0.2 mIU/mL for FSH, and 8 pg/mL for E2.

Statistical Analyses

Frequency tables were used for qualitative variables, and descriptive statistics were used for quantitative variables. The χ^2 test was used to assess the association between 2 qualitative variables. The Mann–Whitney test was used to compare independent variables. Receiver operating characteristic (ROC)[17] curves were used to estimate cutoffs for baseline LH, 3-hour LH, and 24-hour E2 levels that optimally distinguished PP from NPP. All statistical analyses were performed using SAS 9 software through PROC FREQ and PROC MEANS.

Table 1
Disorders of Pubertal Development and Their Prevalence, in Number of Patients, Over 6 Months (N = 60)

Clinical Condition	Prevalence over 6 Months, n (%)
Premature thelarche, n = 30	Nonprogressive puberty, 19 (63.3) Progressive puberty, 11 (36.7)
Premature pubarche, n = 12	Nonprogressive puberty, 6 (50.08) Progressive puberty, 6 (50.0)
Premature thelarche + pubarche, n = 18	Nonprogressive puberty, 5 (27.8) Progressive puberty, 13 (72.2)

Results

Sixty subjects completed the 6-month follow-up; 1 patient in the PP group dropped out of the study. At initial evaluation, the subjects ranged in age from 11 to 96 months, with 14 (23%) having a gap of more than 2 years between bone and chronological age, but none showing alterations in the central nervous system on CT. We found that 30 subjects presented with premature thelarche; of these, 11 girls exhibited secondary sexual characteristics such as breast development and pubic hair. Twelve subjects presented with premature pubarche, with 6 girls exhibiting progression of the secondary sexual characteristics. A total of 18 girls presented with both thelarche and pubarche; of these, 13 girls exhibited progression of the secondary sexual characteristics (Table 1).

There was no association between isolated premature thelarche or pubarche with puberty progression; signs of precocious puberty, such as thelarche and pubarche, did not determine the progression of the disease ($P = .17$). Clinical condition was associated with several disease variables, including bone age/chronological age ratio ($P = .01$), basal LH ($P < .01$), 3-hour LH ($P = .02$), LH/FSH indices at baseline ($P < .01$) and after 3 hours ($P < .01$), and E2 at 24 hours ($P = .02$) (Table 2).

Baseline LH, 3-hour LH, and 24-hour E2 concentrations were analyzed using ROC curves. The best area under the curve for LH surge was observed after 3 hours from the

Table 2
Clinical Characteristics of the Subjects with Progressive and Nonprogressive Precocious Puberty

	NPP Group (n = 30)	PP Group (n = 30)	P^*
Age, mo	55.50 ± 31.47	64.82 ± 28.62	.36
BA/CA ratio	1.12 ± 0.04	1.32 ± 0.05	<.01
Baseline LH level, mIU/mL	0.07 ± 0.01	0.21 ± 0.04	<.01
3-hr LH level, mIU/mL	3.17 ± 0.35	9.69 ± 1.97	<.02
Baseline FSH level, mIU/mL	2.90 ± 0.46	4.11 ± 1.00	.28
3-hr FSH level, mIU/mL	30.29 ± 3.66	35.68 ± 6.14	.45
Baseline LH/FSH ratio	0.03 ± 0.01	0.06 ± 0.01	<.01
3-hr LH/FSH ratio	0.17 ± 0.03	0.41 ± 0.11	<.01
24-hr E2 level, pg/mL	53.50 ± 11.11	72.67 ± 9.77	.02

BA/CA, bone age/chronological age; E2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; NPP, nonprogressive puberty; PP, progressive puberty; SD, standard deviation
Results reported as mean ± SD.
* Mann–Whitney *U* test.