

Evaluation of Gonadotropin Responses and Response Times According to Two Different Cut-Off Values in Luteinizing Hormone Releasing Hormone Stimulation Test in Girls

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Abstract

Background: In this study, we aimed to evaluate FSH, LH responses obtained during LHRH-ST according to two different cut-off values, to determine the diagnostic response times, and to find the optimal blood collection times that could reduce the economic and time burden of LHRH-ST. **Materials and Methods:** Patients who underwent LHRH-ST in our clinic with the preliminary diagnosis of precocious puberty (PP) between 01/08/2016 and 31/12/2017 were retrospectively enrolled to the study. In this study 207 girls with PP were included and some of them (102 according to C1 and 139 according to C2) had central PP (CPP). Test response and response times were evaluated according to both cut-off values of stimulated peak LH pubertal responses as 5 mIU/ml (the 1st cut-off = C1) and 3.3 mIU/ml (the 2nd cut-off = C2). **Results:** Totally, 207 girls with a mean age of 7.5 ± 1.22 (3.4-9.5) years were included in the study. With LHRH-ST; 49.2% ($n = 102$), 67% ($n = 139$) of the cases were in pubertal period according to C1, C2, respectively. According to C1; pubertal LH was present in 94.1% ($n = 96$) of 102 patients who reached pubertal LH value in 45th minutes. The highest pubertal response was obtained in the 45th minute. According to C2, of 139 patients who reached pubertal LH; pubertal LH was determined in 98.5% ($n = 137$) in the 45th minute. Pubertal LH levels were determined according to both cut-off values in all 27 patients with baseline LH ≥ 0.31 mIU/ml. **Conclusion:** It was determined that measuring LH at 45th minutes during LHRH-ST was sufficient in 94.1% of the cases according to C1 and 97.1% of the cases according to C2. It was concluded that the 30th, 45th, and 60th minute samples were enough to assess pubertal LH response in 100% of the cases. If the basal LH is found to be ≥ 0.31 mIU/ml in girls with puberty findings, we recommend that the diagnosis of precocious puberty would be made without performing LHRH-ST.

Keywords: Central Precocious Puberty, LH, LHRH test, 45th minute

INTRODUCTION

Precocious puberty (PP) is defined as the onset of puberty at an age that is two standard deviations younger than the mean age at which secondary sex characters begin to appear in normal population. Although this limit is known as 8 years for girls and 9 years for boys, there are also literature revealing that this age has shifted earlier.^[1-6] The onset of puberty findings before 8 years of age in Turkish girls should be considered as precocious puberty.^[1] The incidence of central precocious puberty (CPP) ranges from 1:5000 to 1:10000. Central precocious puberty is more common in girls. The age at onset of puberty may vary due to genetic characteristics, nutritional status, obesity, stress, and environmental factors. Today, puberty findings begin earlier and the number of

patients admitted to endocrinology clinics due to PP has increased.^[1-7]

In central precocious puberty, the diagnostic value of basal gonadotropins is low due to pulsatile release, but evaluation often begins with basal measurement.

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Commercial immunofluorometric assay (IFMA) and immunochemiluminometric assay (ICMA) methods are used for LH and FSH analyzes. ICMA is more advantageous due to its higher accuracy, precision, and reproducibility and it requires a lower amount of reagent, thus reducing cost per test. In cases where basal gonadotropins are inadequate in diagnosis; Luteinizing hormone releasing hormone stimulation test (LHRH-ST) is the gold standard.^[8] In this test; FSH-LH levels are measured at certain times after LHRH administration and the activation of the hypothalamus-pituitary-gonad (HHG) axis is evaluated. There are various studies with different results on the time when the stimulated FSH-LH values should be recorded and when the most adequate diagnostic value is available.^[9-12] Such studies are important for reducing cost and invasiveness.

In our study; we aimed to evaluate basal and stimulated FSH-LH responses with the LHRH-ST protocol that Carrillo *et al.*^[13] have proposed, to determine the diagnostic value of the current response time and to determine the optimal blood collection times at which LHRH-ST can reduce the economic, time, and invasiveness burden.

MATERIALS AND METHODS

The clinical and laboratory findings of the patients who were older than 3 years of age who had undergone LHRH-ST test between 01/08/2016 and 31/12/2017 with the complaint of onset of puberty findings in clinics of pediatric Endocrinology were evaluated retrospectively. Informed consent was obtained from all patients and their parents. The study was approved by the Medical Specialization and Education Board of our hospital on 15.05.2018 with the approval number of 77799008.

Anthropometric measurements of the patients were performed in our clinic; in the morning, with shoes and clothing removed, by the same trained staff. Body weight, height, and BMI standard deviation score (SDS) values were calculated based on the Turkish population data of Neyzi *et al.*^[14-16] Pubertal staging is performed according to Marshall and Tanner method.^[17,18] FSH, LH, E2 levels are studied by ICMA method. The intra-measurement deviation of the ICMA kit is 2.3-2.9% and the deviation between measurements is 1.5-2.4% (AdviaCentaur ©). Assessment of bone age is evaluated according to the Greulich-Pyle Radiographic Atlas.^[19]

The LHRH-ST protocol

After taking basal LH-FSH levels, 2.5 µg/kg (maximum 100 µg) LHRH (Ferring®, 0.1 mg/mL ampoule) is given intravenously in the morning at 08: 00-09: 00 after fasting at night. LH-FSH levels are taken at 15th, 30th, 45th, 60th, and 90th minutes after intravenous LHRH pushing.

The study groups

ICMA-stimulated LH was evaluated according to two cut-off values. According to the first cut-off value (C1) levels ≥ 5 mIU/ml^[10,20] and according to the second cut-off values (C2)

levels >3.3 mIU/ml^[12] were accepted as pubertal response. Age, basal, and peak LH values (PLH), PLH times and LH/FSH ratios were evaluated according to these two cut-off values, to pubertal and prepubertal status.

The sample size calculation was based on the pubertal C1 proportion at 45th minute. A sample size of 193 participants is required to estimate a proportion of 43.1% in an infinite population with a confidence interval of 95% and a margin of error of 0.07 (<http://homepage.divms.uiowa.edu/~rlenth/Power/>).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences, version 20 for Windows (SPSS Inc., Chicago, IL). Data are given as mean \pm SD. Frequencies were compared using the χ^2 -test. Friedman test was used for repeated measurements. Wilcoxon rank test and Mann-Whitney U test were used to compare the means. A *P* value less than 0.05 was considered statistically significant.

RESULTS

A total of 207 girls with a mean age of 7.5 ± 1.22 (3.4-9.5) years were included in the study. With LHRH test; pubertal response was obtained in 49.2% ($n = 102$) of the cases according to C1 (LH ≥ 5 mIU/ml) and in 67% ($n = 139$) of the subjects according to C2 (LH >3.3 mIU/ml). Based on the two cut-off values and pubertal and prepubertal state; age, basal and peak LH values (PLH) and PLH times are presented in Table 1 [Table 1].

The mean peak LH levels of the patients whose stimulated LH peak was pubertal according to C1 were 9.33 ± 6.60 (5-42.5 mIU/ml). The mean age of this group was 7.65 ± 1.2 (3.4-9.5) years, the mean Δ bone age was $1, 0167 \pm 0.98$ [-1.5 - (+ 3.4)] years, the mean basal LH was 0.32 ± 0.49 (0.07-2,7) mIU/ml, mean height SDS was 0.52 ± 0.95 [-1.80 - (+ 3)] SDS and the mean BMI SDS was 0.58 ± 0.88 [-1.9 - (+ 2.3)].

According to C1; pubertal LH was present in 94.1% ($n = 96$) of patients who reached pubertal LH value in 45th minutes, 83.3% ($n = 85$) in 30th minutes, 79.4% ($n = 81$) of patients in 60 minutes. The highest percentage of pubertal responses (94.1%) was found to be between 4.7 and 4.9 mIU/ml of the 6 patients who did not have a LH value of ≥ 5 mIU/ml at 45th min (at 30th or 60th min, they had pubertal LH level). When the LH values were evaluated according to the intraassay CV of the kit, pubertal response could be increased to 96% in 45 minutes. According to C1, there were 4 patients with pubertal LH level at only 30 minutes, 8 patients with pubertal LH level in 45 minutes, and 2 patients with pubertal LH level in 60 minutes. Table 2 shows the distribution of treatment according to LHRH test results according to both cut-offs.

According to C1, the rate of obtaining pubertal LH was 98% when the 30th and 45th minute samples were evaluated together;

Table 1: According to the two cut-off values and pubertal and pre-pubertal status; age, basal and peak LH values, peak LH times of the subjects

	Pubertal according to C1	Pre-pubertal according to C1		Pubertal according to C2	Pre-pubertal according to C2	
n (%)	102 (49.2)	105 (50.8)		139 (67)	68 (33)	
Age (year)	7.65±1.2 (3.4-9.5)	7.36±1.24 (3.9-9.4)		7.67±1.24 (3.4-9.5)	7.17±1.13 (4.3-9.3)	
Basal LH (mIU/ml)	0.32±0.49 (0.07-2.7)	0.078±0.03 (0.07-0.3)		0.26±0.43 (0.07-2.7)	0.074±0.02 (0.07-0.2)	
Peak LH (mIU/ml)	9.3±6.6 (5-42.5)	2.77±1.3 (0.2-4.99)		7.9±6.08 (3.4-42.5)	1.94±0.82 (0.2-3.3)	
	% (N)	% (N)	P	% (N)	% (N)	P
PeakLH time % (n)						
15 th min	0 (0)	1 (1)	0.000	0.7 (1)		0.001
30 th min	47.1 (48)	19 (20)	0.000	40.3 (56)	17.6 (12)	0.000
45 th min	43.1 (44)	57.1 (60)	0.000	46.8 (65)	57.4 (39)	0.000
60 th min	9.8 (10)	21 (22)	0.000	12.2 (17)	22.1 (15)	0.000
90 th min	0 (0)	1.9 (2)	0.000	0 (0)	2.9 (2)	0.000

Table 2: Treatment distribution according to LHRH test result

LHRH test	GnRH analog treatment started	No treatment	Total
Peak LH ≥5 mIU/ml n (%)	102 (100)	0 (0)	102 (100)
Peak LH <5 mIU/ml n (%)	9 (8,5)	96 (91,5)	105 (100)
Peak LH ≥3,3 mIU/ml n (%)	111 (80)	28 (20)	139 (100)
Peak LH <3,3 mIU/ml n (%)	0 (0)	68 (100)	68 (100)

92,1%, when the 30th and 60th minute samples were obtained together and 96% when the 45th and 60th minute samples were obtained together.

The mean peak LH levels of the patients who received pubertal LH peak according to C2 was 7.99 ± 6,085 (3.40-42.50 mIU/ml). The mean age of this group was 7,6741 ± 1,24 (3,40-9,50) years, the mean Δ bone age was 1,008 ± 0,97 [-1,5 - (+ 3,5)] years, the mean basal LH was 0.26 ± 0.43 (0.07-2.7) mIU/ml, the mean height SDS was 0.54 ± 0.97 [-2.0 - (+ 3.9)] and the mean BMI SDS was 0.557 ± 0.957 [-1.9] - (+ 2,9)].

Of the 139 cases who reached pubertal LH value according to C2, it was determined that pubertal LH was measured in 98.5% (n = 137) in 45th minutes, 96.4% (n = 134) in 60th minutes, 94.2% (n = 131) in 30th minutes.

Pubertal LH levels were determined according to both cut-off values in all 27 patients with baseline LH ≥0.31 mIU/ml. According to C1, the sensitivity was 26.4% and the specificity was 100%. According to C2, sensitivity was 19.4% and specificity was 100%. Table 3 shows the evaluation of basal LH values and peak LH levels according to both cut offs.

Of the 30 patients with baseline LH ≥0.3 mIU/ml; pubertal LH levels were detected in 96.6% (n = 29) according to C1 and 100% with respect to C2. The sensitivity and specificity of C1 were 29.4% and 96.6%, respectively. The sensitivity and specificity of C2 were 21.5% and 100%, respectively. In 36 patients with baseline LH ≥0.2 mIU/ml, 94.4% (n = 34) according to C1 and 97.2% (n = 35) according to C2, pubertal

response revealed. In 53 patients with baseline LH ≥0.1 mIU/ml, pubertal response was detected in 81% (n = 43) of patients with C1 and 94.3% (n = 50) of patients with C2.

According to both cut-off values, there was no PLH value at 90th minutes in pubertal patients. According to C2, pubertal PLH was not detected except one case at 15th minutes. In this case, the LH value was in pubertal level at 15th and 30th minutes.

According to C2, when the 30th and 45th-minute samples were evaluated together, the rate of catching pubertal response was 99.2%, and the 30th and 60th-minute samples were 99.2%, and the 45th and 60th-minute samples were 99.2%.

According to C1 and C2, the percentages of pubertal and pre-pubertal PLH were found statistically significant at all minutes (P < 0.05). When 30th, 45th and 60th minutes were evaluated together according to both cut-off values; 100% pubertal LH was detected.

In our study, the basal FSH level of pubertal patients according to C1 was 3.75 ± 2.16 (0.50-10.7) mIU/ml, and the basal FSH level of pre-pubertal patients according to C1 was 2.03 ± 1.21 (0.2-5.8) mIU/ml. The basal FSH level of pubertal patients according to C2 was 3.45 ± 2.01 (0.50-10.7) mIU/ml. The basal FSH level of pre-pubertal patients according to C2 was 1.72 ± 1.1 (0.2-5.8) mIU/ml. The difference was statistically significant (P < 0.05).

The mean LH/FSH ratio of the patients who were in the pubertal stage according to C1 was 0.52 ± 0.46 (0.15-3.1). The mean LH/FSH ratio of patients with pubertal LH peak according to C2 was 0.455 ± 0.419 (0.04-3.1), and the mean LH/FSH ratio of pre-pubertal patients was 0.13 ± 0.06 -0.39). The difference between pubertal and pre-pubertal groups according to both cut-off values was statistically significant (P < 0.0001). All 30 girls with peak LH/FSH ≥0.6 were pubertal according to both cut-off values. Forty (97.5%) of 41 cases with peak LH/FSH ≥0.48 were found to be pubertal according to C1 and 100% of 41 cases were pubertal according to C2. Of the 55 cases with peak LH/FSH ≥0.4, 50 (90.1%) were found to

Table 3: Evaluation of both cut-offs according to basal LH levels

	C2				C1			
	Peak LH <3.3 mIU/ml		Peak LH ≥3.3 mIU/ml		Peak LH <5 mIU/ml		Peak LH ≥5 mIU/ml	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Basal LH categories								
Basal LH <0.2	64	37.4	107	62.6	103	60.2	68	39.8
Basal LH 0.2-0.29	1	16.7	5	83.3	1	16.7	5	83.3
Basal LH ≥0.3	0	0.0	30	100.0	1	3.3	29	96.7
Chi-Square	17.220				35.928			
<i>P</i>	<0.001				<0.001			

Table 4: Evaluation of both cut-offs according to Peak LH/FSH ratio

	Peak LH/FSH categories								Chi-Square	<i>P</i>
	<0.4		0.4-0.47		0.48-0.59		0.6 and above			
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%		
C2										
Peak LH <3.3 mIU/ml	65 ^a	100.0	0 ^b	0.0	0 ^b	0.0	0 ^b	0.0	34.286	<0.001
Peak LH ≥3.3 mIU/ml	87 ^a	61.3	14 ^b	9.9	11 ^b	7.7	30 ^b	21.1		
C1										
Peak LH <5 mIU/ml	100 ^a	95.2	4 ^b	3.8	1 ^{b,c}	1.0	0 ^c	0.0	55.061	<0.001
Peak LH ≥5 mIU/ml	52 ^a	51.0	10 ^b	9.8	10 ^{b,c}	9.8	30 ^c	29.4		

be pubertal according to C1 and all of 55 cases were pubertal according to C2. The evaluation of the peak LH / FSH ratio according to both cut-off is given in Table 4.

DISCUSSION

In our study, we evaluated the relationship between basal LH level, peak LH, peak LH time and peak LH/FSH ratio of 207 female patients who underwent the LHRH-ST. Pubertal LH levels were found in all 27 patients with baseline LH ≥0.31 mIU/ml according to both cut-off values. The basal LH level ≥0.31 mIU/ml was considered to be pubertal, with low sensitivity according to C1 and C2 (26.4% and 19.4%, respectively). Pubertal LH values were obtained in 45th minute in 94.1% of 102 cases who reached pubertal LH according to C1 and in 98.5% of 139 cases who reached pubertal LH according to C2. In the LHRH-ST, taking blood samples only at the 45th minute is sensitive in detecting pubertal cases while reducing cost and workload.

The peak LH/FSH ratios were found to be 0.52 ± 0.46 (0.15-3.1) and 0.455 ± 0.419 (0.04-3.1), in cases with pubertal LH values for C1 and C2, respectively. These pubertal values were significantly higher according to both cut-off values than that of the pre-pubertal cases. All 30 girls with LH/FSH ≥0.6 were pubertal according to both cut-off values. The LHRH-ST is the gold standard for the diagnosis of CPP. Baseline LH levels of ≥0.3 mIU/ml with ICMA and ≥0.6 mIU/ml with IFMA decreased sensitivity, while increasing the positive predictive value and specificity for CPP to 100%^[11,19] Kandemir *et al.*^[10] found that the sensitivity and specificity of basal LH were

low (69.1% and 79.6%, respectively) in the diagnosis of CPP. When the basal LH level of 1 IU/L was accepted as the cut-off level for the diagnosis of CPP, the positive predictive value of basal LH was 96.4% and the negative predictive value of it was 61.8%.^[10] Houk *et al.*^[20] suggested that the use of basal LH >0.83 IU/L as the cut-off value could be used in the diagnosis of CPP with high sensitivity (93%) and specificity (100%). In the study of Resende *et al.*,^[12] basal LH >0.2 IU/L level was taken as an indicator of activation of HPG axis in girls. However, the frequency of overlapping LH values between girls in first (T1) and other stages of Telarche was 10.4% (8/77). Progressive bone age and baseline LH greater than 0.2 IU/liter show evidence of the maturity of the HPG axis in girls.^[12] In our study, it was found that pubertal response was obtained in 94.4% (34/36) and 97.2% (35/36) of 36 patients with basal LH ≥0.2 mIU/ml (34/36) according to C1 and C2, respectively. Pubertal peak LH levels were detected in 81% (43/53) and 94.3% (50/53) of 53 patients with baseline LH ≥0.1 mIU/ml according to C1 and C2, respectively. Neely *et al.*^[19] reported that basal LH >0.1 IU/L was diagnostic for CPP with 94% sensitivity and 88% specificity. In this study, they showed that a cut off value of >0.3 IU/L increased the specificity to 100%, although sensitivity decreased. Brito *et al.*^[11] found a basal LH pubertal cut-off value of >0.6 IU/L for both sexes. The sensitivity of basal LH was 62.7% (32/51), the specificity was 100% (13/13), the positive predictive value was 100% (32/32) and the negative predictive value was 40.6% (13/32) in girls. In our study, 96.6% (*n* = 29) and 100% of 30 patients with baseline LH value ≥0.3 mIU/ml had a pubertal response according to C1 and C2, respectively.

Pubertal LH levels were found in all 27 patients with baseline LH ≥ 0.31 mIU/ml according to both cut-off values. We conclude that if the basal LH value is ≥ 0.31 mIU/ml, the diagnosis of precocious puberty can be made without LHRH testing in girls with evidence of puberty.

In many studies, the peak LH response in each LHRH test was found between 30th and 60th minutes in all groups.^[9,11,12,19,21] In the study of Resende *et al.*,^[12] pubertal LH level was taken as 3.3 IU/L by ICMA. Considering this cut-off in girls, no significant difference was found between Telarche stage 1 (T1) and 2 (T2) groups. Because 46.1% (6/13) of the girls at Telarche stage 2 reported overlapping values with the Telarche stage 1 group. In the literature, studies with peak pubertal cut-off LH values of ≥ 3.3 mIU/ml were published,^[12] but most of the current studies^[10,19] have taken the cut-off LH as ≥ 5 mIU/ml. In the study of Kandemir *et al.*,^[10] it was reported that CPP could be diagnosed with high sensitivity (98%) and specificity (100%) by performing LHRH-ST (PLH > 5 was taken) and measuring the LH level in the sample taken at 40th minute. In our study, LHRH-ST results were examined for the first time in the literature according to two cut-off values. Peak LH values in our study were determined in between 30th and 60th minutes. According to C1, pubertal LH values were determined in 45th minute in 94.1% (96/102) of the samples. The highest percentage of pubertal responses (94.1%) was at 45th minutes. At this minute, the LH value of 6 patients with the LH value of ≥ 5 mIU/ml ranged from 4.7 to 4.9 mIU/ml (pubertal LH level was obtained in the 30th and 60th minutes). Considering the clinical and laboratory findings and the intraassay CV of the LH kit, taking the blood sample at 45th minute in the LHRH test seems sufficient for the diagnosis of CPP. A total of 98.5% (137/139) of 139 patients who reached pubertal LH according to C2 had pubertal LH at 45 minutes. Further studies are needed especially regarding the diagnostic reliability of the first cut-off value.

Basal FSH levels were largely overlapping between all pubertal stages, and basal and evoked FSH levels had poor diagnostic value in the diagnosis of CPP.^[8,9] The importance of FSH measurement is closely related to differences in hormone profile between pre-pubertal and pubertal children. A decrease in FSH and an increase in LH occur simultaneously. From a physiological point of view, this observation confirms that FSH is the main hormone secreted in the pre-pubertal period and LH in the pubertal period.^[8] In our study, mean basal FSH levels were significantly different between pubertal and pre-pubertal groups according to both cut-off values ($P < 0.05$). The pubertal basal FSH level was 3.75 ± 2.16 (0.50-10.7) mIU/ml, pre-pubertal basal FSH level was 2.03 ± 1.21 (0.2-5.8) mIU/ml according to C1. According to C2; the pubertal basal FSH level was 3.45 ± 2.01 (0.50-10.7) mIU/ml and the pre-pubertal basal FSH level was found to be 1.72 ± 1.1 (0.2-5.8) mIU/ml.

Jiang *et al.*^[21] reported that LH/FSH > 0.9 may be diagnostic at the 15th minute of the test, but with lower sensitivity and specificity (80% and 90%, respectively). In another

study, peak LH/FSH > 1 had the highest positive predictive value (93.8%). In this study, basal and stimulated LH/FSH ratios were shown to have lower sensitivity and specificity than the peak LH level in the diagnosis of CPP.^[22] In our study, the difference in peak LH/FSH ratios between pubertal and pre-pubertal groups according to both cut-off values was statistically significant ($P < 0.0001$). According to C1, the pubertal peak LH/FSH ratio was 0.52 ± 0.46 (0.15-3.1) and the pre-pubertal peak LH/FSH ratio was 0.17 ± 0.09 (0.02-0.58). According to C2, the pubertal peak LH/FSH ratio was 0.455 ± 0.419 (0.04-3.1) and pre-pubertal peak LH/FSH ratio was 0.13 ± 0.06 (0.02-0.39). In our study, all 30 girls with peak LH/FSH ≥ 0.6 were pubertal according to both cut-off values. Forty (97.5%) of 41 cases with peak LH/FSH ≥ 0.48 were found to be pubertal according to C1 and all according to C2. Of the 55 cases with peak LH/FSH ≥ 0.4 , 50 (90.1%) were found to be pubertal according to C1 and all according to C2. The high LH/FSH ratio (> 0.66) should be accepted in favor of progressive CPP.^[23] In our study, we found that the peak LH/FSH ≥ 0.6 could be evaluated in favor of precocious puberty in girls.

CONCLUSION

We concluded that the LHRH test was not necessary for the diagnosis of CPP in girls who were clinically thought to have puberty and had basal LH value ≥ 0.31 mIU/ml and it was sufficient for the diagnosis of CPP in these patients to take a blood sample at the 45th minute and to evaluate the clinical findings. We recommend taking blood samples in 30-45th and 60th minutes and to perform a 45-minute blood sample analysis at first stage and if the diagnostic value cannot be obtained, then to perform the 30th and 60th-minute blood sample analyses. We consider that this approach will reduce the cost of the test. We also think that the intra-assay CV ratio of the kits may be taken into consideration when evaluating the test results. Further studies are needed for the diagnostic value of PLH.

Ethical approval

The study was approved by the Medical Specialization and Education Board of University of Health Sciences Dr. Sami Ulus Training and Research Hospital on 15.05.2018 with the approval number of 77799008.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/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.

REFERENCES

- Berberoglu M. Precocious puberty and normal variant puberty: Definition, etiology, diagnosis and current management. *J Clin Res Pediatr Endocrinol* 2009;1:164–74.
- Kaplowitz PB, Oberfield SE. Therapeutics Pediatric Endocrine Society. Reexamination of the age limit for defining when puberty is precocious in the girls in the United states. Implications for evaluation and treatment. *Pediatrics* 1999;104:936-41.
- Grumbach MM. The neuroendocrinology of human puberty revisited. *Horm Res* 2002;57(Suppl 2):2-14.
- Hermann-Giddens ME, Slora EJ, Wasserman RC, Bourdony CJ, Bhapkar MV, Koch GG, *et al.* Secondary sexual characteristics and menses in young girls seen in Office practice. *Pediatrics* 1997;99:505-12.
- Bundak R, Darendeliler F, Günöz H, Baş F, Saka N., Neyzi O. Puberty and pubertal growth in healthy Turkish girls: No evidence for secular trend. *J Clin Res Pediatr Endocrinol* 2008;1:8-14.
- Tato L, Savage MO, Antoniazzi F, Buzi F, Di Maio S, Oostdijk W, *et al.* Optimal therapy of pubertal disorders in precocious/earlypuberty. *J Pediatr Endocrinol Metab* 2001;14:985-95.
- Kletter GB, Klein KO, Wong YY. A pediatrician's guide to central precocious puberty. *Clin Pediatr (Phila)* 2015;54:414-24.
- Lee PA. Laboratory monitoring of children with precocious puberty. *Arch Pediatr Adolesc Med* 1994;148:369-76.
- Cavallo A, Richards GE, Busey S, Michaels SE. A simplified gonadotrophin-releasing hormone test for precocious puberty. *Clin Endocrinol* 1995;42:641-6.
- Kandemir N, Demirbilek H, Özön ZA, Gönç N, Alikasıfoğlu A. GnRH stimulation test in precocious puberty: Single sample is adequate for diagnosis and dose adjustment. *J Clin Res Pediatr Endocrinol* 2011;3:12-7.
- Brito VN, Batista MC, Borges MF, Latronico AC, Kohek MB, Thirone AC, *et al.* Diagnostic value of fluorometric assays in the evaluation of precocious puberty. *J Clin Endocrinol Metab* 1999;84:3539-44.
- Resende EA, Lara BH, Reis JD, Ferreira BP, Pereira GA, Borges MF. Assessment of basal and gonadotropin-releasing hormone-stimulated gonadotropins by immune chemilumino metric and immune fluorometric assays in normal children. *J Clin Endocrinol Metab* 2007;92:1424–9.
- Carrillo AA, Bao Y. Hormonal dynamic tests and genetic tests used in pediatric endocrinology. In: Lifshitz F, editor. *Pediatric Endocrinology*. Newyork: Informa Healthcare; 2007. p. 737-67.
- Neyzi O, Bundak R, Gökçay G, Gunoz H, Furman A, Gokcay G, *et al.* Reference values for weight, height, head circumference, and body mass index in Turkish children. *J Clin Res Pediatr Endocrinol* 2015;7:280–93.
- Neyzi O, Furman A, Bundak R, Gunoz H, Darendeliler F, Bas F. Growth references for Turkish children aged 6 to 18 years. *Acta Paediatr* 2006;95:1635-41.
- Bundak R, Furman A, Gunoz H, Darendeliler F, Bas F, Neyzi O. Body mass index references for Turkish children. *Acta Paediatr* 2006;95:194-8.
- Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 1970;45:13-23.
- Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in girls. *Arch Dis Child* 1969;44:291-303.
- Neely EK, Hintz RL, Wilson DM, Lee PA, Gaultier T, Argente J, *et al.* Normal ranges for immune chemiluminometric gonadotropin assays. *J Pediatr* 1995;127:40-6.
- Houk CP, Kunselman AR, Lee PA. Adequacy of a single unstimulated luteinizing hormone level to diagnose central precocious puberty in girls. *Pediatrics* 2009;123:1059-63.
- Jiang YJ, Liang L, Zhouliang ZC, Fu JF, Li Y, Hong F, *et al.* Simplified gonadorelin stimulation test in diagnosis of precocious puberty. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 2004;33:452-5.
- Wacharasindhu S, Srivuthana S, Aroonparkmongkol S, Shotelersuk V. A cost-benefit of GnRH stimulation test in diagnosis of central precocious puberty (CPP). *J Med Assoc Thai* 2000;83:1105-11.
- Carel JC, Eugster EA, Rogol A, Ghizzoni L, Palmert MR; ESPE-LWPES GnRH Analogs Consensus Conference Group, Antoniazzi F, Berenbaum S, Bourguignon JP, Chrousos GP, Coste J, Deal S, de Vries L, Foster C, Heger S, Holland J, Jahnukainen K, Juul A, Kaplowitz P, Lahlou N, Lee MM, Lee P, Merke DP, Neely EK, Oostdijk W, Phillip M, Rosenfield RL, Shulman D, Styne D, Tauber M, Wit JM. Consensus statement on the use of gonadotropin-releasing hormone analogs in children. *Pediatrics* 2009;123:e752-62.