The diagnostic value of first-voided urinary LH compared with GNRH-stimulated gonadotropins in differentiating slowly progressive from rapidly progressive precocious puberty in girls

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Abstract

Objective: Characterization of pubertal progression is required to prevent unnecessary intervention in unsustained or slowly progressive (SP) precocious puberty (PP), while delivering hormonal suppression in rapidly progressive (RP) PP. We aimed to assess the diagnostic value of first-voided urinary LH (ULH) compared with GNRH-stimulated gonadotropins in differentiating these forms of PP.

Methods: A total of 62 girls with PP underwent both GNRH stimulation and ULH assay. Fifteen girls with peak LH ≥ 10 IU/l started treatment immediately, whereas the other 47 girls were evaluated after 6 months for pubertal advancement, height acceleration, and bone-age maturation. Based on these criteria, the participants were assigned to five subgroups: pubertal regression, no progression or progression by one, two or three criteria. The first three subgroups were defined as SP-PP ($n=29$), while the other two subgroups were defined as RP-PP ($n=18$). An additional 23 prepubertal girls were evaluated for ULH.

Results: ULH but not serum gonadotropins could distinguish girls with two and three criteria from less progressive subgroups. By comparison with SP-PP (i.e. regression group and groups 0 and 1), those with RP-PP (group 2 + 3) had lower peak FSH (9.28 ± 2.51 vs 12.57 ± 4.30 ; $P=0.007$) and higher peak LH:FSH ratio (0.42 ± 0.30 vs 0.22 ± 0.12 ; $P=0.022$) and ULH (1.63 ± 0.65 vs 1.05 ± 0.26 IU/l; $P<0.001$). Based on receiver operating characteristics analysis, a ULH cutoff of 1.16 IU/l had a better sensitivity (83%) and positive and negative predictive values (65 and 88% respectively) than the other two parameters, with a specificity of 72%.

Conclusions: ULH assay is a noninvasive, reliable method that can assist in the distinction between SP- and RP-PP.

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Introduction

Precocious puberty (PP) in girls is classically defined by the onset of secondary sexual characteristics before 8 years of age (1), but subsequent pubertal maturation can be quite varied (2). In many girls, PP takes a rapid course of progression (rapidly progressive PP; RP-PP) with an early menarche and fusion of the epiphyseal growth plates, leading eventually to a reduced final height if not treated. In a subset of girls with PP, however, the growth rate

slows to normal for age, skeletal maturation progresses in accordance with chronological age and there is little to no risk of impairment of final height (slowly progressive PP; SP-PP) (3, 4, 5). Other conditions of nonprogressive PP include premature thelarche (6, 7) and unsustained PP that is characterized by a regression of sexual precocity (4). Due to their benign course, SP-PP and other nonprogressive forms of PP do not warrant therapy

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with gonadotropin-releasing hormone agonist (GNRHa). Differentiating these forms from RP-PP is therefore essential to prevent unnecessary intervention in a population that accounts for at least 50% of girls with PP (7, 8). A distinction between these forms of PP may be difficult on clinical grounds, however, because all these patients may present initially as isolated breast development.

The gold standard for the diagnosis of true (central) PP is the measurement of gonadotropins following GNRH stimulation test (1, 2), where peak luteinizing hormone (LH) and peak LH:follicle-stimulating hormone (FSH) ratio are the most valuable diagnostic parameters. There is however an overlap between prepubertal and early pubertal values (1, 9) and between girls with premature thelarche and progressive PP (6, 10). It was suggested therefore that pubertal progression and growth acceleration should be documented over a 3- to 6-month period before GNRHa therapy is initiated.

More than a decade ago, along with the development of high-sensitive immunoassay for gonadotropins that replaced the RIA, Demir *et al.* (11) have shown that urinary gonadotropins are age related and significantly increased during puberty (12). It has been suggested that urinary gonadotropins measurements can be used for differential diagnosis of pubertal disorders (13). This is based on the assumption that gradual elevation of nocturnal LH secretion before and at the onset of puberty can be reflected by first-voided urinary LH (ULH). In our prospective study, we aimed to evaluate the value of first-voided ULH measurements in predicting pubertal course and differentiating SP-PP from RP-PP, by comparison with GNRH-stimulated gonadotropins. In our study, the term SP-PP denotes for all kinds of non-RP-PP, namely SP, nonprogressive, and unsustained PP which share in common a benign course of puberty.

Subjects and methods

Population

The study comprised girls who were referred to the pediatric endocrinology unit at Kaplan Medical Center for suspected PP between June 1, 2006 and December 31, 2011. A total of 68 girls with breast development before 8 years but later than 3 years of age were eligible for the study, and only 64 girls were recruited (four girls declined to participate in the study). All participants underwent an initial work-up that included puberty staging, auxological measurements, bone age evaluation, combined GNRH–ACTH (Synacten) stimulation test, and first-voided

ULH measurement. The participants were instructed to empty their bladders just before bedtime and to refrain from drinking until providing first-voided ULH the next morning. First-voided urine sample was collected in the morning shortly before the performance of GNRH–ACTH stimulation test. ACTH stimulation test was carried out to exclude nonclassical congenital adrenal hyperplasia (NC-CAH) in our population that included Ashkenazi Jews, an ethnic group with high prevalence of CAH due to 21-hydroxylase deficiency (14). Reportedly, true PP was found to be quite frequently (27%) a presenting sign of NC-CAH among Israeli children, mostly girls (15). The exclusion criteria were non-central PP, CAH, and nocturnal enuresis. As most children under 3 years of age lack full urinary control (16), a lower age limit of 3 years was set for participation in the study. In addition, this age limit excluded girls with the benign form of breast budding in the neonatal period (infantile mammoplasia) that gradually regresses over the first 2 years of life (17). Mothers of participants reported on their age of menarche. Two girls that were diagnosed with NC-CAH (both presented with breast budding and pubic hair) were excluded, leaving 62 participants in the study.

A control group of 23 healthy prepubertal girls aged 3.1 to 6.5 years (mean 4.6 ± 1.1 years) from hospital staff families and patients in the pediatric orthopedic unit before discharge provided first-voided urine.

The study was approved by the Ethics Committee of Kaplan Medical Center and the Israeli Ministry of Health. Informed consent was obtained from parents of all participants.

Study design

Following GNRH stimulation test, 15 girls with peak LH of ≥ 10 IU/l were considered to have advanced puberty and immediately started treatment with monthly injections of GNRHa (group LH > 10). Although these girls may well be in a process of pubertal progression, this cannot be proven and so they were excluded from the study analysis. In total, 47 girls with peak LH of < 10 IU/l were followed over 6 months without treatment and then a progression of puberty was assessed by a scoring method of three criteria: advancement of breast Tanner stage, height velocity SDS of ≥ 2 , and a ratio of skeletal age to chronological age advancement of > 1 (i.e. the difference between the first- and second-skeletal age readings divided by 6-month period). Based on the puberty progression score, the girls were divided into five subgroups: girls with complete regression of thelarche (regression group); girls with sustained puberty but no criteria

supporting pubertal advancement (group 0); and girls presented with one (group 1), two (group 2), or three (group 3) criteria of pubertal advancement.

Subsequently, we combined the regression group and groups 0 and 1 into SP-PP group and groups 2 and 3 into a RP-PP group.

For each group, we assessed the diagnostic value of the following variables: basal and peak-stimulated gonadotropins, LH:FSH peak ratio, and first-voided ULH.

Clinical examination

Breast and pubic hair staging was determined by experienced pediatric endocrinologists according to Marshall & Tanner (18). Height was measured by a nurse using a wall-mounted Harpenden stadiometer, and the average of three measurements was calculated. Weight was expressed as BMI. At the time of clinical examination, both the nurse and the endocrinologists were blinded to the test results. Height SDS, BMI, and BMI-SDS were calculated by Growth analyzer 3 software (The Dutch Growth Research Foundation, Rotterdam, The Netherlands) based on CDC growth charts published in 2000 (19). Height velocity was calculated by Auxology software version 1.0 b17 (Pfizer, New York, NY, USA) based on Tanner–Davies standards in North-American children (20).

Skeletal age examination

A radiological assessment of skeletal age was carried out according to Greulich & Pyle (21) by one endocrinologist (AZ). Each radiograph was assessed twice or more (where the time spanned between two readings was at least 1 month), until two readings differed by 3 months or less. The reader was blinded to previous skeletal age evaluation(s) and to the clinical and laboratory data of each patient. The average of two similar readings was calculated. Bone age was transformed to SDS by dividing the difference between chronological age and bone age by age-related s.d., as reported by Greulich & Pyle. The intra-observer variability was 3.0 ± 3.2 months based on the difference between the first and second skeletal age readings of 109 radiographs.

Hormone analysis

A combined GNRH–ACTH (Synacten)-stimulated test was carried out in all participants. Serum LH and FSH levels were determined at baseline and 30, 45, 60, and 90 min after i.v. administration of $50 \mu\text{g}/\text{m}^2$ surface area (up to $100 \mu\text{g}$) of LHRH (Gonadorelin; Aventis Pharma, Frankfurt

Am Main, Germany). LH and FSH serum levels were measured by two-site immunochemiluminescence assay (ICMA) (ADVIA Centaur, Siemens Healthcare Diagnostics, Erlangen, Germany). The detection limits for LH and FSH were 0.07 and 0.3 IU/l and maximal intra-assay coefficients of variance (CV) were 3.0 and 2.9% by manufacturer report. The interassay CV for LH ranged between 9.9 and 8.8% for levels of 1.5 and 64.0 IU/l respectively, and 7.8 and 6.7% for FSH levels of 7.8 and 48 IU/l respectively (by our laboratory study).

Urine LH was measured either at the day of collection or the following day by the same assay (ICMA) used for serum LH. In order to validate the assay that was originally planned for serum LH measurements, we conducted some preliminary studies.

- 1 Spiking recovery test. Varying amounts of recombinant LH (Luveris 75 IU) were added to ten urinary samples with endogenous LH levels of 0.78–5.92 IU/l. The recoveries ranged from 93.0 to 115.0% with a mean of 103.5%.
- 2 Functional sensitivity. Forty-six urine samples were measured five times each at the same run and the mean LH levels were plotted against CV ($R^2=0.73$). The functional sensitivity of the ULH assay was 0.76 IU/l based on ULH level corresponded to a CV of 20%.
- 3 Intra-assay variability. Five urine samples were assayed ten times each at the same run. The intra-assay CV values were 19.5, 6.3, 6.4, 6.4, and 5.2% for mean urine LH levels of 0.78, 1.73, 3.70, 5.05, and 7.80 IU/l respectively.
- 4 Interassay variability. Three urine samples were assayed ten times each at ten different runs over 11 consecutive days (skipping 1 weekend day). Urine samples were stored at 4°C until assayed. The interassay CV values were 9.7, 5.5, and 4.7% for mean urine LH levels of 1.23, 4.64 and 7.24 IU/l respectively.
- 5 Effect of storage temperature and duration. A single urine sample was divided into three groups of ten vials each and stored either in room temperature, at 4°C , or at -20°C . Three urine samples, one from each group, were assayed consecutively over 11 days. The CV of the three groups were 9.3, 6.7, and 4.7% for samples that were stored in room temperature, at -20°C , or at 4°C respectively. In addition, two urine samples that were stored at -70°C and assayed after 3 and 6 months, yielded LH levels that were 94 and 93% respectively of the original LH concentration obtained in the first day of collection.

6 Reproducibility of ULH. First-voided urine samples were provided twice (in 2 consecutive days) by 27 healthy children and adolescents aged 3–18 years (22 females). The first sample of each participant was kept in the home refrigerator for 24 h, and both samples were assayed at the day of the second sample collection. By regression analysis, the two samples were highly correlated ($R=0.862$; $P<0.001$).

Statistical analysis

For comparison of two variables, we used *t*-test with Mann–Whitney rank sum test for non-parametric data. One-way ANOVA was used for a comparison of many variables, with Kruskal–Wallis ANOVA on ranks applied for nonparametric data. In cases of significant difference by ANOVA, we performed multiple comparisons by Dunn's method or by Bonferroni *t*-test in order to identify group or groups that differ from the others.

Correlations between ULH and serum gonadotropins were performed by regression analysis.

The variables found to be significant in the distinction between SP- and RP-PP were analyzed by receiver operating characteristics (ROC) curves to select cutoff points. The ROC curve is used to discriminate diseased cases from normal cases. In the ROC curve, the true-positive rate (sensitivity) is plotted in function of the false-positive rate (1-specificity) in order to identify the cutoff value of a parameter (22).

In addition, ROC analysis was also carried out for basal and peak LH, two parameters commonly used to detect PP. Sensitivity and specificity were calculated for each variable using the area under the ROC curve.

Data are presented as mean $\pm$ s.d. A *P* value of <0.05 was considered significant. Statistical analysis was carried out by SigmaPlot/SigmaStat software and SPSS version 18.

Results

Forty-seven girls with breast development before 8 years of age and GNRH-stimulated peak LH of <10 IU/l were followed for 6 months without treatment. Based on the predefined clinical and radiological criteria (detailed in study design section), nine girls were assigned to the regression group, seven to group 0, 13 to group 1, 15 to group 2, and three patients were assigned to group 3. Due to the small number of girls in group 3, groups 2 and 3 were combined to a single group 2+3.

Table 1 Maternal history of menarche, auxological measurements, pubertal staging and bone-age assessment in 62 girls, divided into groups based on their clinical outcome. Data are presented as mean $\pm$ s.d. with range of values in parentheses.

	Regression group	Group 0	Group 1	Group 2 + 3	Group LH > 10	Significant differences
Number	9	7	13	18	15	
Maternal menarche (years)	12.6 $\pm$ 1.9 (4) ^a (10.0 to 14.0)	12.6 $\pm$ 0.5 (4) (12.0 to 13.0)	12.4 $\pm$ 0.9 (11) (11.0 to 14.0)	12.1 $\pm$ 0.9 (16) (11.0 to 14.0)	13.0 $\pm$ 1.7 (12) (11.0 to 16.0)	NS
Breast budding age (years)	6.6 $\pm$ 1.1 (5.1 to 7.8)	6.0 $\pm$ 1.7 (4.0 to 7.9)	7.2 $\pm$ 0.9 ^d (5.8 to 7.9)	6.4 $\pm$ 1.1 (3.9 to 7.8)	7.4 $\pm$ 0.7 (6.0 to 7.9)	d: vs group 2 + 3
Age of first visit (years)	6.8 $\pm$ 1.1 (5.6 to 8.1)	7.1 $\pm$ 1.2 (4.8 to 7.9)	7.5 $\pm$ 0.8 (6.6 to 8.6)	7.2 $\pm$ 1.0 (4.8 to 8.6)	8.0 $\pm$ 0.8 (6.3 to 8.5)	NS
Height-SDS	0.52 $\pm$ 1.29 (–1.74 to 2.58)	0.91 $\pm$ 0.83 (–0.86 to 1.50)	0.76 $\pm$ 0.72 (–0.59 to 1.87)	1.17 $\pm$ 0.94 (–0.65 to 2.80)	0.58 $\pm$ 1.24 (–1.65 to 2.81)	NS
BMI-SDS	0.02 $\pm$ 0.91 ^c (–1.59 to –1.48)	1.83 $\pm$ 0.75 (0.94 to 2.81)	1.42 $\pm$ 0.70 (0.07 to 2.48)	1.35 $\pm$ 0.99 (–1.23 to –2.48)	0.46 $\pm$ 0.81 ^f (–0.95 to –1.19)	e: vs all groups; f: vs groups 0, 1 and 2 + 3
Breast stage	9/0/0 ^b	3/4/0	5/8/0	9/9/0	4/10/1	
Pubic hair stage	7/2/0 ^c	6/1/0	12/1/0	12/5/1	6/6/3	
Bone age (BA) (years)	7.6 $\pm$ 1.0 (6.3 to 9.5)	8.6 $\pm$ 1.5 (6.3 to 10.1)	9.0 $\pm$ 1.2 (7.3 to 10.5)	8.6 $\pm$ 1.5 (6.4 to 11.0)	9.1 $\pm$ 1.5 (6.5 to 10.9)	NS
BA-SDS	1.2 $\pm$ 1.4 (–0.8 to –2.4)	2.2 $\pm$ 1.1 (0.1 to 3.2)	2.1 $\pm$ 1.7 (–0.5 to 5.2)	1.9 $\pm$ 1.4 (–0.5 to 4.2)	1.5 $\pm$ 1.3 (–1.7 to 2.9)	NS

Comparisons between groups were performed by ANOVA, with statistically significant differences ($P<0.05$) marked by letters.

^aNumber of mothers that provided data on their age of first menarche.

^bNumber of girls with breast Tanner stage II, III, and IV.

^cNumber of girls with pubic hair Tanner stage I, II, and III.

The clinical characteristics of the patients in each group (including 15 patients with peak LH ≥ 10 IU/l) are described in Table 1.

All girls in this study had breast Tanner stage of II or III except for one girl with peak LH > 10 IU/l who presented with breast Tanner stage IV. Thirteen girls underwent brain MRI and two of them demonstrated incidental findings: arachnoid cyst in one girl and mild dilatation of brain ventricles in the other (probably related to a history of prematurity).

GNRH stimulation test

In a cohort of 47 girls who were followed over 6 months, basal and GNRH-stimulated LH as well as peak LH:FSH ratio were similar in the four subgroups (regression, 0, 1 and 2+3 groups) (Fig. 1A, B and D). Peak FSH levels tended to decrease as the number of pubertal criteria increased, reaching the highest levels in the regression group (14.6 ± 4.9 IU/l) and lowest levels in group 2+3 (9.3 ± 2.5 IU/l) ($P=0.016$ between these two groups) (Fig. 1C).

Figure 1

Basal LH (A), peak LH (B), peak FSH (C), and peak LH:FSH ratio (D) levels in girls with regression of pubertal signs (Reg), no progression (Gr 0), or a progression by one (Gr 1), or two or three (Gr 2+3) criteria of pubertal advancement. Data are displayed as box-plots with median, upper and lower quartiles; minimal and maximal nonextreme values (error bars); and extreme values (circles). * $P=0.016$ (group 2+3 vs regression group; Fig. 1C).

Girls in the RP-PP group had significantly lower FSH peak and higher LH:FSH peak ratio in comparison with the SP-PP group, whereas basal gonadotropins and peak LH levels were not different between these groups (Table 2).

First-voided ULH

First-voided ULH levels were evaluated in the four subgroups and in the control group of prepubertal girls. Mean ULH levels in group 2+3 was significantly higher than in all other groups (ANOVA with multiple comparisons study) (Fig. 2A).

Mean ULH levels was significantly higher in RP-PP than in the SP-PP (Table 2) and prepubertal girls (Fig. 2B). By regression analysis that included the entire PP cohort ($n=62$), ULH levels were positively correlated both with GNRH-stimulated LH ($R=0.589$; $P<0.001$) and peak LH:FSH ratio ($R=0.717$; $P<0.001$), but not with other gonadotropins values.

Differentiating SP from RP-PP

Based on the ROC curve (Fig. 3), we determined the optimal cutoff points for the parameters that distinguished SP- from RP-PP, namely peak FSH, peak LH:FSH ratio, and first-voided ULH (Table 4). ULH cutoff point (≥ 1.16 IU/l) had better sensitivity and positive and negative predictive value than all other parameters, and a specificity that was equal to that of peak LH:FSH ratio. The area under the ROC curve was the largest in the ULH curve (Table 3).

Discussion

Measurement of first-voided ULH to detect pubertal changes was suggested over a decade ago (12, 13, 23, 24), but was not adopted in the clinical practice of pediatric endocrinology. Apparently, lack of normative data for normal and disordered puberty prevented the implementation of this measure so far. In our study we assessed the diagnostic value of ULH by comparison with GNRH-stimulation test in predicting the outcome of girls with PP. We found that peak FSH, peak LH:FSH ratio, and first-voided ULH are significantly different between SP-PP and RP-PP, but ULH had better sensitivity, positive and negative PV than the other two parameters, and a specificity that was equal to that of peak LH/FSH.

Pulsatile secretion of gonadotropins, especially LH, starts as early as mid- and late-childhood, especially during sleep-time, with accentuation of gonadotropin secretion at the onset of puberty (25, 26, 27). As repeated

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Table 2 Serum and urinary gonadotropins in slowly progressive vs rapidly progressive precocious puberty. Data are presented as mean $\pm$ s.d. with range of values in parentheses. Comparisons between groups were performed by *t*-test.

	Slowly progressive group (n=29)	Rapidly progressive group (n=18)	P value
Basal LH (IU/l)	0.12 $\pm$ 0.05 (0.1–0.3)	0.24 $\pm$ 0.33 (0.1–1.4)	NS
Peak LH (IU/l)	2.78 $\pm$ 1.78 (0.3–9.0)	3.69 $\pm$ 2.60 (0.3–9.0)	NS
Basal FSH (IU/l)	1.17 $\pm$ 0.89 (0.1–3.5)	1.78 $\pm$ 1.48 (0.4–6.5)	NS
Peak FSH (IU/l)	12.57 $\pm$ 4.30 (4.1–21.5)	9.28 $\pm$ 2.51 (4.1–14.2)	0.007
Peak LH/peak FSH	0.22 $\pm$ 0.12 (0.06–0.56)	0.42 $\pm$ 0.30 (0.03–1.06)	0.022
ULH (IU/l)	1.05 $\pm$ 0.26 (0.76–1.79)	1.63 $\pm$ 0.65 (0.78–3.32)	<0.001

NS, not significant.

serum sampling of gonadotropins during sleep is impractical as a routine diagnostic tool, it has been suggested that first-voided urine levels of gonadotropins can reflect their nocturnal secretion (13). Initial studies that examined the change in urinary gonadotropins from childhood to puberty were limited however by the poor sensitivity of the assay (RIA) (23, 24), and only the introduction of highly-sensitive immunoassays has allowed a reliable measurement of even low concentrations of gonadotropins in urine (28). Unlike Demir *et al.* (12) who used ultrasensitive immunofluorometric assay (IFMA), we used ICMA for ULH measurement, but the intra- and interassay variations were comparable between the studies.

For evaluating the diagnostic value of first-voided ULH compared with GNRH-stimulated gonadotropin in the prediction of the natural history of PP (SP-PP vs RP-PP), we assigned the participating girls to several subgroups, based on a clinical and radiological evaluation after 6 months of follow-up. Currently, there is a lack of consensus criteria for determining SP-PP and RP-PP. Pescovitz *et al.* (6) had divided girls with premature breast development into six clinically distinct groups that ranged from premature thelarche to RP-PP based on various combinations of clinical (pubic hair, growth acceleration) and radiological (skeletal age) features.

Others have used various terms such as 'thelarche variant', 'non-classical premature thelarche', and 'atypical premature thelarche' to describe intermediate forms of PP on the continuum between premature thelarche and RP-PP (29, 30). Unlike these descriptive terms of PP variants, we used a simple, numerical system whereby progression of clinical and radiological variables was scored from 0 (nonprogressive PP) to 3 (RP-PP with a progression of Tanner stage, skeletal age, and height velocity). All three criteria were equally important for the categorization of the patients. An exception was a group of girls with GNRH-stimulated LH $\geq$ 10 IU/l who were not evaluated for pubertal progression and started treatment immediately. By all reports this level of peak LH is associated with advanced puberty (1, 9, 10, 31, 32, 33, 34, 35); therefore, for ethical reasons we decided not to defer treatment for 6 months, a delay that could have yielded rapid progression toward menarche and further advancement of skeletal maturation. Furthermore, 11 out of 15 girls in this group presented with breast Tanner stage III or more, which makes an observational follow-up unnecessary according to the consensus statement on treatment of PP (1).

Within the four groups of girls with peak LH of <10 IU/l (regression group and groups 0, 1, and 2+3),

Table 3 Area under the curve, sensitivity, specificity, and positive and negative predicted values of the ROC-based optimal cutoff values that predict RP precocious puberty. Basal and peak LH values were added, although these parameters did not distinguish SP- from RP-PP. The best variables are in bold letters.

	Peak LH:FSH ratio	Peak FSH	First-voided ULH	Basal LH^a	Peak LH
Cutoff value (IU/l)	$\geq$ 0.24	$\leq$ 10.45	$\geq$ 1.16		$\geq$ 2.35
Area under the ROC curve	0.701	0.736	0.835	0.595	0.594
Sensitivity (%)	72	78	83		61
Specificity (%)	72	66	72		48
Positive predictive value (%)	62	58	65		42
Negative predictive value (%)	81	83	88		67

^aBasal LH has no cutoff based on ROC analysis.

Figure 2

First-voided ULH levels in prepubertal girls (Pre-Pub), in girls with regression of pubertal signs (Reg), no progression (Gr 0), a progression by one (Gr 1), or two or three (Gr 2+3) criteria of pubertal advancement and in girls with advanced puberty defined by peak LH ≥ 10 IU/L. * $P < 0.001$ (group 2+3 and group LH > 10 vs prepubertal group) (A). After combining groups Reg, 0 and 1 into SP-PP, and group 2+3 into RP-PP, ULH levels are compared between prepubertal group, SP-PP, and RP-PP.

* $P < 0.001$ (rapidly progressive group vs other groups) (B).

Data in both figures are displayed as box-plots with median, upper, and lower quartiles; minimal and maximal nonextreme values (error bars); and extreme values (circles).

mean basal and peak LH levels as well as mean peak LH:FSH ratio were similar and lacked a diagnostic value. Mean stimulated FSH level gradually declined as puberty progressed, reflecting the transition from FSH-

predominant to LH-predominant response to GNRH stimulation that is observed as puberty progressed (6). By comparison, mean levels of first-voided ULH clearly differed the group that demonstrated pubertal progression (group 2+3) from those with nonprogressive course. In addition, levels of serum and urinary gonadotropins in the nonprogressive groups were indistinguishable from levels in the control group of prepubertal girls. These observations are of clinical importance because most equivocal cases of PP lie within the range of stimulated LH below 10 IU/L.

Figure 3

ROC curve for the analysis of basal and peak LH, peak LH:FSH ratio, ULH (A), and peak FSH (B). ROC curve for peak FSH was depicted separately because the peak FSH curve has opposite direction compared with other parameters, i.e. peak FSH is lower in RP-PP and higher in SP-PP.

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Table 4 Sensitivity and specificity of various peak LH cutoff values that predict RP-PP (compared with first-voided ULH). Peak LH values were previously reported as cutoff levels that distinguish pre-pubertal from pubertal girls (reference are in parentheses).

Cutoff value (IU/l)	Sensitivity (%)	Specificity (%)
> 3.3 (9)	44	72
> 4.2 (9)	28	86
> 5.0 (32)	28	90
> 6.9 (31)	17	97
> 8.0 (32)	11	97
ULH > 1.16	83	72

While combining the subgroups into two main groups, we found that first-voided ULH and peak LH:FSH ratio were higher, whereas peak FSH was lower in RP-PP than SP-PP. Although GNRH-stimulated LH and peak LH:FSH ratio are considered the most useful parameters in detecting true, progressive PP, a paucity of normative data has hampered the development of consensus cutoffs (1). Based on GNRH-stimulated LH levels in the 95th percentile of normal prepubertal girls, Brito *et al.* (31) suggested a peak LH cutoff of > 6.9 IU/l, whereas Resende *et al.* (9) suggested a cutoff of 3.3 and 4.2 IU/l when LH was assayed by ICMA and IFMA respectively. Neely *et al.* (32) have suggested a peak LH cutoff of > 5 IU/l which reflects a level of 2 s.d. above the mean in prepubertal girls, or alternatively a more stringent threshold of > 8 IU/l that corresponds to a level of 4 s.d. above the mean in the same reference group. Many authors adopted the > 5 IU/l threshold (7, 10, 32, 33, 34). While applying this cutoff of peak LH in our study, 13 out of 18 girls (72%) in the RP group and two out of 29 girls (7%) in the SP group were wrongly assigned to the opposite group. We found that these peak LH values that have been previously published had better specificity in most cases but poor sensitivity in differentiating RP- from SP-PP, by comparison to first-voided ULH levels (Table 4). Although peak LH (and in some studies basal LH (35, 36)) is commonly used to detect true PP, we found that neither basal nor GNRH-stimulated LH holds a diagnostic value in distinguishing SP- from RP-PP. These seemingly contrasting results derive from differences in methodology. While previous studies compared LH levels in prepubertal vs pubertal girls in order to define a cutoff for serum LH, our cohort of patients comprised only pubertal girls, and a comparison of LH levels was done between girls with regression or no progression of puberty and those with RP-PP. Expectedly, a comparison between girls before and during puberty

supposes to yield significant differences. However, our study is more relevant to the common case of a girl with premature breast budding that undergoes GNRH stimulation not to differentiate her from prepubertal girls but in order to define what course of pubertal progression she is going to take. Similarly, different values of peak LH:FSH ratio (0.66; 1.0) have been suggested for the diagnosis of true PP (33, 37), but neither of them was clinically perfect (6). We found a significantly lower cutoff value for peak LH:FSH ratio (0.24), which probably reflects differences in mean FSH values (the denominator in the ratio) between the studies. While previous studies compared prepubertal girls with early pubertal girls, we evaluated the difference in peak LH:FSH ratio between girls with RP-PP and those with SP-PP who demonstrated elevated FSH levels and subsequently lower LH:FSH ratio.

In addition to its superior diagnostic value over GNRH-stimulation test, ULH reflects physiological secretion of LH rather than supraphysiological secretion of gonadotropins by GNRH-stimulation. Technically it is a simple, noninvasive procedure that saves time in the outpatient clinic and reduces inconvenience to the young patients. Other procedures such as pelvic ultrasonography were suggested as a noninvasive measure to detect PP (10), but compared with ULH it is a time-consuming procedure and operator-dependent, which probably accounts for contradictory reports on its utility (38).

There are several limitations to our study. First, although we created normative data of ULH for prepubertal girls, normative ULH levels in girls at various stages of puberty are still needed. Demir *et al.* (12) found an increase of 40-fold in ULH levels in girls from breast stages 1 to 5, using IFMA for ULH measurements. Second, although our results among 47 PP girls are promising, the diagnostic value of ULH should be confirmed in larger studies.

In conclusion, first-voided ULH is a reliable and accurate assay that may assist in the distinction between SP- and RP-PP. Being noninvasive, this test can potentially be repeated along the clinical follow-up both before pubertal suppression and during GNRHa therapy to assure adequate suppression of the hypothalamic–pituitary–gonadal axis. Nevertheless, it cannot replace the careful clinical monitoring aimed to define the course of PP and the need for therapy.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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