Full Title: Clinical findings influencing time to menarche post gonadotropin-releasing hormone agonist therapy in central precocious puberty

Running Title: Outcomes post GnRHa therapy in central precocious puberty

Vickie Wu, MD¹; Victoria Zhao²; Rula Issa²; Meredith Wilkes, MD²; Elizabeth Wallach, MD²; Robert Rapaport, MD²; Christopher Romero, MD²; Mabel Yau, MD².

¹Department of Pediatrics, ²Department of Pediatric Endocrinology, Icahn School of Medicine at Mount Sinai, New York, New York, USA.

Address manuscript correspondence to:

Vickie Wu, MD, Department of Pediatrics, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, Box 1512, New York, NY 10029

Phone: 718-928-8967      Fax: 212-241-4309      Email: vickie.wu@mountsinai.org

Research Support: None
Conflict of Interest: None
Word Count: 2526
Abstract

**Purpose:** This study aimed to evaluate the time interval to menarche after gonadotropin-releasing hormone agonist (GnRHa) treatment in females with central precocious puberty (CPP) and identify factors contributing to timing of menarche.

**Methods:** We retrospectively reviewed medical records of 39 females with CPP who reached menarche after GnRHa treatment (leuprolide or histrelin). CPP diagnostic criteria were: breast development <8 years, pubertal luteinizing hormone and/or estradiol concentrations, and bone age advancement. Indications to treat are advanced bone age and psychosocial concerns. Descriptive summaries were reported as frequency and proportion for categorical variables and mean and standard deviation for continuous measures. Linear regression models were performed to evaluate the association between clinical factors with the time interval to menarche.

**Results:** Mean age was 9.4±1.6 years at treatment onset and treatment duration was 2.2±1.4 years. Menarche occurred at 12.6±1.1 years, which was 1.04±0.5 years after treatment discontinuation. This was negatively associated with Tanner stage of breast development and bone age at treatment onset, and change in bone age during treatment. No association was seen between time interval to menarche and treatment duration, medication, or body mass index.

**Conclusions:** We found the average time interval to menarche after GnRHa treatment in our population of female patients with CPP was 1.04±0.5 years and this is in agreement with other reports. Tanner stage of breast development and bone age at treatment onset, and change in bone age were negatively associated with time interval to menarche. This data provide clinical correlates that assist providers during anticipatory guidance of patients with CPP after GnRHa treatment.

**Key Words:** precocious puberty, gonadotropin-releasing hormone, menarche
INTRODUCTION

Precocious puberty in females is defined clinically as any sign of puberty before 8 years old. 95% of cases are due to premature activation of the hypothalamic-pituitary-gonadal (HPG) axis, known as central precocious puberty (CPP).1) Sustained activation can lead to rapidly progressive puberty in a patient who may not be ready for physical changes including menarche. In addition, CPP leads to premature fusion of epiphyseal growth plates, therefore compromising adult height potential.2,3)

Gonadotropin-releasing hormone agonists (GnRHa) are used as the main treatment for CPP. GnRHa binds to GnRH receptors in the anterior pituitary, promoting endocytosis and down-regulation of GnRH receptors.4) This decreases the release of gonadotropins, which leads to decreased estrogen production to prepubertal levels. This results in halting the progression of breast development, preventing early menarche, and slowing advanced skeletal maturation and early epiphyseal closure.5)

Prior studies have shown that menarche begins 0.5 – 2.5 years after the discontinuation of GnRHa.6-10) However few studies have evaluated auxological factors that may predict the time interval to menarche after treatment cessation. The aims of this study were to evaluate the time interval to menarche after GnRHa discontinuation and identify factors that contribute to timing of menarche.

METHODS

Study Design

We performed a retrospective chart review of females with idiopathic CPP who were treated with GnRHa from May 2005 to November 2017 at an academic pediatric endocrinology clinic. The diagnostic criteria for CPP were: 1) breast development before age 8 years by history or by clinical exam (Tanner stage 2 or above), 2) pubertal luteinizing hormone and/or estradiol concentrations at
baseline or in response to GnRH stimulation test, and 3) significant bone age advancement defined as a bone age 2 standard deviations older than chronological age. Patients with an identified etiology of CPP (such as central nervous system pathology) and coexisting endocrinopathies (such as thyroid, ovarian, or adrenal diseases) were excluded (Figure 1). Luteinizing hormone, follicle-stimulating hormone, and estradiol were measured by two-site electrochemiluminescence at Esoterix Laboratory Services. No patient reached menarche prior to starting GnRHa treatment.

Of 450 female patients diagnosed with CPP between May 2005 to November 2017, 67 patients were treated with GnRHa and had menarche documented in their medical record (Figure 1). 22 of them had coexisting endocrinopathies and 6 had identified etiology of CPP, leaving 39 patients with idiopathic CPP in this study.

Indications for CPP treatment with GnRHa included: poor height prediction, advanced bone age, and psychosocial concerns. Patients were treated with histrelin acetate (50 mg subcutaneous implant yearly, Supprelin LA, Endo Pharmaceuticals) or leuprolide (intramuscular injections 15 mg every 1 month or 30 mg every 3 months, Lupron Depot-PED, Abbvie). Decision on which GnRHa was used depended on the endocrinologist and family’s preference. All forms of treatment have been validated as adequate treatment modalities in CPP.11) Chronologic age, bone age, and pubertal stage measurements were compared at the start and end of GnRHa treatment. Height, weight, and body mass index (BMI) were expressed as z-scores and were calculated based on the Center for Disease Control and Prevention (CDC) growth charts.12) Pubertal progression was staged on the Tanner scale. Bone age was assessed from radiographs of the left hand and wrist and interpreted by a pediatric radiologist using the Greulich-Pyle method.13) Predicted adult height (PAH) was calculated using the Bayley-Pinneau method.14) Mid-parental height (MPH) was calculated for these patients using the average of the parental heights minus 6.5 cm. After GnRHa treatment, patients were followed until at least menarche.
The calculation of the time interval to menarche differed for each medication. For histrelin the time interval started at removal of implant; for leuprolide the time interval started after the expected duration after the last injection.

The study was approved by Icahn School of Medicine Institutional Review Board (IRB-19-02148). The IRB waived the need for informed consent.

Statistical Analysis

Descriptive summaries of patient demographics and clinical characteristics were reported as frequency and proportion for categorical variables and mean and standard deviation for continuous measures. Linear regression models were performed to evaluate the association between various clinical factors with the time interval to menarche after the end of treatment. Models that assessed for the change in physical characteristic were adjusted for the starting measures at the time of treatment. Multiple regression analysis was performed to determine factors influencing time to menarche after the end of treatment. P-values <0.05 were considered statistically significant. All analyses were performed using SAS version 9.4 (SAS Institute Inc, Cary, NC).

RESULTS

Clinical and auxological characteristics of patients at start of treatment and end of treatment

Clinical and auxological characteristics of the patients are depicted in Table 1. The majority of the patients had pubertal LH levels prior to starting GnRHa treatment (mean LH 2.0 ± 1.8 mIU/mL). Six patients required GnRH stimulation testing to confirm CPP biochemically; their mean peak LH level was 7.6 mIU/mL (range 1.1 – 15 mIU/mL) and mean peak estradiol level was 107.5 pg/mL (range 4.9 – 405 pg/mL). The distribution of the patients’ races are reported in Table 1. There were no
significant difference in the distribution of race between medication groups, however there is a larger percentage of non-Hispanic white females who were treated with leuprolide than with histrelin.

The mean chronologic age and bone age at the start of GnRHa treatment were 9.4 ± 1.6 years and 11.2 ± 1.3 years, respectively (Table 1). The difference between bone age and chronologic age was 1.8 ± 1.4 years at start of treatment. At the start of treatment, the mean height was 139.0 ± 9.5 cm and the mean weight was 39.5 ± 10.6 kg. Of the 39 patients, 13 of them met CDC diagnostic criteria for pediatric obesity. The mean PAH at the start of treatment was 154.4 ± 7.6 cm and the mean MPH was 159.7 ± 7.2 cm.

At the end of treatment, the mean chronologic age and bone age were 11.6 ± 1.2 years and 12.5 ± 0.9 years, respectively. The difference between bone age and chronologic age by the end of treatment was 0.9 ± 1.2 years.

A majority of the patients at the start of treatment had breast development measured at Tanner stages 2 (30.8%) and 3 (33.3%). There was a slight incremental increase in Tanner stage of breast development by the end of treatment but most were still in stages 2 (25.6%) and 3 (46.2%).

Of the 39 patients, 18 (46%) were treated with leuprolide and the other 21 patients were treated with histrelin. Of the patients treated with leuprolide, 13 received the 1-month formulation and 5 received the 3-month formulation. Table 1 includes a comparison between the patients treated with histrelin and patients treated with leuprolide. Patients treated with leuprolide were at a significantly higher chronologic age at the end of treatment than those treated with histrelin ($P=0.02$). There were no significant differences between these two groups in the following: chronologic age at start of treatment, bone age at start or end of treatment, bone age-chronologic age at start or end of treatment, Tanner
stage at start or end of treatment, height/weight/BMI at start or end of treatment, baseline LH, or
treatment duration (all $P > 0.05$).

*Treatment duration and time to menarche*

Patients were treated with GnRHa for a mean duration of 2.2 ± 1.4 years. Mean age of
menarche was 12.6 ± 1.1 years. Patients reached menarche 1.04 ± 0.5 years after the end of GnRHa
treatment (Figure 2). 54% of patients reached menarche by 1 year after the end of GnRHa treatment
and an additional 44% reached menarche by 2 years.

Clinical factors associated with the time interval to menarche from the end of treatment are
reported in Table 2. Time interval to menarche was found to be negatively associated with bone age at
start of treatment, Tanner stage of breast development at start of treatment, and change in bone age
from start to end of treatment (all $P < 0.05$). As bone age at the start of treatment advanced, the interval
to menarche decreased ($\beta = -0.16$, 95%CI -0.27 to -0.05; $P=0.005$). Similarly as Tanner stage increased
prior to treatment, the shorter the interval to menarche was observed. Moreover, after adjusting for
initial bone age, a greater change in bone age over the course of the treatment period was significantly
associated with a shorter interval to menarche ($\beta = -0.25$, 95%CI -0.44 to -0.07; $P=0.009$). No
association was seen between time to menarche and treatment duration, medication, or BMI.

In the multiple regression analysis, clinically relevant variables at the start of treatment such as
Tanner stage, age, height, weight, BMI, bone age, and height prediction were explored and considered
as potential candidates. We performed backward selection with a p-value threshold of <0.15 to obtain
the final model for time to menarche after medication. In the final model, the overall model F-statistic
$P$-value was 0.01 (R-squared = 0.36). This suggests an association in at least one of the parameters
however, the more parsimonious model would be with only Tanner stage or only bone age (see Table 2). Table 3 shows that as Tanner stage increases and bone age increases at the start of treatment, the time interval from end of treatment to menarche is shorter in duration.

**DISCUSSION**

We performed a retrospective chart review of 39 females with CPP who were treated with GnRHa and evaluated age of menarche, time interval to menarche after cessation of GnRHa treatment, and factors that influence time to menarche. Menarche in our patients treated with GnRHa occurred at 12.6 ± 1.1 years which is comparable to the general population. Given that one of the goals of treatment in females with CPP is to realign pubertal development to that of their peers, GnRHa was able to help achieve this goal in our patients. This is similar to previous research that examined age of menarche post-GnRHa treatment (Baek et al. reported menarche at 11.9 ± 0.7 years, Heger et al. at 12.3 ± 1.4 years, Lee et al. at 12.6 ± 0.6 years).6,15,16

We found the average time interval to menarche after GnRHa treatment in our population of female patients with CPP was 12 ± 6 months. This was similar to previous studies: Baek et al. observed the time interval to be 14 ± 5.6 months after stopping leuprolide injection, Gillis et al. observed 9.3 ± 1.5 months after histrelin implant removal, and Neely et al. observed 18 ± 6 months after stopping leuprolide.6-8 In our study there was no difference in time interval to menarche between patients who received leuprolide and those who received histrelin; this is a novel finding, as no other study has reported comparisons between leuprolide and histrelin. Gillis et al. performed a similar study but compared histrelin implant versus triptorelin depot.7 We found this to be a valuable observation in regards to patient counseling as efficacy of treatment regardless of GnRH agonist therapy is equal and allows puberty to appropriately resume. Time interval to menarche was found to be negatively
associated with bone age at start of treatment, Tanner stage of breast development at start of treatment, and change in bone age from start to end of treatment. Thus, females reached menarche sooner after discontinuation of GnRHa treatment when treatment was started later in puberty. In contrast, there was no association between time to menarche and treatment duration, medication, or BMI. Aside from reports from Baek et al. and Arrigo et al., this is the only other study that examines auxological factors that may predict time interval to menarche post-GnRHa treatment.\(^{6,17}\) We believe these are very important findings with clinical utility. These auxological factors and effects of treatment are typically counseling points parents seek at the time of starting treatment. Although the goal is to safely pause puberty, the provider needs to be prepared to appropriately advise when parents seek counsel on GnRH agonist therapy during and post-treatment. These questions relate to timing of menarche and what factors may affect this duration to menarche, and if treatment duration affects restoration of HPG axis function.

One of the treatment indications of GnRHa for females with CPP is to delay physical changes and subsequent menarche to minimize psychosocial distress. Precocious puberty has been associated with increased delinquency and physical aggression, and females with early puberty have been suggested to face social isolation and bullying compared to their peers.\(^{18,19}\) Some studies have shown increased stress and anxiety in females with precocious puberty.\(^{20,21}\) These data on time interval to menarche after GnRHa treatment and clinical factors that influence this time interval can aid clinicians in counseling parents and reduce some of the parental and patient concerns. We believe having data on time interval to menarche after GnRHa treatment and an evidence-based discussion on factors that influence this time interval will reduce many of the parental and patient concerns at the start and stop of therapy.
Precocious puberty can also lead to stunted growth as a consequence of premature growth plate fusion. However, there are conflicting reports on the height outcomes in females with CPP who were treated with GnRHa.\textsuperscript{22} Lazar et al. observed little or no increase in final adult height (FAH) if GnRHa treatment was started after age 8 years.\textsuperscript{23} In contrast, other studies showed GnRHa treatment improved height outcomes even in slightly older patients.\textsuperscript{16,24-26} This suggests that other factors such as the degree of bone age advancement, age of pubertal onset and height are better predictors of final height than age of treatment onset.\textsuperscript{16} Heger et al. reported that females with CPP who received GnRHa treatment had final adult height that were significantly higher than the pretreatment PAH (FAH was 160.6 ± 8.0 cm, pretreatment PAH 154.9 ± 9.6 cm), as did Jung et al. (FAH was 160.4 ± 4.2 cm, pretreatment PAH 156.6 ± 3.9 cm).\textsuperscript{15,27} In contrast, Korkmaz et al. found no significant difference in final heights between treated and non-treated females, in addition to PAH being greater than final heights.\textsuperscript{28} Analysis of height outcome was not performed in this study, as 36\% of the patients were treated with growth hormone therapy and FAH was available in only 44\% of our patients.

One limitation of our study was a small sample size compared to most other studies. A larger sample size could improve the statistical power of the results and also allow the researchers to more robustly compare medications. As well, we did not have an untreated control group of females with CPP who could be followed until they reached menarche. While the patients in this study started GnRHa treatment at a late age, the timing of presentation is dependent on pediatricians’ referrals and therefore impacts the age of starting treatment. Only 49\% of our patients had maternal age of menarche available. If more data were available, the researchers would have been interested to study any correlation between patients’ age of menarche and maternal age of menarche.
In conclusion, the time from the end of treatment to menarche ranged from 0.5 to 1.5 years, with the following factors being negatively associated with the time interval to menarche: Tanner stage of breast development at treatment onset, bone age at treatment onset, and change in bone age. These data provide clinical correlates that assist providers during anticipatory guidance of patients after treatment of CPP with GnRHa.
ACKNOWLEDGEMENTS

The authors wish to thank Stephanie Pan, MS, for her assistance in statistical support.
REFERENCES


Figure 1’s Legend:

Study design schematic. CPP, central precocious puberty; GnRHa, gonadotropin-releasing hormone agonist.
Figure 2’s Legend:

Distribution of time intervals from end of GnRHa treatment to menarche.
Table 1. Clinical and auxological characteristics at start and end of GnRHa.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All patients (N=39)</th>
<th>Histrelin patients (N=21)</th>
<th>Leuprolide patients (N=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronologic age (year)</td>
<td>9.4 ± 1.6</td>
<td>11.6 ± 1.2</td>
<td>9.0 ± 1.5</td>
</tr>
<tr>
<td>Bone age (year)</td>
<td>11.2 ± 1.3</td>
<td>12.5 ± 0.9</td>
<td>11.0 ± 1.5</td>
</tr>
<tr>
<td>Bone age-Chronologic age (year)</td>
<td>1.8 ± 1.4</td>
<td>0.9 ± 1.2</td>
<td>2.0 ± 1.4</td>
</tr>
<tr>
<td>Tanner stage breast development</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>139.0 ± 9.5</td>
<td>149.4 ± 7.1</td>
<td>138.2 ± 9.2</td>
</tr>
<tr>
<td>Height z-score</td>
<td>0.5 ± 1.2</td>
<td>0.2 ± 1.2</td>
<td>0.7 ± 1.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>39.5 ± 10.6</td>
<td>50.6 ± 13.2</td>
<td>38.3 ± 11.1</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>0.9 ± 1.1</td>
<td>0.84 ± 1.2</td>
<td>0.9 ± 1.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.2 ± 3.7</td>
<td>22.4 ± 4.6</td>
<td>19.7 ± 3.9</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>0.9 ± 1.0</td>
<td>0.9 ± 1.1</td>
<td>0.8 ± 1.2</td>
</tr>
<tr>
<td>Baseline LH (mIU/mL)</td>
<td>2.0 ± 1.8</td>
<td>n/a</td>
<td>2.0 ± 1.8</td>
</tr>
<tr>
<td>Treatment duration (year)</td>
<td>2.2 ± 1.4</td>
<td>2.1 ± 1.2</td>
<td>2.3 ± 1.6</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>20 (51.3)</td>
<td>8 (38.1)</td>
<td>12 (66.6)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>8 (20.5)</td>
<td>5 (23.8)</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>8 (20.5)</td>
<td>5 (23.8)</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td>Asian</td>
<td>3 (7.7)</td>
<td>3 (14.3)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation or N (%).  
GnRHa, gonadotropin-releasing hormone agonists; n/a, not applicable.
Table 2. Univariable analysis between time interval to menarche and multiple factors.

<table>
<thead>
<tr>
<th>Variable</th>
<th>β Estimate (95% Confidence Interval)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medication</td>
<td></td>
<td>0.70</td>
</tr>
<tr>
<td>Histrelin</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Leuprolide</td>
<td>-0.07 (-0.40, 0.27)</td>
<td></td>
</tr>
<tr>
<td>Tanner Stage Start</td>
<td></td>
<td>0.006</td>
</tr>
<tr>
<td>2</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-0.36 (-0.74, 0.01)</td>
<td></td>
</tr>
<tr>
<td>4/5</td>
<td>-0.66 (-1.06, -0.27)</td>
<td></td>
</tr>
<tr>
<td>Age Start</td>
<td>-0.09 (-0.19, 0.01)</td>
<td>0.08</td>
</tr>
<tr>
<td>Treatment duration</td>
<td>-0.11 (-0.27, 0.06)</td>
<td>0.19</td>
</tr>
<tr>
<td>Height Start</td>
<td>-0.01 (-0.03, 0.01)</td>
<td>0.17</td>
</tr>
<tr>
<td>Δ Height*</td>
<td>0.003 (-0.04, 0.05)</td>
<td>0.91</td>
</tr>
<tr>
<td>Weight Start</td>
<td>-0.02 (-0.03, 0.0001)</td>
<td>0.05</td>
</tr>
<tr>
<td>Δ Weight*</td>
<td>-0.01 (-0.04, 0.03)</td>
<td>0.09</td>
</tr>
<tr>
<td>BMI Start</td>
<td>-0.04 (-0.09, 0.002)</td>
<td>0.06</td>
</tr>
<tr>
<td>Δ BMI*</td>
<td>-0.01 (-0.10, 0.08)</td>
<td>0.09</td>
</tr>
<tr>
<td>BMI Z-Score Start</td>
<td>-0.09 (-0.26, 0.07)</td>
<td>0.25</td>
</tr>
<tr>
<td>Δ BMI Z-Score*</td>
<td>-0.02 (-0.34, 0.31)</td>
<td>0.92</td>
</tr>
<tr>
<td>Bone Age Start</td>
<td>-0.16 (-0.27, -0.05)</td>
<td>0.005</td>
</tr>
<tr>
<td>Δ Bone Age*</td>
<td>-0.25 (-0.44, -0.07)</td>
<td>0.009</td>
</tr>
<tr>
<td>PAH Start</td>
<td>-0.01 (-0.04, 0.01)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

*Adjusts for the respective starting values measured at the time of medication.
Table 3. Multiple regression analysis on time interval from end of GnRHa treatment to menarche.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate (95% Confidence Interval)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanner Stage Start</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Reference</td>
<td>0.08</td>
</tr>
<tr>
<td>3</td>
<td>-0.30 (-0.66, 0.07)</td>
<td>0.11</td>
</tr>
<tr>
<td>4/5</td>
<td>-0.47 (-0.88, -0.06)</td>
<td>0.03</td>
</tr>
<tr>
<td>Bone Age Start</td>
<td>-0.09 (-0.21, 0.03)</td>
<td>0.13</td>
</tr>
</tbody>
</table>
450 females diagnosed with CPP between May 2005 to November 2017 at an academic pediatric endocrinology clinic

67 females treated with GnRHa and had menarche documented in their medical record

22 females with coexisting endocrinopathies

6 females with identified etiology of CPP

39 females with idiopathic CPP
Figure 2

Time interval from end of treatment to menarche (years)