The Use of Morning Urinary Gonadotropins and Sexual Hormones in the Management of Early Puberty in Chinese Girls

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Author contributions

Junfen Fu, Yan Ni, and Guanping Dong contributed to the conception of this manuscript. Shumin Zhan contributed to the data acquisition, data analyses and drafted the manuscript. Danni Zhang and Robert M. Dorazio analyzed data and generated figures. Ana Liu contributed to sample collection. Jianrong Shi contributed to sample assays. Ke Huang, Wei Wu, and Rahim Ullah critically revised the manuscript for important intellectual content. Robert M. Dorazio finished English language editing. Li Zhang, and Jinling Wang contributed to the clinical evaluation of participants. All authors were involved in writing the paper and had final approval of the submitted and published versions.
Funding

This study was supported by the National Key Research and Development Program of China (No. 2016YFC1305300), Fundamental Research Funds for the Central Universities (2020XZZX002-22), Research Fund of Zhejiang Major Medical and Health Science and Technology & National Ministry of Health (WKJ-ZJ-1804), Zhejiang Provincial Key Science and Technology Project （LGF21H070004）, and Zhejiang Science and Technology Plan Project (2020C03121)

Conflict of interest

No potential conflict of interest exists regarding to this article.

Clinical Trial Registration Number

ChiCTR2100047299
ABSTRACT

Context: Although gonadotropin-releasing hormone stimulation test (GnRHST) is the gold standard in diagnosing central precocious puberty (CPP), it is invasive, expensive, and time-consuming, requiring multiple blood samples to measure gonadotropin levels.

Objective. We evaluated whether urinary hormones could be potential biomarkers for pre- or post-puberty, aiming to simplify the current diagnosis and prognosis procedure.

Design, Setting, and Participants: We performed a cross-sectional study of a total of 355 girls with CPP in National Clinical Research Center for Child Health in China, including 258 girls with positive and 97 girls with negative results from GnRHST. Twenty patients received GnRH analogue (GnRHa) treatment and completed a six-month follow up.

Main Outcome Measures: We measured luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol, prolactin, progesterone, testosterone, and human chorionic gonadotropin in the first morning voided urine samples.

Results: Their urinary LH levels and the ratios of LH: FSH increased significantly with the advancement in Tanner Stages. uLH levels were positively associated with basal and peak LH levels in the serum after GnRH stimulation. A cut-off value of 1.74 IU/L for uLH reached a sensitivity of 69.4% and a specificity of 75.3% in predicting a positive GnRHST result. For the combined threshold (uLH≥1.74+uLH: uFSH ratio >0.4), the specificity reached 86.6%. After 3 months of GnRHa therapy, the uLH and uFSH levels decreased accordingly.

Conclusions: uLH could be a reliable biomarker for the initial CPP diagnosis and screening; uLH also could be an effective marker for evaluating the efficacy of clinical treatment.

Key words

precocious puberty, GnRH stimulation test, urinary gonadotropins and sexual hormones test, GnRH analogue, non-invasive, diagnosis and screening
INTRODUCTION

Central precocious puberty (CPP) is the result of an early activation of the hypothalamic pituitary gonadal axis (HPG-axis)\(^1\). Because CPP impairs both physical and psychosocial growth in children\(^2\), testing based on specially hormone measurements is very important for early diagnosis and treatment. The gonadotropin releasing hormone (GnRH) stimulation test (GnRHST) has been the gold standard for confirming the activation of the HPG-axis\(^3\). GnRHST requires out-patient hospitalization and a sequence of blood collections that are not only time-consuming but are also costly and painful. Therefore, an updated proposal and international consortium suggested the use of un-stimulated luteinizing hormone (LH) levels in random blood samples instead of GnRHST\(^4\).

Analysis of urinary gonadotropins for differentiating pre-pubertal and post-pubertal subjects was initially carried out almost 50 years ago. This study revealed that sexual maturation is accompanied with more excretion of LH and FSH in the urine\(^5\). Owing to a marked increase in the night-time amplitude and frequency of gonadotropins\(^6\), gonadotropins concentrations in the first morning voided (FMV) urine samples may reflect the nocturnal secretion and can be used as one of the potential alternative approaches for assessing pubertal development. Even small amounts of intact gonadotropins can now be detected owing to the development of third-generation assays with improved sensitivity\(^7\). These assays have led to the use of urine as a sample of choice, rather than use of serum.

Various studies have reported consistency between serum and urinary gonadotropins. FMV uLH correlates with the basal and GnRH stimulated serum LH (sLH) and gives a sensitivity of 75% and a specificity of 92% according to the GnRHST results\(^8\). Assessment of puberty signs with urinary gonadotropins helps to distinguish the slowly-progressive and rapidly-progressive precocious puberty\(^9\). In addition, a recent study reported that FMV uLH provides accurate information about the puberty suppression and can be used to monitor the adequacy of GnRH analogue (GnRHa) therapy\(^10\).

Therefore, in the present study we conducted analyses to validate the use of FMV gonadotropins and sexual hormones in the diagnosis of early puberty in girls and to establish cut-off values of these biomarkers. We also validated whether FMV sampling is adequate for monitoring the clinical efficacy of GnRHa therapy.
PARTICIPANTS AND METHODS

Participants

The study was approved by the Medical Ethics Committee of the Children’s Hospital, Zhejiang University School of Medicine (No. 2020-IRB-167). Chinese girls with clinical signs of precocious puberty and concerns were admitted to the Department of Endocrinology, Children’s Hospital, Zhejiang University School of Medicine. Detailed explanations of the study were conveyed to each subject and their parents, and informed consents were obtained.

Anthropometry, pubertal staging, and imaging

Height, weight, and body mass index (BMI) were recorded at enrollment. BMI was calculated as weight (kg) divided by height squared (m^2). All of these parameters were converted into Z-scores using Child Growth Standards from the World Health Organization. Pubertal status was staged according to Tanner Breast (Tanner B) and Pubic Hair (Tanner PH). To improve the validity, bone age (BA) was calculated by the Tanner-Whitehouse (TW3) method.

Pelvic ultrasound (PHILIPS IU Elite) was performed on all patients to assess uterine volume, cervical length, and follicular volume. Pituitary magnetic resonance imaging (MRI) (PHILIPS Prodiva CX 1.5T) was performed routinely to exclude intracranial causes.

Study Design

The GnRHST was used to determine CPP patients. GnRH (2.5-3μg/kg, maximum 100ug, Triptorelin Acetate, Ipsen Pharma Biotech) was administered intravenously, and serum LH and FSH levels were measured at 0, 30-, 45-, 60-, and 90-minute intervals. Patients were diagnosed as CPP cases when they had peak LH level exceeding 5 IU/L in response to GnRHST and early puberty signs before 8 years old, including progressive breast development accompanied by rapid growth (> 6-7 cm/year) and bone maturation. Patients with an evidence of tumor, adrenal disease, gonadal disease or central nervous system disorder were excluded from this study. Plasma thyroxin and thyroid-stimulating hormone (TSH) concentrations were measured to exclude hypothyroidism.

The FMV urine specimens were collected in the morning on the day of GnRHST and were stored at 4°C immediately. After centrifuging at 3,000 rpm for 10 minutes, urinary supernatants were collected and stored at -80°C until the hormones assay. Total urine volumes were recorded.

CPP patients were treated with either leuprolide acetate or triptorelin acetate, at a dose of 3.75 mg every 28 days. Stimulated peak serum LH, FSH and estradiol concentrations were evaluated 45 minutes after GnRHa injection at 3 and 6 months. Baseline and post-treatment FMV urine samples were collected from 20 girls who were diagnosed with CPP in this cohort.
Hormones assays

Gonadotropins and other sex hormones levels in urine and serum were measured by immunoochemiluminescence assays (ICMA) (Siemens Healthcare Diagnostics Products Limited, Llanberis, United Kingdom). The estradiol (E2) levels were measured by ICMA (Siemens Healthcare Diagnostics Inc., East Walpole, Massachusetts, USA). Urinary creatinine was measured by a biochemical analyzer (Mindray BS-820) and was used as adjustment factor for urine dilution.

The intra-assay coefficients of variation (CVs) and the inter-assay CVs were less than 3.7% and 7% for LH, 3.4% and 4.8% for FSH, 4% and 4.3% for estradiol (E2), 3.3% and 4.8% for prolactin (PRL), 9.7% and 12.5% for progesterone (P), 11.7% and 10.3% for testosterone (T), 6.6% and 7.4% for human chorionic gonadotropin (HCG), respectively. The limits of detection (analytical sensitivity) for LH, FSH, E2, PRL, P, T and HCG were 0.1 IU/L, 0.1 IU/L, 43.6 pmol/L, 10.6 mIU/L, 0.64 nmol/L, 0.69 nmol/L and 1 IU/L, respectively.

Statistical analyses

For statistical evaluation, concentrations below the detection limits were given a value of the lowest standard. To minimize the possible error in renal function and wide difference in body size, hormone values were multiplied by the corresponding total urine volume (×mL) or were divided by creatinine excretion levels (/uCr).

Statistical analyses were performed using SPSS software (IBM, version 23) and figures were made by GraphPad Prism (Version 8) and by the R programming language (version 3.6.0). Normally distributed data were expressed as mean ± standard deviation. Otherwise, the data were represented as median (P25, P75). The Student t test, Mann-Whitney U test, and Chi-square test were performed separately to compare measurements between girls with or without peak LH > 5 IU/L (i.e., to compare GnRHST positives versus GnRHST negatives). The urinary gonadotropins medians at different pubertal stage groups were compared using a Kruskal-Wallis test. The relationships between urinary and serum hormone levels were further compared using a heat map of correlation coefficients.

The diagnostic value of the urine hormones’ concentrations was evaluated using receiver operating characteristic (ROC) analysis. Optimal cut-off values for biomarkers were computed using sensitivity and specificity. Sensitivity is defined as the probability of detecting positive cases in GnRHST positive subjects, and specificity is defined as the probability of detecting negative cases in GnRHST negative subjects. The Youden index, a summary measure of diagnostic accuracy, is computed by subtracting one from the sum of sensitivity and specificity. The optimal cut-off value for a single biomarker or for the linear combination of biomarkers in the ROC model (a logistic regression model) was computed using the probability of detecting positive cases that maximizes the Youden index.

A Wilcoxon signed-rank test was performed to compare hormone concentrations between pre-treatment and post-treatment groups. Differences were considered statistically significant if P < 0.05.
RESULTS

1. General Characteristics
   A total of 355 girls who presented early signs of puberty before 8 years old were recruited, and they were aged between 3.92 and 9.83 years during the first hospitalization. 258 patients with peak LH > 5 IU/L were considered as the GnRHST positive (+) group, and the rest were considered as the GnRHST negative (-) group. Baseline clinical and radiological characteristics of the participants are shown in Table 1. The average age of the GnRHST (+) group was 8.23 years, whereas that of the GnRHST (-) group was 7.82 years. The GnRHST (+) group had advanced Tanner stages, larger uterine volume, and longer cervical length. Significant increases in the basal sLH and sFSH levels were observed in the GnRHST (+) group.

2. Urinary gonadotrophins in relation with pubertal status
   All girls in this study were of breast Tanner stage 2, 3 or 4. Overall, the levels of urinary gonadotrophins increased with pubertal advancement (Figure 1). Significant differences in levels of uLH, uLH: uCr, and uLH: uFSH were observed in girls with advanced Tanner stages. However, overlapping hormone levels were observed among the Tanner stages in both the original and creatinine-normalized hormones.

3. Correlation between urinary and serum hormone levels
   Pearson’s correlation coefficient was calculated to evaluate the correlation between urinary hormone levels and blood indexes or ovary maturation (Figure 2). Positive correlations were found between uLH and sLH in both the basal (r=0.451, p < 0.001) and peak levels (r=0.326, p < 0.001). These correlations also were confirmed using normalized uLH levels adjusted by the total volume of urine or urinary creatinine. Positive correlations were found between uLH: uFSH ratio and basal LH, sum LH, peak LH, IGF-1, uterine volume and uterine length.

4. ROC analysis
   Using the 355 cases who received GnRHST, we performed a ROC analysis to estimate the optimal cut-off value for predicting a positive GnRHST result (peak LH >5 IU/L) (Figure 3). uLH or urinary creatinine-adjusted uLH had the best performance of any individual urinary hormone as indicated by having the largest area under the ROC curve (AUC). However, it was interesting that the AUC of the uLH: uFSH ratio was even larger and reached a value of 0.853.

   With a sensitivity of 95%, which could possibly detect the great majority of positive cases, the optimal uLH cut-off value for screening was 0.55 IU/L (Table 2). The cut-off value for diagnosis was 1.74 IU/L with a sensitivity of 69.4% and a specificity of 75.3%. Based on AUC for a single marker, a uLH cut-off value of 1.74 IU/l combined with uLH: uFSH ratio >0.4 had a higher specificity of 86.6%.

   We then created a scatter-plot in which uLH and uFSH were used for a single data series. The GnRHST positive cases were marked in blue and the negative cases were marked in red. Logistic regression was applied to calculate the predictive probability based on using both uLH and uFSH for
the diagnosis of CPP (Figure 4). An ROC curve for the combination of biomarkers was constructed based on logistic regression, and the resulting AUC was higher than the AUCs obtained using single biomarkers. Table 2 lists the logistic regression analysis parameters for CPP diagnosis compared with single markers. The results showed that when $0.399\times uLH - 0.17\times uFSH > -0.087$, the patient was predicted with GnRHST positive results. The results showed that the sensitivity of CPP diagnosis was 78.8% and specificity was 80.4% when calculated based on the maximum Youden index.

5. Follow-up study

A total of 23 girls received GnRHa therapy, and 20 of them were enrolled in our study. Before each injection, FMV urine samples were collected from 20 participants at 3 months and at 6 months of the treatment. After the subcutaneous administration of GnRHa, blood samples were collected 45 minutes later and serum LH, FSH, and E2 levels were checked.

All post-peak LH levels throughout this follow-up study were under 1.7 IU/L, which indicates that HPG-axis was well suppressed due to the GnRHa treatment. The mean $uLH$ was $0.37 \pm 0.20$ with a range of 0.16-0.84 IU/L at 3 months, and at 6 months the mean was $0.50 \pm 0.25$ with a range of 0.24-1.07 IU/L. Stimulated peak LH was highest at the baseline and declined significantly after 3 months of GnRHa therapy (Figure 5). The $uLH$ and $uFSH$ levels decreased at 8 weeks, indicating good efficacy of the therapy; however, the levels increased slightly at 6 months (Table 3).

DISCUSSION

In this study we evaluated the diagnostic value of urinary hormones in CPP girls. We found that $uLH$ and $uLH: uFSH$ ratio were associated with $sLH$ levels and Tanner stages, which could predict puberty status. We concluded that $uLH$ could be a convenient and non-invasive biomarker as compared to the serum peak LH of GnRHST. The optimal cut-off values of $uLH$ were 0.55 IU/L for screening and 1.74 IU/L for diagnosis, respectively.

With advanced techniques of immunochemiluminescence assay and electrochemiluminescence immunoassay, several studies measured urinary gonadotropins in children undergoing through puberty and reported that urinary LH performed well in predicting the pubertal process (Table 4). Sex hormones in the urine, including LH, T, and E2, correlated cross-sectionally and longitudinally with age, anthropometry and Tanner stages. Urinary gonadotrophins reflected the clinical/physical status using the ratio of LH:FSH. Furthermore, FMV $uLH$ levels were strongly correlated with stimulated LH levels ($r = 0.91$) and with basal LH levels in blood ($r = 0.65$). FMV $uLH$ and $uLH$: $uFSH$ performed equally well as GnRHST in distinguishing early puberty and pre-puberty status. Similarly, another study found that $uFSH$: $uCr$ ratio and $uLH$/uCr ratio correlated with $sFSH$ and $sLH$ levels respectively; the authors recommended that $uLH$: uCr might play a role in identifying CPP and that $uFSH$: uCr assessment could be used to recognize germ cell failure. Based on Zung’s analysis, a FMV $uLH$ cutoff value of 1.16 IU/l had higher sensitivity (83%) and predictive values than that of serum basal LH. Likewise, it was reported that a cut-off SD score of 2 for $uLH$ had a sensitivity of 75% and a specificity of 92% in the COHENHAGEN puberty study with a large population. In another important study random urinary gonadotropins were recommended for the initial assessment of CPP in girls because there was no difference between FMV and random urinary gonadotropins levels. We consider that the main reason for various cut-off values was difference in sample size and hormone assay.
In general, our results are consistent with the previous findings, and we recommend urinary LH as a superior alternative to GnRHST. A significant increase in levels of uLH, uLH: uCr, and uLH: uFSH with the advanced Tanner stages was observed in girls. A cut-off value of 1.74 IU/L for FMV uLH reached a sensitivity of 69.4% and a specificity of 75.3% in predicting a positive GnRHST; however, when 0.399*ulH-0.17*uFSH > -0.087, the patient was predicted with GnRHST positive results with a sensitivity of 78.8% and a specificity of 80.4%. For the combined threshold of the urinary gonadotropins (uLH≥1.74+uLH: uFSH ratio >0.4), the specificity was 86.6%. A cut-off value of 0.55 IU/L for uLH showed the sensitivity of 95%, indicating the potential value of uLH for screening in large-scale population and diagnosis in clinical use. We suggest that girls who reach the cut-off level should receive further physical examination to confirm Tanner stage. Compared to uLH, urinary E2, T, HCG, and PRL levels overlapped among Tanner stages and therefore had poor correlation with puberty advancement.

The gold-standard biochemical diagnosis of CPP is mainly based on the assessment of peak gonadotropin levels in GnRHST. The disadvantages of GnRHST are the high cost and invasiveness. Other alternatives to GnRHST are serum basal gonadotropin levels, imaging and gene expression (for familial PP). Basal serum LH levels were suggested by Heo et al (2019) as the most sensitive parameter for screening CPP and early puberty. Other investigators recommended basal LH levels instead of GnRHST, but the cut-off values of basal LH varied from 0.1 to 0.83 IU/L (ICMA) with 64% to 93% diagnostic sensitivity. Similarly, pelvic ultrasonography or pituitary MRI could not distinguish pre-pubertal and pubertal stages. As a major inhibitor of hypothalamic GnRH secretion, serum MKRN3 concentration declines before puberty onset in girls and might be useful for the assessment of pubertal development; however, it is also invasive. In comparison, urinary gonadotropins can provide a simple, inexpensive, non-invasive, and well-accepted alternative to assess pubertal stages and the efficacy of CCP therapy; thus, urinary gonadotropins may even help to build a monitoring/screening system for pediatric growth in a large population.

During the follow-up period of our study, pituitary suppression during GnRHa therapy was optimally assessed by GnRH stimulation, and suppressed gonadotropin levels indicated the effectiveness of GnRHa therapy in CPP patients. In clinical practice the common method to monitor the efficacy of CPP therapy is to inject GnRHa monthly and to examine the plasma LH levels every 3-4 months. A single serum sample obtained 30-60minutes after leuprolide injection is an accurate way to evaluate treatment efficacy. Peak plasma LH levels less than 2 IU/L are considered as indicative of biochemical suppression. Efforts were made to simplify this test by using pre-infection serum basal LH, but they failed; so researchers tried to replace GnRHST by evaluating FMV urinary hormone levels before the administration of GnRHa. Zung et al found that 8% of uLH levels in 36 CPP patients with adequate gonadotropins suppression were above the pre-pubertal threshold. Their results suggested that uLH could not fully replace the serum LH in monitoring CPP. Another study conducted by Yuce et al showed a significant difference in FMV uLH levels between suppressed and unsuppressed participants. In the COHENHAGEN puberty study, urinary concentrations of LH decreased after three months of GnRH treatment to levels below +2 standard deviation. In our study the pronounced difference in uLH levels between pre- and post-treatment time points indicated the good efficacy. Several cases showed elevated uLH levels at 20 weeks; however, their uLH levels were not higher than baseline. Pubertal development and body growth should be examined every 3-6 months, and repeated blood tests are not necessary if the uLH or uLH: uFSH ratio is not elevated.

By no means should clinical manifestations of pubertal regression or arrest be given priority over hormonal tests because the probability of a positive GnRHST response after GnRHST increases with the increasing difference between bone age and chronological age.
To summarize, the relatively large number of 355 participants enrolled in our study provided convincing cut-off values of uLH for screening and diagnosis of CPP in clinical practice. A few studies found that uLH degrades with time when stored at \(-20^\circ C\) \(^9,20\); therefore, we stored our samples at \(-80^\circ C\) after centrifugation and extraction. We did comprehensive assays that cover all sorts of sexual hormones in urine. Furthermore, we also recorded the total urine volume and urinary creatinine levels to check whether urine volumes or urinary creatinine levels affected normalized hormone values. However, the performance of the tests used to recognize precocious puberty differs in younger age groups especially below 3 years of age\(^30\) because of the mini-puberty. The present study does not have this younger age group so further studies are needed to explore the association of urinary hormones and sexual development. Although 20 participants were followed during the 6 months of treatment, none showed inadequate suppression of HPG function. The value and reference of uLH in well-suppressed patients should be confirmed in large-scale and multi-center studies. In addition, randomized urinary gonadotropins were promising in clinical practice \(^16\); so further work is required to clarify whether elevated randomized uLH is sufficiently sensitive to replace FMV uLH.

**CONCLUSION**

Based on our results, we recommend urinary LH as a reliable biomarker for initial diagnosis and effective screening for CPP in suspected populations. In well-suppressed CPP patients, uLH and uFSH decreased significantly as indicators of the effective therapy.
DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.
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Figure 1. Distribution of unadjusted and adjusted hormone levels in different Tanner Breast stages

Changes of uLH (A), uLH: uCr (B), uFSH (C), uFSH: uCr (D), uE2 (E), uE2: uCr (F), uP (G), uP: uCr (H), uPRL (I), uPRL: uCr (J), uHCG (K), uHCG: uCr (L), uT (M), uT: uCr (N), and uLH: uFSH ratio (O) in advanced Tanner Breast stages respectively.

uLH, urinary luteinizing hormone; uLH: uCr, adjusted uLH by urinary creatinine; uFSH, urinary follicle-stimulating hormone; uFSH: uCr, adjusted uFSH by urinary creatinine; uE2, urinary estradiol; uE2: uCr, adjusted uE2 by urinary creatinine; uP, urinary progesterone; uP: uCr, adjusted uP by urinary creatinine; uPRL, urinary prolactin; uPRL: uCr, adjusted uPRL by urinary creatinine; uHCG, urinary human chorionic gonadotropin; uHCG: uCr, adjusted uHCG by urinary creatinine; uT, urinary testosterone; uT: uCr, adjusted uT by urinary creatinine; uLH: uFSH ratio, unadjusted uLH divided by uFSH.

The lines in the boxes represent the median values, the lower and upper limits of the boxes correspond to the 25th and 75th percentiles, and the whiskers represent the 95% confidence interval limits. Note the logarithmic scale of the Y-axes. (*, P < 0.05. **, P < 0.01. ***, P < 0.001)
Figure 2. Correlations between urinary hormone concentrations and blood hormone concentrations, uterine indexes

Sum, the summation of corresponding gonadotropin concentrations measured at measured at 0, 30-, 45-, 60-, and 90-minute intervals in GnRH stimulation test.
Figure 3. ROC analysis to calculate the optimal cut-off point for a single biomarker

(A) ROC curve of unadjusted hormone concentrations and uLH: uFSH ratio
(B) ROC curve of adjusted urinary hormone concentrations
Figure 4. ROC analysis to calculate the optimal cut-off point for the linear combination of uLH and uFSH

(A) Scatter plot of uLH and uFSH
(B) ROC curve of combination of uLH and uFSH
Figure 5. The changing tendency of serum and urinary hormone levels during the follow-up assessment.
Table 1. Comparison of auxological and laboratory data between GnRHST (+) and (-) groups

| Characteristics                        | Total      | GnRHST (+) | GnRHST (-) | P value |
|----------------------------------------|------------|------------|------------|---------|
| Patient number                         | 355        | 258        | 97         | -       |
| CA (years)                             | 8.12±0.77  | 8.23±0.67  | 7.82±0.94  | <0.001  |
| BA-CA (years)                          | 1.6±0.93   | 1.65±0.97  | 1.50±0.82  | 0.233   |
| Weight Z-score                         | 0.67±0.92  | 0.67±0.88  | 0.67±1.03  | 0.933   |
| Height Z-score                         | 0.77±0.93  | 0.81±0.90  | 0.65±1.00  | 0.143   |
| BMI Z-score                            | 0.34±0.96  | 0.31±0.93  | 0.41±1.04  | 0.388   |
| Tanner B (1/2/3/4)                     | 0/217/133/5| 0/144/110/4| 0/73/23/1  | 0.004   |
| Tanner PH (1/2/3)                      | 331/23/1   | 244/13/1   | 87/10/0    | 0.167   |
| Uterine Volume (cm³)                   | 3.91(2.66,5.61) | 4.57(3.15,6.22) | 2.72(2,02,3.44) | <0.001 |
| Uterine+ cervical Length (cm)          | 4.20(3.80,4.6) | 4.3(3.9,4.7) | 4.0(3.7,4.2) | <0.001 |
| Fundo-cervical ratio                   | 1.41(1.28,1.56) | 1.43(1.31,1.58) | 1.36(1.22,1.53) | 0.01   |
| Basal LH (IU/L)                        | 0.28(0.13,0.89) | 0.50(0.19,1.19) | 0.13(0.10,0.22) | <0.001 |
| Basal FSH (IU/L)                       | 2.70(1.66,4.18) | 3.12(2.00,4.57) | 1.75(1.34,2.68) | <0.001 |
| Basal E2 (pmol/L)                      | 171.0(121.0,229.9) | 181.5(123.5,241.8) | 151.5(110.0,184.0) | 0.055   |
| Basal PRL (mIU/L)                      | 45.5(106.0,237.0) | 146.5(108.5,237.8) | 139.0(104.23,250.6) | 0.089   |

Values are presented as mean ± standard deviation for normal data which were compared by Student t test and the median (25%,75%) for non-normal data which were compared by Mann-Whitney U test.

CA, chronological age; BA, bone age; BMI, body mass index; Tanner B, Tanner breast stage; Tanner PH, Tanner pubic hair; LH, luteinizing hormone; FSH, follicle-stimulating hormone; E2, estradiol; PRL, prolactin.
Table 2. Different cut-off values of uLH, uFSH, their ratio and combination algorithm on GnRHST results.

| Cut-off value | Sensitivity | Specificity | Youden Index |
|---------------|-------------|-------------|--------------|
| uLH (IU/L)    |             |             |              |
| >0.55         | 95.0%       | 25.8%       | 0.207        |
| >1.74         | 69.4%       | 75.3%       | 0.446        |
| >20.1         | 7.4%        | 99%         | 0.06         |
| uFSH (IU/L)   |             |             |              |
| >1.23         | 95.0%       | 5.2%        | 0.02         |
| >4.09         | 56.6%       | 66.0%       | 0.226        |
| >21.05        | 4.3%        | 99.0%       | 0.032        |
| uLH: uFSH     |             |             |              |
| >0.416        | 78.68%      | 80.41%      | 0.591        |
| uLH≥1.74 IU/L+ uLH: uFSH >0.4 | | | | |
| 65.50% | 86.60% | 0.521 |
| 0.399·uLH-0.17·uFSH > -0.087 | 78.8% | 80.4% | 0.591 |
Table 3. Comparison of serum and urinary gonadotropins between pre-treatment and post-treatment levels.

|                        | Serum Peak LH | uLH   | uFSH  | uE2    |
|------------------------|---------------|-------|-------|--------|
| **3 months (compare to initial treatment)** |                |       |       |        |
| z score                | -3.920***     | -3.92*** | -3.92*** | -2.464* |
| P value                | < 0.001       | < 0.001 | < 0.001 | 0.014  |
| **6 months (compare to 8 weeks)** |                |       |       |        |
| z score                | -0.543*       | -2.375* | -2.875*** | -1.269* |
| P value                | 0.587         | 0.018  | 0.004  | 0.204  |

a. based on positive ranks; b. based on positive ranks.

*, P < 0.05. **, P < 0.01. ***, P < 0.001
Table 4. uLH cut-off values used to diagnose PP

| Assay Description | Sensitivity, % | Specificity, % | Subjects number (sex) | Publication time | Assay | Study |
|-------------------|----------------|----------------|-----------------------|-----------------|-------|-------|
| FMV uLH > 1.01 IU/L to predict RP-PP | 83 | 72 | 47 (F) | 2014 | two-site immunochemiluminescence assay | Zung et al.9 |
| FMV uLH > 1.75 IU/L | 91.5 | 82.7 | 138 (M) | 2016 | sandwich fluoroimmunoassays | Demir et al.10 |
| FMV uLH > 1.2 IU/L | 80 | 74 | 52 (F) | | | |
| Non-timed uLH-uCr > 0.05 IU/mmol | 86 | 71 | 41 (M) | 2016 | chemiluminescent microparticle immunoassays | Lucaccioni et al.11 |
| Random uLH > +2SD | 75 | 92 | 12 in 479 (F) | 2017 | time-resolved immunofluorometric assays | Kolby et al.8 |
| FMV uLH > 0.58 IU/L | 91.9 | 63.2 | 100 (F) | 2019 | electrochemiluminescence immunoassays | Shim et al.12 |
| Random uLH > 0.2 IU/L | 77.4 | 73.7 | | | | |
| FMV uLH > 1.01 mIU/mL | 92.3 | 100 | 68 (F) | 2020 | electrochemiluminescence assays | Yüce et al.13 |

RP-PP, rapidly progressive precocious puberty

SD, standard deviation