Original Article

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

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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.[1] Differentiation between CPP and PPP is important because the management differs.[1] 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.[2] The other extreme of pubertal disorder is delayed puberty which can be either due to hypogonadotropic-hypogonadism (HH) or hypergonadotropic–hypogonadism (Hyper-Hypo).[3] 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.[3] The CDGP is often confused with HH and it is sometimes very difficult to differentiate between them.[3]

The CPP and PPP are usually differentiated by combination of clinical, radiological, and hormonal parameters.[1] The early morning serum basal gonadotropins and GnRH stimulated gonadotropins are the hormonal parameters used to differentiate between these two.[4] However, GnRH is currently not available in India, but GnRH analogues like leuprolide[5] and triptorelin[6] 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

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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 gonadotropin 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.[10-13] 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/m^2 (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,[16] and post-hCG testosterone value ≥260 ng/dL[17] were classified as CDGP. Patients with delayed puberty with basal LH <0.3 IU/L,[16] post-triptorelin LH <5.3 IU/L,[16] and post-hCG testosterone value <260 ng/dL[17] 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].

Table 1: Baseline characters of healthy controls ($n=35$)

Parameters	Prepubertal ($n=15$)	Pubertal ($n=20$)	*P*
Median age in year (IQR)	9.20 (8-10.1)	13.05 (11.62-14.72)	<0.001
Female (*n*)	7	8	0.76
Male (*n*)	8	12	0.76
SMR			
A	1 (1-1)	1 (1-1)	0.49
P	1 (1-1)	2 (2-3)	<0.001
B	1 (1-1)	3 (2-3.75)	<0.001
SPL	4.95 (4.25-5.15)	6.5 (5.6-7.5)	<0.001
TV	2 (2-2)	7 (5.25-9.5)	<0.001
Weight in kg (IQR)	26 (21-35)	44 (34.5-53.2)	0.002
Height in cm (IQR)	126 (115-137)	150 (135.7-157.5)	<0.001
BMI in kg/m^2 (IQR)	16.25 (15.14-19.78)	19.44 (17.84-21.81)	0.03
US: LS Ratio (IQR)	0.94 (0.9-1.1)	0.89 (0.86-0.94)	0.016
Arm Span in cm (IQR)	127 (115-135)	149 (135.2-157.5)	<0.001

SMR - Sexual Maturity Rating, A - Axillary hair stage, P - Pubic hair stage, B - Breast stage, SPL - Stretched penile length, TV - Testicular volume, US: LS Ratio - Upper segment to lower segment ratio

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].

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

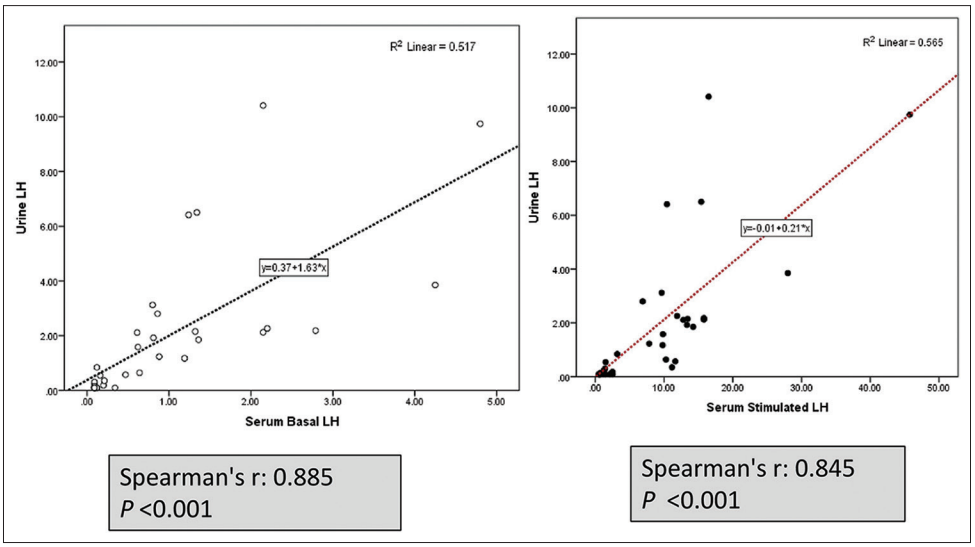

Figure 1: Correlation between urinary LH and serum LH in healthy controls

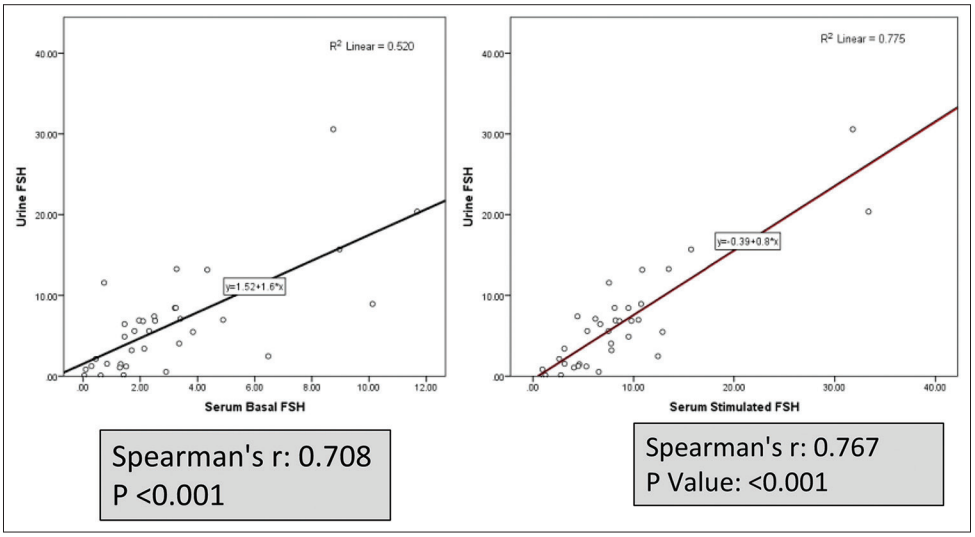

Figure 2: Correlation of urinary FSH with serum FSH in healthy controls

Table 2: Serum and urinary gonadotropins in prepubertal vs pubertal controls

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

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).

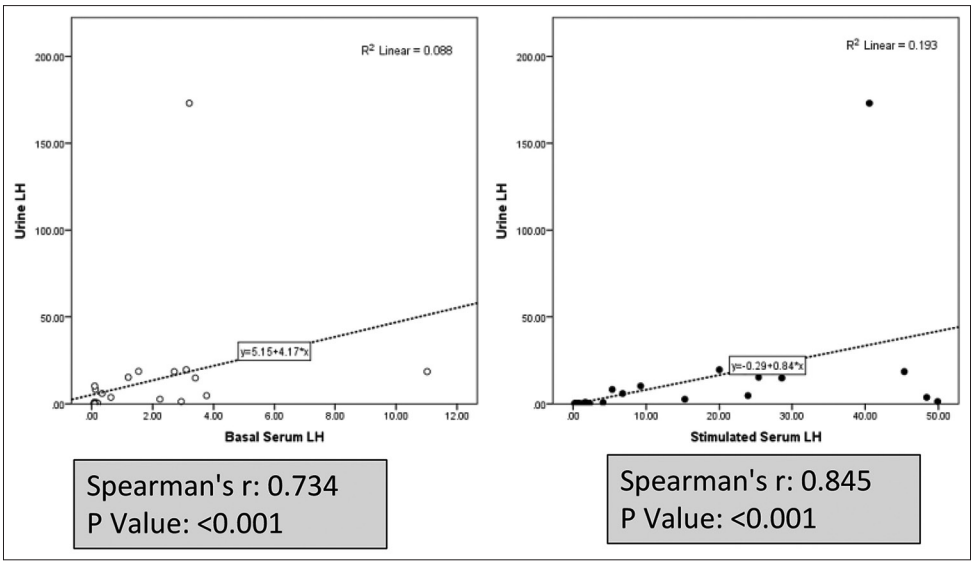

Figure 3: Correlation between urinary LH and serum LH in early puberty

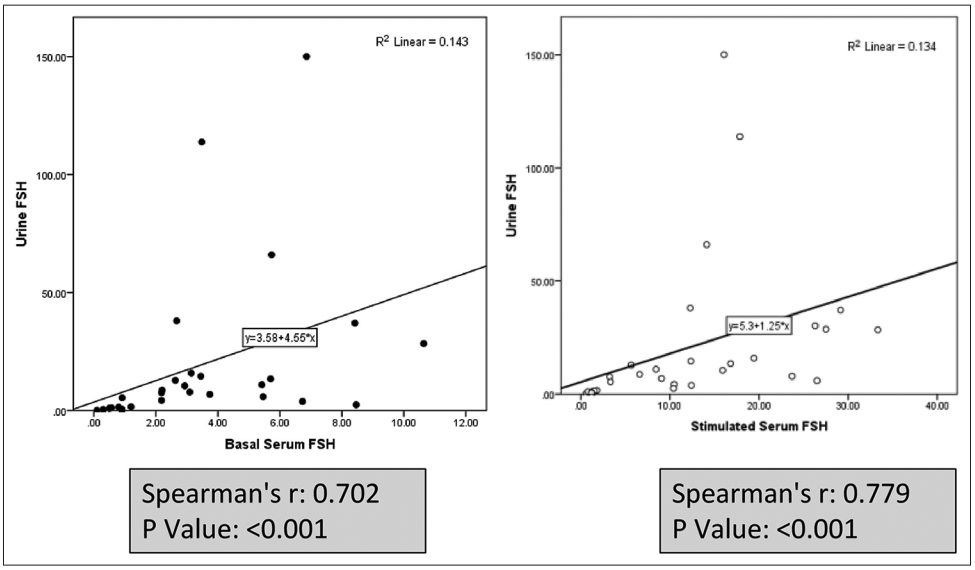

Figure 4: Correlation between urinary FSH and serum FSH in early puberty

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%**

The median uLH (1.63 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 ≥0.13 IU/L had 100% sensitivity and 86.7% specificity to identify those who had nonsuppressed stimulated LH levels,

Table 4: Baseline characters of patients with early puberty

Parameters Median (IQR)	CPP (*n*=14)	PT (*n*=7)	PPP (*n*=10)	*P* trend	*P* CPP vs PT	*P* CPP vs PPP
Age at onset of puberty (in year)	3.25 (1.5-5.78)	1.2 (0.6-4.0)	3.25 (1.98-5.78)	0.078	0.052	0.666
Age of presentation (in year)	4.95 (2.93-6.6)	2.1 (1.3-4.8)	4.0 (2.88-6.25)	0.215	0.146	0.796
Female (percentage)	10 (71.4%)	7 (100%)	7 (70%)			
SMR at presentation						
A	1 (1-1)	1 (1-1)	1 (1-1.25)	0.367	0.48	0.358
P	1 (1-2.25)	1 (1-1)	2.5 (1.75-3.0)	**0.006**	0.08	**0.047**
B	3 (3-4)	3 (2-3)	1 (1-2)	**0.002**	0.272	**0.002**
SPL in cm	7.8 (6.4-8)	NA	8 (8-8)	0.119	NA	0.119
TV in mL	5.5 (4.25-6)	NA	3 (2-3)	**0.031**	**NA**	**0.031**
Height SDS	0.8 (0.23-1.9)	0.3 (−0.5-1.0)	1.4 (−0.8-2.28)	0.521	0.294	0.883
Bone age SDS	2.93 (2.09-6.68)	−0.6 (−0.68-0.3)	4.23 (2.17-6.9)	**0.01**	**0.003**	1
Left Ovarian Volume in mL	2.6 (0.68-4.05)	0.5 (0.31-0.84)	0.5 (0.1-54.0)	0.115	0.019	0.434
Right Ovarian Volume in mL	2.1 (1.23-4.60)	0.3 (0.29-0.98)	0.4 (0.08-60.0)	**0.047**	**0.007**	0.157
Average Ovarian Volume in mL	2.4 (0.76-4.29)	0.42 (0.30-0.71)	0.45 (0.09-57.0)	**0.03**	**0.003**	0.222
Uterine Volume in mL	6.65 (5.10-10.25)	1.6 (1.4-2.0)	1.1 (0.3-30)	0.653	**0.001**	0.143

SMR - Sexual Maturity Rating, A - Axillary hair stage, P - Pubic hair stage, B - Breast stage, SPL - Stretched penile length, TV - Testicular volume

Table 5: Comparison of serum and urinary gonadotropins among different causes of early puberty

Parameters Median (IQR)	CPP	PT	PPP	*P*	*P* for CPP vs PT	*P* for CPP vs PPP
Serum basal LH (IU/L)	2.48 (0.56-3.25)	0.09 (0.09-0.12)	0.1 (0.09-0.12)	**<0.001**	**0.001**	**<0.001**
Serum Basal FSH (IU/L)	3.6 (2.86-5.71)	3.48 (2.18-6.50)	0.85 (0.45-2.58)	**0.02**	0.911	**0.008**
Serum Stimulated LH (IU/L)	24.66 (13.77-41.76)	1.74 (1.19-2.29)	0.65 (0.30-0.80)	**<0.001**	**<0.001**	**<0.001**
Serum Stimulated FSH (IU/L)	15.03 (7.98-20.49)	17.84 (10.47-27.50)	1.33 (1.1-3.95)	**0.001**	0.456	**0.001**
Urine LH (IU/L)	12.54 (4.46-18.54)	0.24 (0.15-0.68)	0.10 (0.09-0.13)	**<0.001**	**<0.001**	**<0.001**
Urine FSH (IU/L)	11.86 (7.77-18.98)	30.13 (5.4-38.0)	1.06 (0.36-1.80)	**<0.001**	0.371	**<0.001**

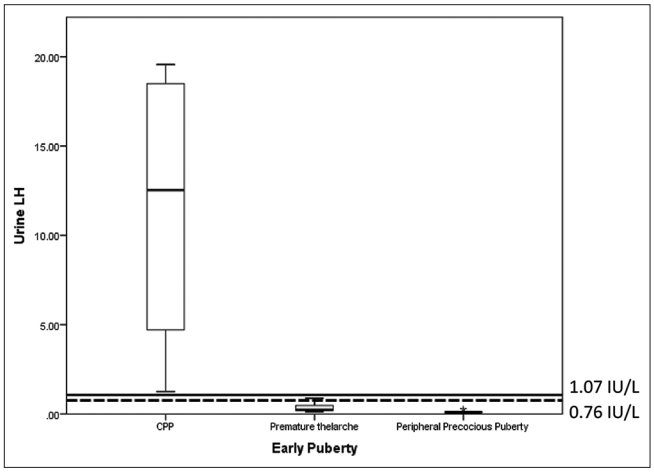

Figure 5: Comparison of urinary LH in different causes of early puberty

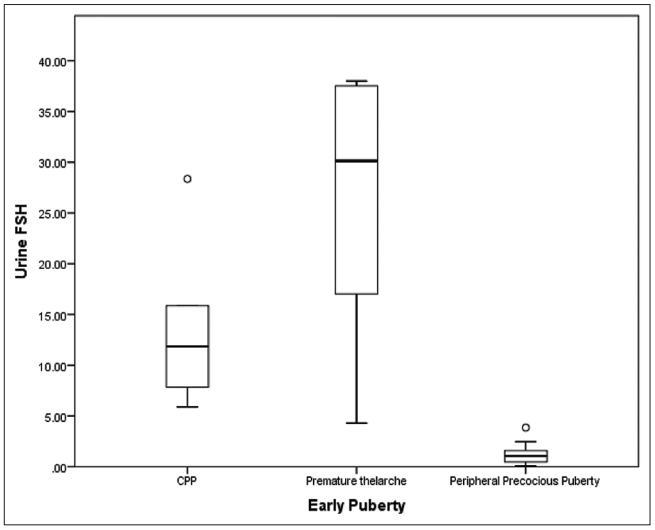

Figure 6: Comparison of urinary FSH in different causes of early puberty

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.

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%

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 prepubertal 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

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

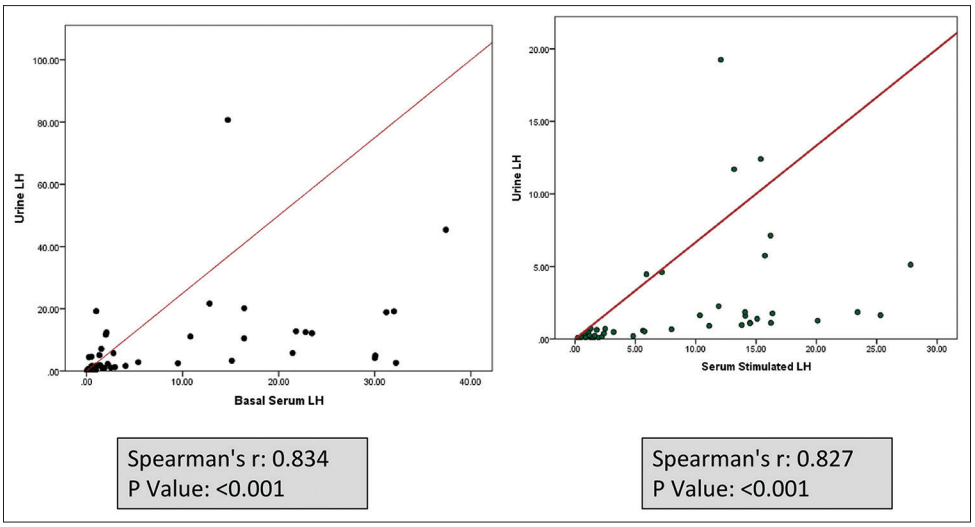

Figure 7: Correlation between urine LH and serum LH in delayed puberty

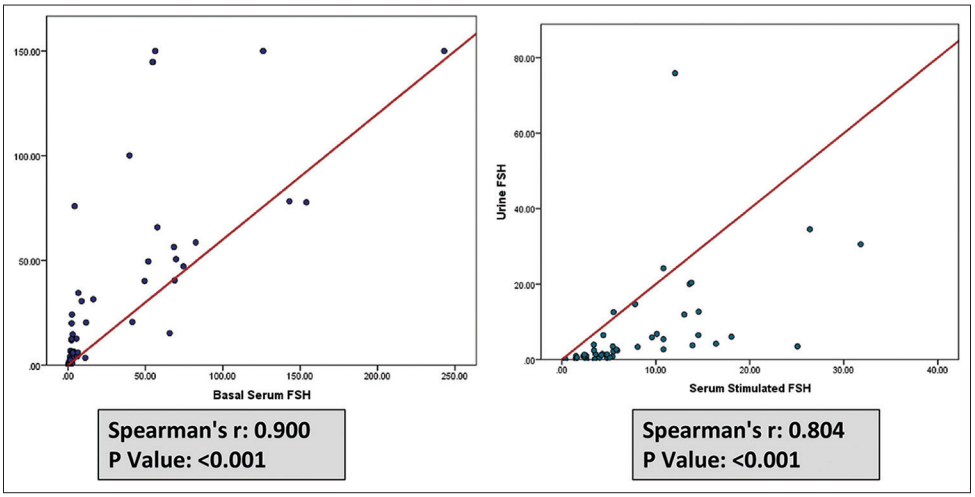

Figure 8: Correlation of urine FSH with serum FSH in delayed puberty

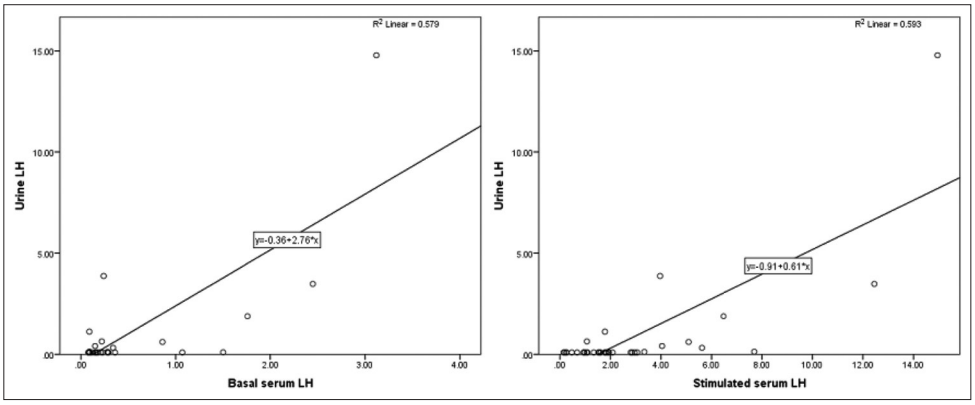

Figure 9: Correlation between urine LH and serum LH in CPP patients on GnRHa therapy

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.

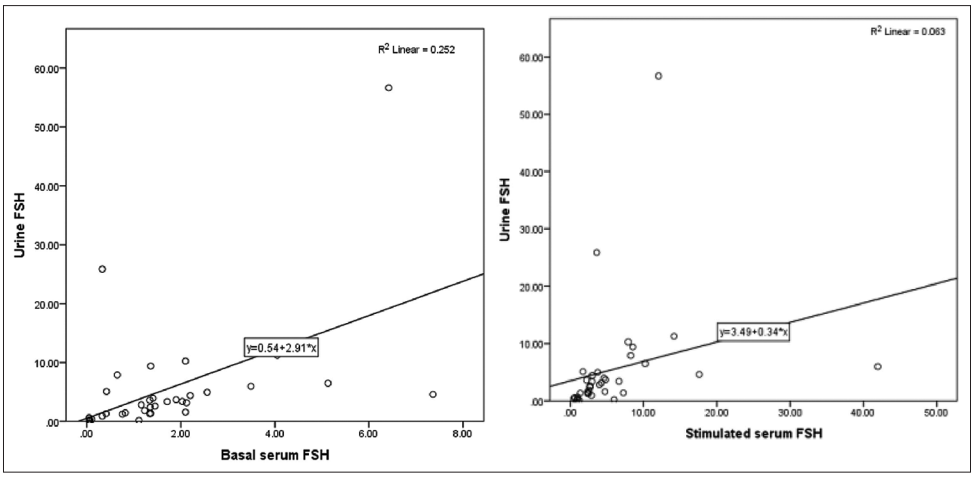

Figure 10: Correlation between urine FSH and serum FSH in CPP patients on GnRHa therapy

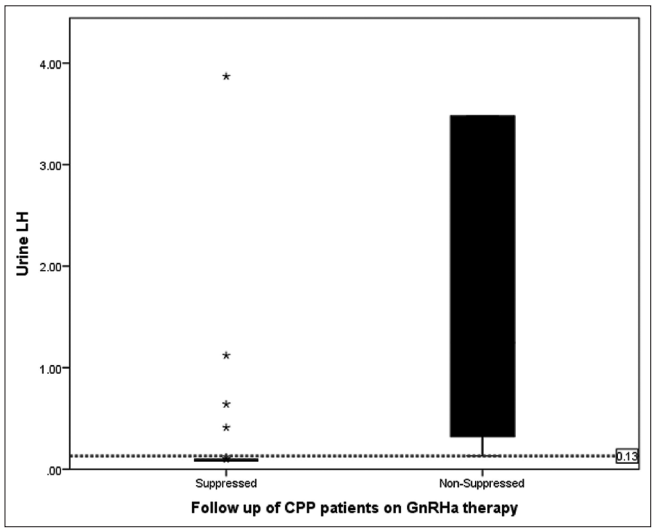

Figure 11: Urinary LH in follow up of CPP patients on GnRHa therapy

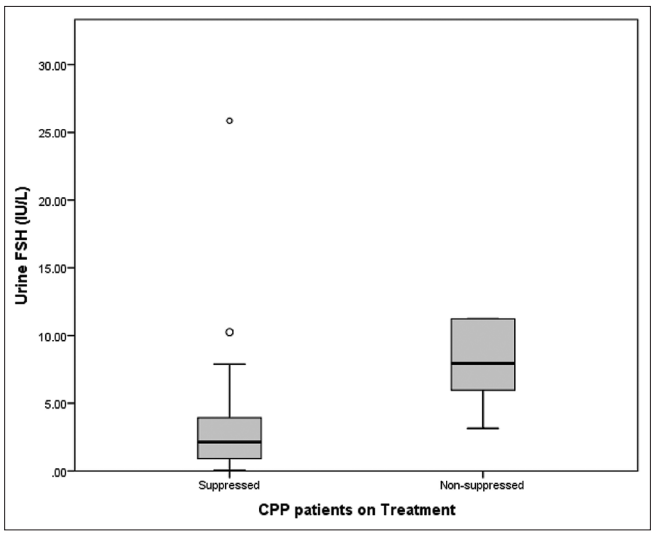

Figure 12: Urinary FSH in follow up of CPP patients on GnRHa therapy

Lucaccioni *et al*.[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 ≥2.38 IU/L had 100% sensitivity and 82.6% specificity for the diagnosis of Hyper-Hypo cases, whereas

Table 10: Cut-off of gonadotropins to differentiate Hyper-Hypo from CDGP + HH

	Cut-off value	Sensitivity	Specificity
Urine LH (IU/L)	**≥2.38**	**100%**	**82.6%**
	≥4.78	73.7%	87%
	≥5.77	68.4%	91.7%
Urine FSH (IU/L)	≥14.99	100%	87%
	≥20.51	**94.7%**	**91.3%**
	≥31.02	89.5%	95.7%

Table 11: Cut-off of Serum and urinary gonadotropins to differentiate CDGP from HH

	Cut-off value	Sensitivity	Specificity
Urine LH (IU/L)	≥0.18	100%	55%
	≥0.50	**96.2%**	**85%**
	≥0.65	88.5%	90%
	≥0.81	**84.6%**	**100%**
Urine FSH (IU/L)	≥1.10	100%	65%
	≥1.35	**96.2%**	**75%**
	≥2.20	88.5%	85%

Table 12: CPP patients on GnRHa therapy

n	36
Female	30
Male	6
Suppressed Group	30
Nonsuppressed Group	6

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

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.

Declaration 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.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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