

First-year efficacy of 3.75 mg versus 11.25 mg depot leuprolide acetate and predictors of biochemical non-suppression in girls with central precocious puberty

Didem Helvacioğlu¹, Elif Keleştemur¹, İlknur Kurt¹, Ahmet Kahveci¹, Büşra Gürpınar Tosun¹, Abdullah Bereket¹

¹Department of Pediatric Endocrinology, Marmara University Faculty of Medicine, İstanbul, Türkiye

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ABSTRACT

Objective: To compare first-year biochemical and auxological outcomes of monthly 3.75 mg and 3-monthly 11.25 mg depot leuprolide acetate (dLA) treatment in girls with central precocious puberty (CPP) and to evaluate factors associated with biochemical non-suppression and first-year growth velocity.

Methods: This single-center prospective quasi-randomized comparative study included 87 girls with CPP treated with dLA for 12 months. Patients received either monthly 3.75 mg (n=46) or 3-monthly 11.25 mg dLA treatment (n=41). Auxological parameters, gonadotropin-releasing hormone (GnRH)-stimulated luteinizing hormone (LH) responses, and LH levels measured 40 minutes after dLA injection (40-minute post-dLA LH) were evaluated. Biochemical non-suppression was defined as a year-one 40-minute post-dLA LH level >3.07 IU/L. Logistic regression was used to identify predictors of biochemical non-suppression, and linear regression was performed to determine factors associated with first-year annual growth velocity.

Results: One year after initiation of treatment, there was no difference in annual growth velocity (5.73 ± 1.01 vs 5.81 ± 0.92 cm/year, $p=0.956$), bone age, Δ bone age, and other auxological and pelvic ultrasonographic parameters between treatment groups. Year-one biochemical suppression was achieved in 33/46 patients (71.7%) in the 3.75 mg group and 33/41 patients (80.5%) in the 11.25 mg group, with no significant between-group difference ($p=0.450$). Biochemical non-suppression was associated with higher annual growth velocity and higher pretreatment GnRH-stimulated peak LH; in logistic regression analysis, pretreatment GnRH-stimulated peak LH was the only independent predictor of biochemical non-suppression (adjusted OR 1.04, 95% CI 1.01–1.07, $p=0.012$). First-year annual growth velocity correlated with 6-month GnRH-stimulated peak LH ($r=0.320$, $p=0.003$), 6-month 40-minute post-dLA LH ($r=0.339$, $p=0.001$), year-one basal LH ($r=0.314$, $p=0.003$), and year-one 40-minute post-dLA LH ($r=0.517$, $p<0.001$). In multivariable analysis, year-one 40-minute post-dLA LH remained independently associated with annual growth velocity ($B=0.39$, 95% CI 0.26–0.52, $p<0.001$), whereas other parameters did not.

Conclusions: Monthly 3.75 mg and 3-monthly 11.25 mg depot leuprolide acetate provided comparable first-year auxological outcomes. Higher pretreatment GnRH-stimulated peak LH predicted biochemical non-suppression, whereas year-one 40-minute post-dLA LH level was independently associated with first-year growth velocity, suggesting that it may represent a more robust parameter for monitoring residual pubertal activity during treatment.

Keywords: puberty, precocious, gonadotropin-releasing hormone, luteinizing hormone, treatment outcome, leuprolide



✉ Didem Helvacioğlu ▪ drdidemh@gmail.com

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INTRODUCTION

Central precocious puberty (CPP) is characterized by premature activation of the hypothalamic–pituitary–gonadal axis, resulting in progressive pubertal development before 8 years of age in girls. Accelerated growth and advanced skeletal maturation may compromise adult height if treatment is not initiated in appropriate patients. The main goals of therapy are to suppress pubertal progression, normalize growth velocity, slow bone age advancement, and preserve height potential.¹ Gonadotropin-releasing hormone (GnRH) agonists (GnRHa) are the standard treatment for CPP. Depot leuprolide acetate (dLA) is available as monthly 3.75 mg and 3-monthly 11.25 mg formulations, and previous studies have reported broadly comparable biochemical and auxological efficacy between these regimens. The 3-monthly formulation may also reduce injection burden during long-term treatment.^{2–5}

Monitoring treatment adequacy during GnRHa therapy remains challenging. Although basal or random luteinizing hormone (LH) measurements are practical, elevated pre-injection or random LH levels may occur despite adequate clinical suppression, limiting their reliability as isolated markers of treatment adequacy.^{6–8} GnRH-stimulated LH testing has traditionally been used to assess biochemical suppression during treatment; however, it requires venous access and repeated sampling, limiting its practicality for routine follow-up. Post-dLA LH sampling has therefore been proposed as a practical dynamic alternative that assesses the acute gonadotropin response to the therapeutic agonist.^{9,10}

Building on our previously identified 40-minute post-dLA LH threshold for monitoring pubertal suppression¹¹, this study compared first-year biochemical and auxological outcomes of monthly 3.75 mg and 3-monthly 11.25 mg dLA treatment in girls with CPP. We also evaluated factors associated with year-one biochemical non-suppression and first-year annual growth velocity, with particular emphasis on the year-one 40-minute post-dLA LH response.

MATERIALS AND METHODS

Study design and patients

This single-center prospective quasi-randomized comparative study was conducted between 2022 and 2025 in a tertiary pediatric endocrinology clinic. Patients were allocated in a 1:1 ratio to receive either monthly dLA 3.75 mg or 3-monthly dLA 11.25 mg according to a

predefined alternating sequence. Treatment allocation was implemented by the principal investigator without allocation concealment.

No a priori sample size calculation was performed. All eligible patients who met the inclusion criteria and provided consent during the predefined study period were enrolled prospectively. A total of 91 patients were allocated to the two treatment groups evaluated in the present study: 46 patients to the monthly 3.75 mg group and 45 patients to the 3-monthly 11.25 mg group. Of the four patients excluded from the 3-monthly 11.25 mg group, one moved to another city, and three did not attend scheduled follow-up visits regularly. We calculated scheduled dates for dLA administration and hormonal testing in advance, and the same experienced pediatric endocrinology nurse performed all injections, blood sampling, and stimulation tests. No procurement or administration problems were documented. Therefore, the final analysis included 87 girls who completed 12 months of follow-up and had complete first-year auxological and hormonal data: 46 patients in the monthly 3.75 mg group and 41 patients in the 3-monthly 11.25 mg group. The analyses were therefore performed according to the per-protocol principle. Patient flow is shown in Figure 1.

CPP was diagnosed according to established criteria: onset of breast development before 8 years of age, accelerated growth velocity and/or advanced bone age, and a pubertal LH response to GnRH stimulation (peak LH ≥ 5 IU/L).¹ Exclusion criteria were peripheral precocious puberty, organic CPP, any chronic systemic disease affecting growth, and incomplete 12-month follow-up data.

Auxological and pubertal assessments

Clinical follow-up visits were scheduled at 3-month intervals throughout the first treatment year. At baseline and at each follow-up visit, height, weight, and body mass index (BMI) were measured and expressed as standard deviation scores (SDS) according to national reference data.¹² Pubertal stage was assessed according to Tanner criteria.¹³ Bone age was evaluated from left hand and wrist radiographs using the Greulich and Pyle method¹⁴, and the bone age/chronological age (BA/CA) ratio and bone age advancement (BA–CA) were calculated. Change in bone age (Δ BA) was calculated as year-one bone age minus baseline bone age. Annualized growth velocity during the first 6 months was calculated from baseline and 6-month height measurements, whereas first-year annual growth velocity was calculated from baseline and 12-month

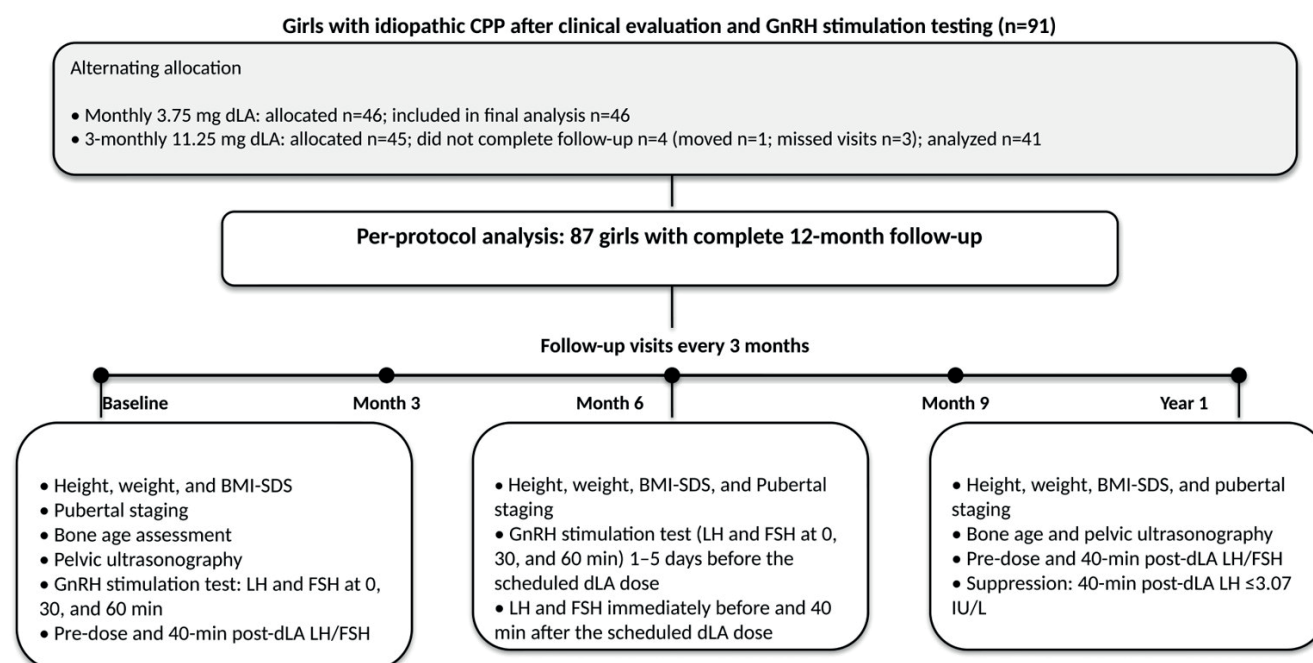


Figure 1. Study flow and follow-up protocol.

CPP, central precocious puberty; dLA, depot leuprolide acetate; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; BMI-SDS, body mass index standard deviation score.

height measurements. Standing height was measured using a calibrated Harpenden stadiometer (Holtain Ltd., Crymych, UK) and recorded to the nearest 0.1 cm. Pelvic ultrasonography was performed at baseline and at year 1 as part of routine clinical assessment.

Hormonal assessments

All blood samples were collected in the morning between 08:00 and 08:30. At baseline, the GnRH stimulation test was performed after insertion of an intravenous cannula. Blood samples were obtained for basal LH and follicle-stimulating hormone (FSH), followed by intravenous administration of gonadorelin acetate at 2.5 µg/kg, up to a maximum dose of 100 µg. Blood samples for LH and FSH were then collected at 30 and 60 minutes, and peak LH was recorded. At treatment initiation, serum LH and FSH were measured immediately before the first dLA injection and 40 minutes after injection. At month 6, a standard GnRH stimulation test was performed 1–5 days before the scheduled dLA dose. On the day of the scheduled dLA injection, LH and FSH were again measured immediately before and 40 minutes after dLA administration. The same pre-injection and 40-minute post-dLA sampling protocol was repeated at the year-one dose visit. All hormonal samples were

analyzed on the day of collection and were not stored for subsequent batch analysis. Serum LH, FSH, and estradiol were measured using a chemiluminescent immunoassay system (Beckman Coulter Diagnostics, CA, USA) according to the manufacturer's protocol. The minimum detectable concentration was 0.07 IU/L for both LH and FSH.

Definition of biochemical suppression

At 6 months, biochemical suppression was assessed using the standard GnRH stimulation test and was defined as a GnRH-stimulated peak LH level <3 IU/L, consistent with previous CPP treatment studies.¹⁵⁻¹⁷ Six-month GnRH-stimulated peak LH and 6-month 40-minute post-dLA LH levels were also evaluated as on-treatment hormonal parameters. As a repeat GnRH stimulation test was not included in the year-one protocol, year-one biochemical suppression was assessed using the previously validated 40-minute post-dLA LH threshold. Year-one biochemical suppression was defined as a year-one 40-minute post-dLA LH level of ≤ 3.07 IU/L, and biochemical non-suppression was defined as a level >3.07 IU/L. This threshold was derived from our group's previously published receiver operating characteristic (ROC) analysis in a prospective cohort of girls with CPP, in which a 40-minute post-dLA LH

level of 3.07 IU/L optimally discriminated between GnRH-stimulated suppressed and non-suppressed patients, with an area under the curve (AUC) of 0.83, sensitivity of 80.8%, and specificity of 75.5%.¹¹

Statistical analysis

The normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables are presented as mean $\pm$ standard deviation or median (25th–75th percentiles), as appropriate. Categorical variables are presented as number and percentage. Between-group comparisons were performed using the independent-samples t-test or Mann–Whitney U test for continuous variables and the chi-square or Fisher’s exact test for categorical variables. We calculated the magnitude of decline in growth velocity for each patient by subtracting the first-year annual growth velocity from the 6-month annualized growth velocity and compared it between treatment groups using an independent-samples t-test. We evaluated within-group changes in growth velocity between 6 months and year one using a paired-samples t-test. We used Pearson or Spearman correlation analyses to assess associations between LH parameters and annual growth velocity. A multivariable logistic regression model including treatment regimen and pretreatment GnRH-stimulated peak LH was used to evaluate factors associated with year-one biochemical non-suppression; adjusted odds ratios (OR) with 95% confidence intervals (CI) are reported. A multivariable linear regression model including treatment regimen, age at treatment initiation, pretreatment GnRH-stimulated peak LH, and year-one 40-minute post-dLA LH was used to evaluate factors associated with annual growth velocity. Variables were selected based on clinical relevance and their associations with the outcomes, while correlated on-treatment LH measurements were not included simultaneously. All analyses were performed using GraphPad Prism version 10.0 (GraphPad Software, Boston, MA, USA). A two-sided p-value < 0.05 was considered statistically significant.

Ethical approval

The study was approved by the institutional Ethics Committee, approval number 09.2022.486, and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from parents or legal

guardians of all participants, and patient assent was obtained where appropriate.

RESULTS

At treatment initiation, 87 girls with CPP were included in the baseline comparison: 46 patients received monthly dLA 3.75 mg and 41 patients received 3-monthly dLA 11.25 mg. Chronological age was higher in the monthly 3.75 mg group than in the 3-monthly 11.25 mg group (8.27 ± 0.60 vs 7.86 ± 0.94 years, $p=0.014$), whereas bone age and the BA/CA ratio did not differ significantly between groups ($p=0.065$ and $p=0.961$, respectively).

Baseline anthropometric parameters, basal and GnRH-stimulated gonadotropin responses, LH/FSH ratios, and pelvic ultrasonographic findings were comparable between groups, except for uterine length and pre-stimulation basal FSH during the pretreatment GnRH test, both of which were higher in the monthly 3.75 mg group (both $p=0.046$) (Table 1). At year one, annual growth velocity was comparable between the monthly 3.75 mg and 3-monthly 11.25 mg groups (5.73 ± 1.01 vs 5.81 ± 0.92 cm/year, $p=0.956$). Other first-year auxological and pelvic ultrasonographic outcomes, including height SDS, BMI-SDS, bone age, BA/CA ratio, BA advancement, uterine measurements, and ovarian volumes, were also similar between treatment regimens. The year-one 40-minute post-dLA LH level did not differ significantly between groups, whereas basal LH, basal FSH, basal LH/FSH ratio, 40-minute FSH, and 40-minute LH/FSH ratio differed between groups (Table 2). In the 3-monthly 11.25 mg group, growth velocity decreased from 8.03 ± 2.12 to 5.81 ± 0.92 cm/year, and in the monthly 3.75 mg group, it decreased from 8.09 ± 2.08 to 5.73 ± 1.01 cm/year (both $p<0.001$). The magnitude of decline in growth velocity was 2.36 ± 2.14 cm/year in the monthly 3.75 mg group and 2.22 ± 2.24 cm/year in the 3-monthly 11.25 mg group, with no significant between-group difference (mean difference, 0.14 cm/year; 95% CI, -0.80 to 1.08 ; $p=0.767$). At the year-one dose visit, using the predefined 40-minute post-dLA LH threshold, biochemical suppression was achieved in 33/46 patients (71.7%) in the monthly 3.75 mg group and 33/41 patients (80.5%) in the 3-monthly 11.25 mg group, with no significant between-group difference ($p=0.450$). Patients with year-one biochemical non-suppression and those with suppression were comparable in age at treatment initiation,

Table 1. Pretreatment clinical, auxological, ultrasonographic, and hormonal characteristics by treatment regimen.

	dLA 3.75 mg (n=46)	dLA 11.25 mg (n=41)	p
Age, years	8.27 ± 0.60	7.86 ± 0.94	0.014
Bone age, years	9.80 ± 1.15	9.71 ± 1.32	0.065
BA/CA ratio	1.18 ± 0.12	1.18 ± 0.14	0.961
Height SDS	0.87 ± 1.11	1.06 ± 1.05	0.394
Weight SDS	1.03 ± 1.19	1.05 ± 0.98	0.929
BMI SDS	0.83 ± 1.17	0.69 ± 1.03	0.569
Basal LH, IU/L	0.84 (0.43–2.00)	0.62 (0.47–0.88)	0.225
Basal FSH, IU/L	3.40 (1.65–4.70)	2.73 (1.95–3.94)	0.401
Uterine length, mm	35.00 (33.00–41.00)	32.00 (29.00–37.00)	0.046
Uterine volume, cm ³	2.80 (1.70–4.70)	2.62 (1.88–4.25)	0.397
Left ovarian volume, cm ³	2.30 (1.70–3.60)	1.60 (1.00–3.25)	0.410
Right ovarian volume, cm ³	2.20 (1.40–3.10)	1.65 (0.90–2.60)	0.065
Basal LH during GnRH test, IU/L	0.94 (0.36–1.62)	0.68 (0.49–1.46)	0.781
Peak LH during pretreatment GnRH test, IU/L	10.80 (6.64–15.75)	10.10 (6.81–21.35)	0.879
Basal FSH during GnRH test, IU/L	3.90 (2.43–5.48)	2.94 (2.05–4.10)	0.046
Peak FSH during pretreatment GnRH test, IU/L	12.73 ± 4.86	13.42 ± 3.70	0.212
Peak LH/FSH ratio during GnRH test	0.91 (0.70–1.54)	0.85 (0.50–1.61)	0.745

Values are presented as mean±SD or median (25th–75th percentiles), according to data distribution. BA, bone age; BMI, body mass index; CA, chronological age; dLA, depot leuprolide acetate; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; SD, standard deviation; SDS, standard deviation score.

Table 2. Year-one auxological, ultrasonographic, and hormonal outcomes by treatment regimen.

	dLA 3.75 mg (n=46)	dLA 11.25 mg (n=41)	p
Height SDS	1.06 ± 1.05	1.24 ± 1.04	0.411
Weight SDS	1.18 ± 1.11	1.20 ± 0.96	0.920
BMI-SDS	0.91 ± 1.07	0.80 ± 1.11	0.643
Bone age (BA), years	11.04 ± 0.95	10.66 ± 1.18	0.094
BA/CA ratio	1.21 ± 0.10	1.22 ± 0.14	0.640
BA advancement (BA–CA), years	1.23 ± 0.69	1.38 ± 0.61	0.191
Uterine length, mm	32.54 ± 7.52	28.96 ± 5.53	0.425
Uterine volume, cm ³	2.45 ± 2.74	1.90 ± 1.80	0.240
Left ovarian volume, cm ³	1.89 ± 1.32	1.74 ± 1.11	0.663
Right ovarian volume, cm ³	1.95 ± 1.16	1.63 ± 1.11	0.201
Annual growth velocity, cm/year	5.73 ± 1.01	5.81 ± 0.92	0.956
Hormonal outcomes at year one			
Basal LH, IU/L	0.68 ± 0.34	0.58 ± 0.40	0.020
Basal FSH, IU/L	1.42 ± 0.67	1.90 ± 1.00	0.005
Basal LH/FSH ratio	0.56 ± 0.32	0.36 ± 0.28	<0.001
40-minute post-dLA LH, IU/L	2.52 ± 1.22	2.63 ± 1.51	0.782
40-minute post-dLA FSH, IU/L	3.31 ± 1.59	4.63 ± 2.21	0.001
40-minute post-dLA LH/FSH ratio	0.87 ± 0.41	0.63 ± 0.34	0.003

Values are presented as mean±SD. BA, bone age; BMI-SDS, body mass index standard deviation score; CA, chronological age; dLA, depot leuprolide acetate; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SD, standard deviation; SDS, standard deviation score.

BA/CA ratio, and BMI-SDS (all $p>0.05$). Pretreatment GnRH-stimulated peak LH was significantly higher in the non-suppressed group than in the suppressed group (15.60 [9.27–25.00] vs 8.91 [6.54–14.84] IU/L, $p=0.004$). In addition, both 6-month GnRH-stimulated peak LH and 6-month 40-minute post-dLA LH were higher in patients with year-one biochemical non-suppression than in those with suppression (2.82 [2.09–4.04] vs 1.63 [1.18–2.22] IU/L and 4.04 [3.11–5.27] vs 2.33 [1.85–2.83] IU/L, respectively; both $p<0.001$). Patients with biochemical non-suppression also had higher annual growth velocity than those with suppression (6.45 [6.00–6.95] vs 5.50 [4.94–5.90] cm/year, $p<0.001$), whereas Δ BA was similar between groups ($p=0.510$) (Table 3). A multivariable logistic regression model including treatment regimen and pretreatment

GnRH-stimulated peak LH was constructed. Pretreatment GnRH-stimulated peak LH remained independently associated with biochemical non-suppression (adjusted OR 1.04, 95% CI 1.01–1.07, $p=0.012$), whereas treatment regimen was not independently associated with this outcome (Table 4). First-year annual growth velocity was positively correlated with both 6-month GnRH-stimulated peak LH ($r=0.320$, $p=0.003$) and 6-month 40-minute post-dLA LH ($r=0.339$, $p=0.001$). Year-one basal LH was also positively correlated with annual growth velocity ($r=0.314$, $p=0.003$); however, the strongest correlation was observed with year-one 40-minute post-dLA LH ($r=0.517$, $p<0.001$) (Table 5). In the multivariable linear regression model, year-one 40-minute post-dLA LH remained independently associated with annual growth velocity after adjustment

Table 3. Clinical and hormonal parameters according to year-one biochemical suppression status.

	Non-suppressed (n=21)	Suppressed (n=66)	p
Age, years	8.35 (7.62–8.83)	8.09 (7.59–8.63)	0.380
BA/CA ratio	1.19 (1.08–1.32)	1.17 (1.09–1.26)	0.510
BMI-SDS	0.91 (–0.24–1.73)	0.66 (0.23–1.52)	0.580
Pretreatment GnRH-stimulated peak LH, IU/L	15.60 (9.27–25.00)	8.91 (6.54–14.84)	0.004
6-month GnRH-stimulated peak LH, IU/L	2.82 (2.09–4.04)	1.63 (1.18–2.22)	<0.001
6-month 40-minute post-dLA LH, IU/L	4.04 (3.11–5.27)	2.33 (1.85–2.83)	<0.001
Δ BA, years	1.20 (1.00–1.90)	1.15 (0.97–2.00)	0.510
Annual GV, cm/year	6.45 (6.00–6.95)	5.50 (4.94–5.90)	<0.001

Values are presented as median (25th–75th percentiles). BA, bone age; BMI-SDS, body mass index standard deviation score; dLA, depot leuprolide acetate; GnRH, gonadotropin-releasing hormone; GV, growth velocity; LH, luteinizing hormone. Biochemical non-suppression was defined as a year-one 40-minute post-dLA LH level >3.07 IU/L, and suppression as ≤ 3.07 IU/L.

Table 4. Baseline predictors of year-one biochemical non-suppression.

	Adjusted OR (95% CI)	p
Treatment regimen, 11.25 mg vs 3.75 mg	0.57 (0.20–1.65)	0.298
Pretreatment GnRH-stimulated peak LH, IU/L	1.04 (1.01–1.07)	0.012

Year-one biochemical non-suppression was defined as a year-one 40-minute post-dLA LH level >3.07 IU/L. Model fit: omnibus likelihood-ratio $\chi^2(2)=8.32$, $p=0.016$; Nagelkerke $R^2=0.136$; Hosmer–Lemeshow $\chi^2(8)=1.17$, $p=0.997$. The maximum variance inflation factor was 1.00. CI, confidence interval; dLA, depot leuprolide acetate; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; OR, odds ratio.

Table 5. Correlations between LH parameters and annual growth velocity during the first year of treatment.

Variable	r	p
Pretreatment GnRH-stimulated peak LH	0.067	0.530
6-month GnRH-stimulated peak LH	0.320	0.003
6-month 40-minute post-dLA LH	0.339	0.001
Year-one basal LH	0.314	0.003
Year-one 40-minute post-dLA LH	0.517	<0.001

dLA, depot leuprolide acetate; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

Table 6. Factors associated with first-year annual growth velocity.

Variable	B coefficient	95% CI	p
Treatment regimen	−0.06	−0.40 to 0.29	0.745
Age at treatment initiation	−0.14	−0.36 to 0.09	0.231
Pretreatment GnRH-stimulated peak LH	−0.01	−0.02 to 0.00	0.213
Year-one 40-minute post-dLA LH	0.39	0.26 to 0.52	<0.001

Model fit: $R^2=0.304$; adjusted $R^2=0.270$; $F(4,82)=8.95$, $p<0.001$. B coefficients are unstandardized regression coefficients. The maximum variance inflation factor was 1.31. B, unstandardized regression coefficient; CI, confidence interval; dLA, depot leuprolide acetate; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

for treatment regimen, age at treatment initiation, and pretreatment GnRH-stimulated peak LH ($B=0.39$, 95% CI 0.26–0.52, $p<0.001$) (Table 6).

DISCUSSION

In this prospective quasi-randomized comparative study of girls with CPP treated with depot leuprolide acetate for 12 months, monthly 3.75 mg and 3-monthly 11.25 mg regimens provided comparable first-year auxological outcomes, with a numerically higher biochemical suppression rate in the 3-monthly regimen. Annual growth velocity, height SDS, BMI-SDS, bone age parameters, and pelvic ultrasonographic findings were similar between groups, and growth velocity declined significantly from 6 months to year one in both regimens. These findings support effective first-year auxological control in the majority of patients with both formulations and are consistent with previous studies reporting comparable clinical efficacy of monthly and longer-acting leuprolide acetate regimens in CPP.³⁻⁵

Beyond regimen comparison, pretreatment GnRH-stimulated peak LH independently predicted year-one biochemical non-suppression in our cohort. Previous studies have evaluated basal, pre-injection, and stimulated LH measurements as biochemical monitoring tools during GnRHa treatment; however, isolated LH elevations do not always correspond to clinical pubertal progression or unfavorable auxological outcomes.^{6,18-20} Although the specific relationship between pretreatment GnRH-stimulated peak LH and subsequent biochemical non-suppression has not been extensively investigated, our finding suggests that greater hypothalamic–pituitary–gonadal axis activation before treatment may be associated with a higher likelihood of incomplete biochemical suppression during follow-up. This finding requires confirmation in independent cohorts. Previous studies have also demonstrated that a single post-leuprolide LH measurement may provide a practical assessment of biochemical suppression, primarily based on

its agreement with stimulated LH criteria.^{9,10} Nevertheless, biochemical LH measurements have not consistently correlated with clinical or growth-related outcomes and should therefore be interpreted together with clinical and auxological parameters.²¹ In the present study, year-one 40-minute post-dLA LH remained independently associated with concurrent annual growth velocity. To our knowledge, this specific association has not previously been reported.

Previous randomized and comparative studies have shown effective pubertal suppression with both monthly and 3-monthly leuprolide acetate formulations, although stimulated gonadotropin responses may differ according to regimen, sampling time, and suppression threshold.^{3-5,22} In a recent comparative study, 3-monthly leuprolide acetate was associated with greater hormonal suppression and lower growth velocity and bone age advancement; however, predicted adult height did not differ significantly between monthly and 3-monthly regimens.²² In our cohort, biochemical suppression rates and first-year auxological outcomes were comparable between regimens, despite slightly different hormonal responses. This supports the interpretation that hormonal differences should be evaluated together with growth velocity and skeletal maturation rather than as isolated endpoints.

Biochemical monitoring during GnRH agonist therapy remains challenging, because basal or random LH measurements are practical but may not reliably reflect clinically relevant residual gonadotropic activity during treatment.^{7,8,18} Although GnRH-stimulated LH levels are considered the gold standard for monitoring hormonal suppression during treatment, they require intravenous cannula insertion and repeated sampling, and are therefore cumbersome and invasive. We have recently demonstrated that 40-minute post-dLA LH levels show close agreement with GnRH-stimulated LH levels and can be used for both diagnostic assessment and treatment monitoring in girls with CPP.¹¹ The physiological rationale for post-dose LH monitoring is supported by pharmacodynamic studies

showing that LH rises shortly after depot leuprolide administration, creating a brief stimulatory window for post-injection assessment.²³ Previous studies also suggested that single post-leuprolide LH measurements may be useful for evaluating gonadotropin suppression during GnRHa therapy.^{9,10} Building on this concept, our previous prospective study identified a 40-minute post-dLA LH threshold of 3.07 IU/L for treatment monitoring using GnRH-stimulated suppression as the reference standard.¹¹ In the present study, year-one basal LH correlated with annual growth velocity, but this association was weaker than that observed for year-one 40-minute post-dLA LH and was no longer independent in multivariable analysis. By contrast, year-one 40-minute post-dLA LH remained independently associated with annual growth velocity. These findings suggest that the 40-minute post-dLA LH response is a better marker than basal LH for evaluating gonadotropic activity during dLA treatment.

Year-one biochemical non-suppression was not associated with treatment regimen, age at treatment initiation, BA/CA ratio, or baseline BMI-SDS. Patients with biochemical non-suppression had higher annual growth velocity than those with suppression, whereas bone-age advancement was comparable between groups. This dissociation may reflect the limited timeframe of first-year follow-up, as changes in growth velocity may precede detectable differences in skeletal maturation. Biochemical suppression is generally accompanied by regression or stabilization of clinical pubertal signs and reduced growth velocity; however, the relationship between the degree of LH suppression and the magnitude of the auxological response is not uniform across studies. In a cohort of 64 girls and seven boys treated with depot leuprolide, peak-stimulated and basal LH levels during treatment were not significantly associated with growth velocity, rate of skeletal maturation, or predicted adult stature²⁴, whereas a separate series of 30 girls reported a significant correlation between lower pre-injection LH levels and greater improvement in predicted adult height.²⁰ This discordance may reflect heterogeneity in cohort composition, assay methodology, and the timing of hormonal sampling relative to the injection interval. Regarding final adult height, long-term studies have primarily focused on clinical and auxological determinants rather than biochemical suppression status during the first treatment year.^{25,26} Consequently, direct evidence that the degree of first-year biochemical suppression predicts final adult height remains to be established. Thus, biochemical

non-suppression defined by a laboratory threshold should be interpreted together with growth velocity, skeletal maturation, and clinical pubertal progression rather than as an isolated indicator of treatment failure. In the present study, year-one 40-minute post-dLA LH was independently associated with concurrent annual growth velocity after multivariable adjustment, suggesting potential auxological relevance. Whether this association translates into differences in predicted or final adult height requires prospective long-term follow-up.

This study has some limitations. No a priori sample size calculation was performed, the sample size was modest (particularly for subgroup analyses), and follow-up was limited to the first treatment year. Therefore, the long-term implications of year-one biochemical non-suppression for predicted adult height and final adult height could not be determined. In addition, the 3.07 IU/L threshold was derived from our previous cohort and requires external validation across different populations, assays, and GnRHa protocols. Furthermore, pelvic ultrasonography was performed by multiple radiologists, and inter-observer reliability was not assessed; therefore, measurement variability between observers cannot be excluded. Finally, despite quasi-randomized allocation, chronological age differed significantly between the treatment groups at baseline, whereas bone age was comparable. This baseline imbalance may have introduced residual confounding when interpreting between-group outcomes. Nevertheless, the prospective design, predefined follow-up protocol, and combined assessment of biochemical and auxological outcomes strengthen the clinical relevance of the findings.

In conclusion, monthly 3.75 mg and 3-monthly 11.25 mg depot leuprolide acetate provided comparable first-year auxological outcomes in girls with CPP. Pretreatment GnRH-stimulated peak LH independently predicted year-one biochemical non-suppression, whereas year-one 40-minute post-dLA LH was independently associated with first-year annual growth velocity. These findings suggest that the 40-minute post-dLA LH response may serve as an adjunctive marker of residual gonadotropic activity when interpreted together with growth velocity and other clinical indicators.

Author contributions

Conception and design: D.H., A.B.; Data acquisition: D.H., E.K., I.K., A.K., B.G.T.; Data analysis: D.H., A.K., A.B.; Data

interpretation: D.H., A.K., A.B.; Drafting of the manuscript: D.H.; Critical revision of the manuscript: E.K., I.K., A.K., B.G.T., A.B. All authors reviewed the results, approved the final version of the manuscript, and agreed to be accountable for all aspects of this study.

Ethical approval

This study was approved by the Marmara University Ethics Committee (Date: 29.04.2022, Decision/Protocol No: 09.2022.486). Informed consent was obtained from all participants involved in this study.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare that this study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Generative AI statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing or preparation of this study.

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