The Role of Urinary LH and FSH in the Diagnosis of Pubertal Disorders

Manoranjan Tripathy, A. K. Baliarsinha, A. K. Choudhury, Upendra K. Das
Departments of Endocrinology and Biochemistry, SCB MCH, Cuttack, Odisha, India

Abstract

Background: Various hormonal parameters used to differentiate between different causes of pubertal disorders are invasive, cumbersome, and has variable sensitivity and specificity. Thus, the use of a noninvasive test like urinary gonadotropin for the diagnosis of pubertal disorders will offer a significant advantage. Objective: To study the role of urinary gonadotropins (uLH, uFSH) for the diagnosis of various pubertal disorders and in the monitoring of Gonadotrophin releasing hormone, Hypothalamic-pituitary-gonadal (GnRHa) therapy in patients with central precocious puberty (CPP). Materials and Methods: We evaluated 35 healthy children and 96 patients with disorders of puberty out of which 31 cases had early puberty and 65 cases had delayed puberty. We used Spearman’s correlation coefficient to evaluate the correlation between the serum and urinary gonadotropins. We used Mann–Whitney U test (for 2 groups) and Kruskal–Wallis test (for > 2 groups) to compare the median urinary and serum gonadotropins of different groups. Results: The urinary gonadotropins correlated strongly with serum gonadotropins in both healthy controls and individuals with pubertal disorders. The uLH level of ≥0.76 IU/L had 100% sensitivity and specificity to differentiate CPP from peripheral precocious puberty, whereas uLH level of ≥1.07 IU/L had 100% sensitivity and specificity for differentiating CPP from PT. In patients with delayed puberty, uFSH of ≥20.51 IU/L had 94.7% sensitivity and 91.3% specificity for the diagnosis of Hyper-Hypo cases and uLH level of ≥0.5 IU/L had sensitivity of 96.2% and specificity of 85% to differentiate constitutional delay in growth and puberty from hypogonadotropic-hypogonadism. In CPP patients on GnRHa therapy, the uLH level of ≥0.13 IU/L had 100% sensitivity and 86.7% specificity to identify those who had nonsuppressed serum LH levels. Conclusion: The urinary gonadotropins can be used as a reliable noninvasive test for the diagnosis of various pubertal disorders and also for monitoring of CPP patients on GnRHa therapy.

Keywords: Delayed puberty, precocious puberty, pubertal disorders, urinary FSH, urinary gonadotropins, urinary LH

INTRODUCTION

Pubertal disorders can be divided into precocious puberty and delayed puberty. Precocious puberty can be either due to central precocious puberty (CPP) or peripheral precocious puberty (PPP) depending upon activation of the HPG axis. Differentiation between CPP and PPP is important because the management differs. In addition to CPP and PPP, there are several benign variants of early puberty like premature thelarche, premature adrenarche, and premature menarche, which usually do not require treatment. The other extreme of pubertal disorder is delayed puberty which can be either due to hypogonadotropic-hypogonadism (HH) or hypergonadotropic–hypogonadism (Hyper-Hypo). Another common cause of pubertal delay in children is constitutional delay in growth and puberty (CDGP), which is a physiological variant of late onset of puberty. The CDGP is often confused with HH and it is sometimes very difficult to differentiate between them. The CPP and PPP are usually differentiated by combination of clinical, radiological, and hormonal parameters. The early morning serum basal gonadotropins and GnRH stimulated gonadotropins are the hormonal parameters used to differentiate between these two. However, GnRH is currently not available in India, but GnRH analogues like leuprolide and triptorelin are available and being used. The major disadvantages of these tests are they are invasive and may require multiple pricks. The CPP patients are usually

Address for correspondence: Dr. Manoranjan Tripathy, C1, Sector 7, CDA, Near Markatnagar Police Station, Cuttack - 753 014, Odisha, India.
E-mail: dr.manoranjan84@gmail.com

Submitted: 02-Feb-2021
Accepted: 14-Jun-2021
Revised: 12-Mar-2021
Published: 08-Sep-2021

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@ wolterskluwer.com

How to cite this article: Tripathy M, Baliarsinha AK, Choudhury AK, Das UK. The role of urinary LH and FSH in the diagnosis of pubertal disorders. Indian J Endocr Metab 2021;25:110-20.
treated with GnRHa and during follow-up various hormonal parameters are checked, which again are invasive.

The basal gonadotropins are usually sufficient to differentiate between HH and Hyper-Hypo.\(^7\) However, it is often difficult to differentiate between CDGP and HH and the hormonal parameters used are invasive and cumbersome.\(^8\) Thus, the use of a noninvasive test like urinary gonadotropins estimation for the diagnosis of pubertal disorders will offer a significant advantage.

The onset of pubertal maturation is marked by increased secretion of gonadotropins at night.\(^9\) Hence, a morning sample of urinary gonadotropins may reflect the integrated secretion of gonadotropins throughout the night. Therefore, it is hypothesized that the first void urinary gonadotropins may be used as a marker of onset of puberty.

There are very few studies that have evaluated the utility of urinary gonadotropins in differentiating various pubertal disorders.\(^10\) To the best of our knowledge, there is no study from India which has evaluated the efficacy of urinary gonadotropin measurement in the diagnosis of various pubertal disorders. Therefore, we planned to study the role of urinary gonadotropins, which is a simple and noninvasive test for the diagnosis of various pubertal disorders, and in the monitoring of therapy in patients with CPP.

**Materials and Methods**

All the patients presenting for the evaluation of pubertal disorders to the department of Endocrinology, SCB Medical College and hospital, Cuttack between November 2017 and November 2019 were enrolled as per the inclusion criteria. However, patients who did not meet inclusion criteria or met the exclusion criteria were excluded from the study.

**Inclusion criteria**

1. Development of secondary sexual characteristics before 8 years of age in girls or before 9 years of age in boys.
2. Nonappearance of secondary sexual characters by 13 years in girls or 14 years in boys.
3. Previously diagnosed cases of CPP on GnRH analogue therapy.

**Exclusion criteria**

1. Children with nocturnal enuresis.
2. Parents or patients not willing to give consent for the test.

**Consent**

Informed consent was taken from patients (≥18 years) or their guardian (<18 years).

**Ethical clearance**

The ethical clearance for the study was obtained from our institute ethical committee.

**Procedure of the tests**

The study was conducted in two phases. In the first phase, 35 healthy children were evaluated for pubertal status. The boys with testicular volume ≥4 cc and the girls with breast stage ≥2 were classified as pubertal. In all the control subjects, investigations like Complete blood count (CBC), Erythrocyte sedimentation rate (ESR), serum urea and creatinine, serum electrolytes, Liver function tests (LFT) and Thyroid function tests (TFT) (T4, TSH) were done. If all these tests were normal, then the first morning void urine was collected for urinary LH and Follicle stimulating hormone (FSH) (uLH and uFSH) estimation along with serum basal LH and FSH in the early morning fasting state. Then injection triptorelin was given subcutaneously at a dose of 100 µg/m² (maximum 0.1 mg) and post-triptorelin LH and FSH samples were obtained after 60 min. The control subjects were classified as pubertal if their basal serum LH levels were ≥0.3 IU/L and post-triptorelin LH levels were ≥6 IU/L.\(^14\),\(^15\) We included 20 individuals in the pubertal group and 15 individuals in the prepubertal group.

In the phase 2 study, we included patients with pubertal disorders. We had a total of 96 patients with disorders of puberty out of which 31 cases had early puberty and 65 cases had delayed puberty.

**Early puberty**

In all these children, TFT and bone age were done. Bone age was considered to be advanced if it was ≥2.5 SD above the chronological age. Samples for uLH, uFSH, basal and 60 min post-triptorelin serum LH, and FSH, and basal serum testosterone (in boys) and serum estradiol (in girls) were also collected in all patients. Patients were categorized as CPP if they had basal LH levels ≥0.3 IU/L or post-triptorelin LH of ≥6 IU/L.\(^14\),\(^15\) They were classified as PPP if basal LH was <0.3 IU/L and post-triptorelin LH was <6 IU/L with pubertal levels of gonadal steroids (serum testosterone level of ≥20 ng/dL in boys and serum estradiol level ≥10 pg/mL in girls). Girls with isolated breast development with serum basal LH <0.3 IU/L, post-triptorelin LH <6 IU/L, and serum estradiol level <10 pg/mL were classified into premature thelarche (PT) group.

**Delayed puberty**

In individuals with delayed puberty, CBC, ESR, serum urea, creatinine, serum electrolytes, LFT, urine and stool microscopy, and TFT were done initially. If these tests were normal, then basal serum LH and FSH and first morning void urinary LH and FSH were done. Patients with elevated basal LH and FSH were classified as Hyper-Hypo. Patients with low basal serum LH and FSH were further evaluated to differentiate CDGP from HH. In these patients, on D1, injection triptorelin 0.1 mg was given subcutaneously and post-triptorelin LH and FSH were obtained after 4 h. Then, from D2 onwards, 72 h hCG-stimulation test was done in which each patient was given injection hCG 1500 IU per day by deep intramuscular route for 3 days and 24 h after the last dose of hCG, serum sample for testosterone was obtained.

Patients with delayed puberty with chronological age of <18 years, and with basal LH ≥0.3 IU/L,\(^16\) post-triptorelin...
LH ≥ 5.3 IU/L and post-hCG testosterone value ≥ 260 ng/dL were classified as CDGP. Patients with delayed puberty with basal LH < 0.3 IU/L and post-triptorelin LH < 5.3 IU/L and post-hCG testosterone value < 260 ng/dL were classified as HH. In patients with overlapping hormonal parameters, the stimulated LH values were taken for classification into a particular group. Patients with history of anosmia and magnetic resonance imaging evidence hypoplastic or absent olfactory bulb were also considered as HH.

**Follow up of CPP patients on GnRHa therapy**

We measured uLH and uFSH in 17 cases of CPP during follow up while they were on GnRHa therapy. We had a total of 36 measurements of urinary gonadotropins in these 17 patients. We also measured serum basal and 60 min post-triptorelin serum LH and FSH in these patients and classified them into two groups depending upon the levels of serum-stimulated LH. Patients who had stimulated serum LH levels > 6 IU/L were classified into the “nonsuppressed group” and those who had stimulated serum LH < 6 IU/L were called “suppressed group.” We compared the urinary LH and FSH levels in these two groups of CPP patients who were on GnRHa therapy.

**Investigation methods**

Serum LH, FSH, uLH, and uFSH levels were estimated by chemiluminescent microparticle immunoassay (CMIA) using Abbott ARCHITECT. Since for LH assay, the limit of quantification (LoQ) was 0.09 IU/L, any LH (serum or urine) value at or below this value was taken as 0.09 IU/L. Similarly for FSH, the lowest value taken was 0.05 IU/L. Serum testosterone was measured by using The Abbott ARCHITECT testosterone assay with a limit of detection of 4.3 ng/dL. Serum estradiol was measured by The ARCHITECT Estradiol assay with the limit of detection of 5 pg/mL.

**Statistical analysis**

Since most of our data were not normally distributed, we calculated the median values of urinary and serum gonadotropins and other baseline parameters. We used Spearman’s correlation coefficient to evaluate the correlation between the serum and urinary gonadotropins. We used Mann–Whitney U test (for 2 groups) and Kruskal–Wallis test (for > 2 groups) to compare the median urinary and serum gonadotropins of different groups.

**Results**

**Control group**

The baseline characteristics of healthy controls are presented in Table 1. The uLH correlated significantly with both basal serum LH (r = 0.885; P < 0.001) and post-triptorelin LH (r = 0.845; P < 0.001) [Figure 1]. Similarly, the uFSH also correlated significantly with basal serum FSH (r = 0.708; P < 0.001) and post-triptorelin FSH (r = 0.767; P < 0.001) [Figure 2]. The pubertal controls had significantly higher levels of median uLH (2.13 IU/L vs 0.09 IU/L; P < 0.001) and uFSH (6.88 IU/L vs 1.54 IU/L; P = 0.003) compared to prepubertal controls [Table 2]. The uLH value of ≥ 0.55 IU/L had 95% sensitivity and 93.3% specificity, whereas uFSH of ≥ 2.30 IU/L had sensitivity and specificity of 90% and 60%, respectively, to identify puberty [Table 3].

**Delayed puberty**

We had 65 cases of delayed puberty out of which 26 patients were diagnosed as CDGP, 20 as HH, and 19 patients were diagnosed as DDGP. 7 cases of CDGP had LH < 0.3 IU/L and 12 post-triptorelin LH < 5 IU/L and post-hCG testosterone value < 260 ng/dL were classified as HH. Similarly, the uFSH also correlated significantly with basal serum FSH (r = 0.702; P < 0.001) and triptorelin-stimulated LH (r = 0.845; P < 0.001) [Figure 3]. Similarly, the correlations between uFSH and serum basal FSH (r = 0.702; P < 0.001) and triptorelin-stimulated FSH (r = 0.779; P < 0.001) were statistically significant [Figure 4].

The median uLH was significantly higher in children with CPP in comparison to PPP (12.54 vs 0.1 IU/L; P < 0.001) or PT (12.54 vs 0.24 IU/L; P < 0.001) [Figure 5]. The median uFSH was significantly higher in CPP than PPP (11.86 vs 1.06 IU/L; P < 0.001) but it was unable to differentiate CPP from PT [Figure 6]. Using ROC curve, we got uLH cut-off value of ≥ 0.76 IU/L had 100% sensitivity and specificity to differentiate CPP from PPP, whereas the uFSH value of ≥ 4.86 IU/L had 100% sensitivity and specificity [Table 6]. For differentiating CPP from PT, uLH value of ≥ 1.07 IU/L had sensitivity and specificity of 100%, whereas uFSH value of ≥ 5.63 IU/L had sensitivity of 100% but specificity of only 28.6% [Table 7].

**Early puberty**

We had 31 cases of early puberty which included 14 cases of CPP, 7 cases of PT, and 10 cases of PPP. The baseline characteristics are represented in Table 4 and the median value of serum and urinary gonadotropins are presented in Table 5.

The uLH strongly correlated with both basal (r = 0.734; P < 0.001) and triptorelin-stimulated LH (r = 0.845; P < 0.001) [Figure 3]. Similarly, the correlations between uFSH and serum basal FSH (r = 0.702; P < 0.001) and triptorelin-stimulated FSH (r = 0.779; P < 0.001) were statistically significant [Figure 4].

The median uLH was significantly higher in children with CPP in comparison to PPP (12.54 vs 0.1 IU/L; P < 0.001) or PT (12.54 vs 0.24 IU/L; P < 0.001) [Figure 5]. The median uFSH was significantly higher in CPP than PPP (11.86 vs 1.06 IU/L; P < 0.001) but it was unable to differentiate CPP from PT [Figure 6]. Using ROC curve, we got uLH cut-off value of ≥ 0.76 IU/L had 100% sensitivity and specificity to differentiate CPP from PPP, whereas the uFSH value of ≥ 4.86 IU/L had 100% sensitivity and specificity [Table 6]. For differentiating CPP from PT, uLH value of ≥ 1.07 IU/L had sensitivity and specificity of 100%, whereas uFSH value of ≥ 5.63 IU/L had sensitivity of 100% but specificity of only 28.6% [Table 7].
Hyper-Hypo. The baseline characteristics of patients with delayed puberty are presented in Table 8.

The uLH and uFSH correlated significantly with both basal and stimulated serum LH and FSH [Figures 7 and 8]. The Spearman’s correlation coefficient was 0.834 ($P < 0.001$) between uLH and basal serum LH; and 0.827 ($P < 0.001$) between uLH and stimulated serum LH. The Spearman’s correlation coefficients between uFSH and basal and stimulated serum FSH for delayed puberty were 0.9 and 0.804 ($P < 0.001$ for both).

| Parameters | Prepubertal ($n=15$) | Pubertal ($n=20$) | $P$ |
|------------|-----------------------|-------------------|-----|
| S. Basal LH (IU/L) (IQR) | 0.09 (0.09-0.09) | 1.21 (0.68-2.15) | <0.001 |
| S. Basal FSH (IU/L) (IQR) | 1.42 (0.45-2.14) | 3.22 (1.76-5.94) | 0.002 |
| S. Stimulated LH (IU/L) (IQR) | 1.27 (0.69-2.14) | 12.35 (9.95-15.70) | <0.001 |
| S. Stimulated FSH (IU/L) (IQR) | 4.5 (2.61-7.5) | 9.49 (6.95-12.75) | <0.001 |
| Urine LH (IU/L) (IQR) | 0.09 (0.09-0.24) | 2.13 (1.31-3.67) | <0.001 |
| Urine FSH (IU/L) (IQR) | 1.54 (0.53-6.8) | 6.88 (4.25-12.11) | 0.003 |
| Serum testosterone in ng/dL (IQR) | 9 (5.25-10.75) | 85.50 (40.63-171.62) | <0.001 |
| Serum estradiol in pg/mL (IQR) | 5 (5-5) | 23.0 (13.25-29.50) | 0.002 |
The median uLH (1.33 vs 0.15 IU/L) and uFSH (6.27 vs 0.89 IU/L) levels were higher in patients with CDGP than HH (P < 0.001 for both) [Table 9]. The uLH level of ≥2.38 IU/L had 100% sensitivity and 82.6% specificity for the diagnosis of Hyper-Hypo cases, whereas the uFSH of ≥20.51 IU/L had 94.7% sensitivity and 91.3% specificity [Table 10]. The uLH level of ≥0.5 IU/L had sensitivity of 96.2% and specificity of 85% to differentiate CDGP from HH. The uFSH value of ≥1.35 IU/L had similar sensitivity (96.2%), but lower specificity (75%) for the same purpose [Table 11].

**Follow up of CPP patients on GnRHa therapy**

We measured uLH and uFSH in 17 cases of CPP during follow up while they were on GnRHa therapy. We had a total of 36 measurements of urinary gonadotropins in these 17 patients. The uLH and uFSH levels significantly correlated with serum LH and FSH levels, respectively [Figures 9 and 10]. The uLH levels of ≥0.15 IU/L had 100% sensitivity and 86.7% specificity to identify those who had nonsuppressed stimulated LH levels,

---

**Table 3: Cut-off values for urinary gonadotropins to differentiate pubertal from prepubertal controls**

| Cut-off value | Sensitivity | Specificity |
|---------------|-------------|-------------|
| Urine LH (IU/L) |  |  |
| ≥0.32 | 100% | 86.7% |
| ≥0.55 | 95% | 93.3% |
| ≥1.00 | 85% | 100% |
| Urine FSH (IU/L) |  |  |
| ≥0.95 | 100% | 33.3% |
| ≥1.37 | 95% | 46.7% |
| ≥2.30 | 90% | 60% |
while uFSH levels of ≥5.53 IU/L had 83.3% sensitivity and 90% specificity for the same [Figures 11 and 12].

In our cohort, one patient had nonsuppressed stimulated LH value with suppressed basal LH but uLH value for that patient was above 0.13 IU/L indicating that the uLH might be a better predictor of nonsuppressed LH levels. The median uLH level was 1.25 IU/L in the nonsuppressed group, which was significantly higher than the median uLH level of 0.09 IU/L in the suppressed group (P < 0.001) [Table 13]. The uLH levels were at or below the detection limit of our assay in 23 out of 30 measurements in the suppressed group and none in the nonsuppressed group [Table 12].

**DISCUSSION**

In this study, we evaluated the role urinary gonadotropins in the diagnosis of pubertal disorders and their role in follow up of CPP patients on GnRHa treatment. In both healthy controls...
and patients with pubertal disorders, the urinary gonadotropins strongly correlated with the basal and post-triptorelin serum gonadotropins.

In healthy controls, the median uLH and uFSH levels were significantly higher in the pubertal than prepubertal children. In the control group, the uLH levels were at the lower limit of detection of the assay in 4 out of 8 prepubertal boys and in 5 out of 7 prepubertal girls. The uFSH levels were detectable in all pubertal controls. In the study by Kolby et al.,[10] the uFSH levels were detectable in 100% of prepubertal girls and 99.6% of prepubertal boys, whereas uLH levels were detectable in 89.9% of girls and 89.5% of prepubertal boys.

We found that the uLH level of ≥0.55 IU/L had sensitivity of 95% and specificity of 93.3%, whereas uFSH of ≥2.30 IU/L had sensitivity and specificity of 90% and 60%, respectively, to identify puberty in healthy children. Demir et al.[11] demonstrated that the uLH values of ≥1.5 IU/L in boys and 1.2 IU/l in girls had optimal sensitivity and specificity for the diagnosis of puberty.

We had a total of 31 cases of early puberty which included 14 cases of CPP, 7 cases of PT, and 10 cases of PPP. We evaluated the role uLH and uFSH in differentiating patients with CPP from PT or PPP. In our study both uLH (≥0.76 IU/L) and uFSH (≥4.86 IU/L) had 100% sensitivity and specificity to differentiate CPP from PPP. For differentiating CPP from PT, uLH (≥1.07 IU/L) had sensitivity and specificity of 100%, whereas uFSH had good sensitivity but poor specificity.

### Table 6: Cut-off values for urinary gonadotropins to differentiate CPP from PPP

| Cut-off value | Sensitivity | Specificity |
|---------------|-------------|-------------|
| Urine LH (IU/L) | ≥0.20 | 100% | 90% |
|               | ≥0.76 | 100% | 100% |
|               | ≥1.93 | 92.9% | 100% |
| Urine FSH (IU/L) | ≥3.15 | 100% | 90% |
|               | ≥4.86 | 100% | 100% |
|               | ≥6.35 | 92.9% | 100% |

### Table 7: Cut-off values for urinary gonadotropins to differentiate CPP from PT

| Cut-off value | Sensitivity | Specificity |
|---------------|-------------|-------------|
| Urine LH (IU/L) | ≥0.78 | 100% | 85.7% |
|               | ≥1.07 | 100% | 100% |
|               | ≥1.93 | 92.9% | 100% |
| Urine FSH (IU/L) | ≥5.63 | 100% | 28.6% |
|               | ≥29.39 | 14.3% | 49.2% |
|               | ≥52.0 | 14.3% | 85.7% |

### Table 8: Baseline parameters of delayed puberty

| Parameters | Median (IQR) | CDGP | Hypo-Hypo | Hyper-Hypo | P for Hyper-Hypo vs CDGP + HH | P for CDGP vs HH |
|------------|--------------|------|-----------|------------|-------------------------------|-----------------|
| Age in years | 14.55 (14.18-15.65) | 16.8 (15.23-19.78) | 19.50 (16.5-23) | <0.001 | <0.001 |
| Male: Female | 21/5 | 14/6 | 11/8 | 0.146 | 0.401 |
| Pubertal Stage A | 1 (1-1) | 1 (1-1) | 1 (1-1) | 0.463 | 0.084 |
| P | 1 (1-1) | 1 (1-2) | 1 (1-4) | 0.019 | 0.334 |
| SPL | 5 (4.58-5.58) | 4.15 (3.28-4.7) | 6.65 (6-9) | 0.001 | 0.004 |
| TV | 3 (3-3) | 2 (1-2) | 2.5 (2-3.25) | 0.954 | <0.001 |
| B | 1 (1-1) | 1 (1-1) | 1 (1-1) | 1 | 1 |
| US: LS | 0.84 (0.81-0.9) | 0.84 (0.81-0.89) | 0.82 (0.75-0.9) | 0.461 | 0.885 |
| Arm Span | 151 (135-163) | 162.5 (141.8-169.8) | 166 (142-179) | 0.114 | 0.14 |
| AS-Ht | 2 (1.38-3.25) | 3.5 (1-4) | 4 (1.5-10) | 0.054 | 0.322 |

SMR - Sexual Maturity Rating, A - Axillary hair stage, P - Pubic hair stage, B - Breast stage, SPL - Stretched penile length, TV - Testicular volume, US: LS - Upper segment to lower segment ratio, AS - Ht: difference between arm span and height.

### Table 9: Hormonal parameters in patients with delayed puberty

| Parameters | Median (IQR) | CDGP | Hypo-Hypo | Hyper-Hypo | P for Hyper-Hypo vs CDGP + HH | P for CDGP vs HH |
|------------|--------------|------|-----------|------------|-------------------------------|-----------------|
| Serum Basal LH (IU/L) | 1.34 (0.67-2.02) | 0.12 (0.09-0.25) | 21.8 (14.7-30.05) | <0.001 | <0.001 |
| Serum Basal FSH (IU/L) | 2.98 (1.94-5.54) | 0.94 (0.35-1.88) | 65.66 (51.9-82.51) | <0.001 | <0.001 |
| Stimulated LH (IU/L) | 14.09 (9.76-16.21) | 1.31 (0.81-1.91) | NA | NA | <0.001 |
| Stimulated FSH (IU/L) | 10.80 (5.48-14.06) | 3.45 (2.33-5.02) | NA | NA | <0.001 |
| Urine LH (IU/L) | 1.63 (1.05-4.73) | 0.15 (0.09-0.42) | 12.17 (4.18-19.20) | <0.001 | <0.001 |
| Urine FSH (IU/L) | 6.27 (2.72-16.03) | 0.89 (0.49-1.45) | 58.59 (40.5-144.74) | <0.001 | <0.001 |
| Serum Basal Testosterone (ng/dL) | 20.3 (14.2-26.69) | 4.3 (4.3-12.18) | 23.92 (13.63-105.8) | 0.034 | <0.001 |
| Serum Estradiol (pg/mL) | 15 (10-15.2) | 10 (10-10) | 10 (10-10.38) | 0.803 | 0.037 |
| hCG stimulated Testosterone (ng/dL) | 21.43 (14.1-40.12) | 290 (206.85-376.65) | NA | NA | <0.001 |
specificity (28.6%). In the study by Kolby et al.,[10] the uLH levels were significantly elevated in 9 out of 12 children with CPP compared to the prepubertal reference ranges, whereas uLH levels were within reference ranges in 12 out of 13 of girls with PT. However, in their study, the uFSH levels were not elevated in girls with CPP and PT.

The role of urinary gonadotropins in the diagnosis of delayed puberty has not been previously evaluated extensively.

**Figure 7:** Correlation between urine LH and serum LH in delayed puberty

**Figure 8:** Correlation of urine FSH with serum FSH in delayed puberty

**Figure 9:** Correlation between urine LH and serum LH in CPP patients on GnRHa therapy
Lucaccioni et al.\textsuperscript{[12]} found that urinary gonadotropins vary widely in patients with delayed puberty and did not help to diagnose the type of delayed puberty. However, they collected random urinary sample and not the first morning void urine which might be responsible for the wide variation of levels. In our study, we found a strong correlation between the urinary gonadotropins and serum gonadotropins in patients with delayed puberty.

The uLH level of \( \geq 2.38 \) IU/L had 100% sensitivity and 82.6% specificity for the diagnosis of Hyper-Hypo cases, whereas
the uFSH of ≥20.51 IU/L had 94.7% sensitivity and 91.3% specificity. To differentiate CDGP from HH, the uLH level of ≥0.5 IU/L had sensitivity of 96.2% and specificity of 85%. The uFSH value of ≥1.35 IU/L had similar sensitivity (96.2%), but lower specificity (75%).

In patients of CPP on GnRHa treatment, the uLH and uFSH levels correlated strongly with stimulated serum LH and FSH levels. The uLH levels were at or below the detection limit of the assay in 23 out of 30 measurements in the suppressed group and none in the nonsuppressed group. This indicates the usefulness of this noninvasive test. In our study, uLH of ≥0.13 IU/L had 100% sensitivity and 86.7% specificity to identify those who had nonsuppressed stimulated LH levels, while urinary FSH levels of ≥5.53 IU/L had 83.3% sensitivity and 90% specificity for the same. In our cohort, one patient had nonsuppressed stimulated LH value with suppressed basal LH but uLH value for that patient was above 0.13 IU/L indicating that uLH might be a better test than basal LH levels. In the study by Kolby et al.,[10] authors found that uLH levels get suppressed after 12 weeks of treatment with GnRHa therapy. Lucaccioni et al.[12] also demonstrated that the median uLH and uFSH levels decreased to prepubertal levels after treatment with GnRHa.

Demir et al.[11] and McNeily et al.[13] showed that creatinine corrected and uncorrected urinary gonadotropins had similar sensitivity and specificity for the diagnosis of pubertal disorders. Kolby et al.[10] demonstrated that the osmolality corrected urinary gonadotropin level and timed urinary collection did not alter the result in their study. Based upon these observations, we measured only the first morning void urine without creatinine or osmolality correction. However, we ensured that patients did not pass urine for at least 5–6 h in the previous night before collecting first morning void urine.

**Conclusion**

In this study, we evaluated the role of urinary gonadotropins in the diagnosis of pubertal disorders and follow up of CPP patients on GnRHa therapy. We found that the urinary gonadotropin levels correlate strongly with serum gonadotropin levels. The uLH is a very good noninvasive test with high sensitivity and specificity for the diagnosis of CPP. The uFSH is also very helpful to differentiate CPP from PPP, but less helpful in differentiating CPP from PT. The urinary gonadotropins can be used to differentiate between different causes of delayed puberty. The uLH can also be used as a reliable noninvasive test for monitoring of CPP patients on GnRHa therapy.

**Declarations of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Carel JC, Leger J. Precocious puberty. N Engl J Med 2008;358:2366‑77.
2. Berberoğlu M. Precocious puberty and normal variant puberty: Definition, etiology, diagnosis and current management. J Clin Res Pediatr Endocrinol 2009;1:164‑74.
3. Seldlmeyer IL, Palmert MR. Delayed puberty: Analysis of a large case series from an academic center. J Clin Endocrinol Metab 2002;87:1613‑20.
4. Job JC, Chausain JL, Garnier PE. The use of luteinizing hormone releasing hormone in pediatric patients. Horm Res Paediatr 1977;8:171‑87.
5. Ibañez L, Potau N, Zampolli M, Virdis R, Gussinyé M, Carrascosa A, et al. Use of leuprolide acetate response patterns in the early diagnosis of pubertal disorders: Comparison with the gonadotropin releasing hormone test. J Clin Endocrinol Metab 1994;78:30‑5.
6. Poomthavorn P, Khlahirit P, Mahachoklertwattana P. Subcutaneous gonadotropin-releasing hormone agonist (tripotrenin) test for diagnosing precocious puberty. Horm Res Paediatr 2009;72:114‑9.
7. Palmert MR, Dunkel L. Delayed puberty. N Engl J Med 2012;366:443‑53.
8. Harrington J, Palmert MR. Distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: Critical appraisal of available diagnostic tests. J Clin Endocrinol Metab 2012;97:3056‑67.
9. Boyar RM, Rosenfeld RS, Kapen S, Finkelstein JW, Roffwarg HP, Weitzman ED, et al. Human pubertal simultaneous augmented secretion of luteinizing hormone and testosterone during sleep. J Clin Invest 1974;54:609‑18.
10. Kolby N, Busch AS, Akselgaard L, Sørensen K, Petersen JH, Andersson AM, et al. Nocturnal urinary excretion of FSH and LH in children and adolescents with normal and early puberty. J Clin Endocrinol Metab 2017;102:3830‑8.
11. Demir A, Voutilainen R, Stenman UH, Dunkel L, Albertsson-Wikland K,

---

**Table 13:** Comparison of serum and urinary gonadotropins in CPP patients on GnRHa therapy on follow up

| Parameters | Median values (IQR) | Suppressed Gonadotropin group | Nonsuppressed Gonadotropin group | P |
|------------|--------------------|-------------------------------|---------------------------------|---|
| Serum Basal LH (IU/L) | 0.14 (0.09-0.23) | 1.31 (0.28-2.62) | 0.006 |
| Serum Basal FSH (IU/L) | 1.2 (0.33-1.76) | 3.77 (1.93-5.45) | 0.002 |
| Serum Stimulated LH (IU/L) | 1.59 (0.96-2.26) | 7.09 (5.49-13.08) | <0.001 |
| Serum Stimulated FSH (IU/L) | 2.78 (1.63-4.75) | 11.13 (7.44-21.09) | 0.001 |
| Urinary LH (IU/L) | 0.09 (0.09-0.09) | 1.25 (0.27-6.30) | <0.001 |
| Urinary FSH (IU/L) | 2.13 (0.84-4.04) | 7.94 (5.27-22.59) | 0.003 |
12. Lucaccioni L, McNeilly J, Mason A, Giacomuzzi C, Kyriakou A, Shaikh MG. The measurement of urinary gonadotropins for assessment and management of pubertal disorder. Hormones (Athens) 2016;15:377-84.

13. McNeilly JD, Mason A, Khanna S, Galloway PJ, Ahmed SF. Urinary gonadotrophins: A useful non-invasive marker of activation of the hypothalamic pituitarygonadal axis. Int J Pediatr Endocrinol 2012;2012:10.

14. Neely EK, Wilson DM, Lee PA, Stene M, Hintz RL. Spontaneous serum gonadotropin concentrations in the evaluation of precocious puberty. J Pediatr 1995;127:47-52.

15. Poomthavorn P, Khairir P, Mahachoklertwattana P. Subcutaneous gonadotropin-releasing hormone agonist (triptorelin) test for diagnosing precocious puberty. Horm Res Paediatr 2009;72:114-9.

16. Binder G, Schweizer R, Blumenstock G, Braun R. Inhibin B plus LH vs GnRH agonist test for distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism in boys. Clin Endocrinol (Oxf) 2015;82:100-5.

17. Degros V, Cortet-Rudelli C, Soudan B, Dewailly D. The human chorionic gonadotropin test is more powerful than the gonadotropin-releasing hormone agonist test to discriminate male isolated hypogonadotropic hypogonadism from constitutional delayed puberty. Eur J Endocrinol 2003;149:23-30.