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Effectiveness of basal LH in monitoring central precocious puberty treatment in girls

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Abstract

Objectives: Treatment of central precocious puberty (CPP) is based on administration of GnRH agonists in order to suppress hypothalamic-pituitary-gonadal axis and thus induce the stabilization or regression of pubertal development. Our aim was to determine whether the single basal serum LH and/or FSH concentration could be an effective tool to assess the efficacy of treatment to suppress activation of hypothalamic-pituitary axis.

Patients and methods: Serum LH and FSH were measured before and after the GnRH injection, as well as E2 basal levels in 60 girls with documented idiopathic CPP at diagnosis and 18 and 30 months after the beginning of therapy.

Results: At diagnosis, peaks of >5 IU/L of LH and of FSH were observed in 100 and 91.6% of girls, respectively, with basal LH values of <1 IU/L in 70% and basal FSH levels of <1 IU/L in 10%. E2 were <20 pg/mL in 36.6%. After 18 months, a suppressed peak (i.e. <3 IU/L) was recorded in 85% of girls (p<0.01) for LH and in 98.3% for FSH (p<0.01). Basal LH <1 IU/L was detected in 85% (p<0.01) and basal FSH ≤1 IU/L in 40% (p<0.01). Serum E2 ≤20 pg/mL was recorded in 61.6% (p<0.01). After 30 months, all patients showed LH suppressed peak (p<0.01) and 98.3% suppressed FSH peak (p<0.01). 100% showed basal LH concentrations <1 IU/L (p<0.01) and 38.3% FSH basal values <1 UI/mL (p<0.01). E2 ≤20 pg/mL was observed in 32.72% (p=NS).

Conclusions: Basal LH values are a reliable indicator of the efficacy of GnRHa therapy after 30 months of GnRHa therapy.

Keywords: FSH; GnRH; GnRH analog (GnRHa); LH; monitoring; precocious puberty; puberty.

Introduction

Precocious Puberty (PP) in females is usually defined as the appearance of breast development before 8 years of age, together with an acceleration of height velocity and advanced bone maturation. PP is classified into two major categories based on its etiology, i.e. central precocious puberty (CPP) or gonadotropin-dependent PP and peripheral precocious puberty (PPP) or gonadotropin-independent PP. In CPP, the most common mechanism of progressive precocious puberty is the early activation of pulsatile gonadotropin-releasing hormone (GnRH) secretion, which in most cases, especially in girls, remains unexplained and thus is defined as idiopathic. Currently, in girls, the diagnosis of CPP is based on clinical, biological and radiological signs, with demonstration of activation of the hypothalamic-pituitary-gonadal (HPG) axis. The onset of puberty is marked by breast development (Tanner stage 2 breast development, best assessed by both inspection and palpation), accelerated growth with advanced bone age and a significant increase in the levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) following GnRH stimulation. Pelvic ultrasonography is

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helpful in evaluating uterine size and ovarian volume. Moreover, magnetic resonance imaging (MRI) of the brain, with particular focus on the hypothalamic/pituitary region, must be performed to exclude secondary causes of CPP [1].

The treatment of CPP consists of the use of GnRH analogues (GnRHa) to suppress gonadotropic secretion and, consequently, to induce the regression or stabilization of pubertal development and slow down skeletal maturation. Prompt and timely treatment can prevent premature epiphyseal maturation from leading to compromised final height. Pubertal progression generally reappears within months after GnRH-agonist treatment has terminated, with a mean time to menarche of 16 months [2]. During treatment, the HPG axis can be evaluated by measuring unstimulated or stimulated serum LH, sex steroids or urinary gonadotropin concentrations [3]. There is no consensus about the routine use of random or stimulated measurements of gonadotropins or sex steroids for monitoring therapy [4]. A recent consensus paper endorses that the lack of correlation between biochemical measurements during treatment and clinical outcome, especially in terms of adult height, do not support routine biochemical testing in all patients [5]. However, in some cases, like in suspicion of treatment failure, biochemical verification should be of interest.

Several authors have evaluated alternative, less invasive and cheaper methods of assessing efficacy. With the development of highly sensitivity laboratory methods, basal LH is one of the most promising candidates [6, 7].

Hence, the aim of this study was to evaluate whether basal gonadotropins levels could be used as a reliable parameter in following up girls with CPP, rather than submitting the patients to a GnRH stimulation test at every follow up. In addition, we verified if E2 levels can be a reliable parameter in demonstrating the effectiveness of the treatment and if the other clinical (height, growth velocity, stabilization or regression of breast development) and instrumental parameters (bone age, pelvic ultrasonography) can reflect gonadotropin inhibition.

Patients and methods

Patients

We reviewed 60 girls referred for evaluation of early pubertal development before the age of 8, who were diagnosed with CPP and treated with GnRHa. Eight of the girls were adopted from African and Asiatic Countries.

Since a precise diagnosis at the real appearance of early puberty may be hindered by the fact that parents do not know the exact time of their daughter's breast development, we indicated the diagnosis of the first pediatric consultation. In fact, previous studies have reported that it takes about 1.5 years from the time parents first notice pubertal symptoms to obtain a diagnosis of CPP [8].

In all 60 patients, the diagnostic and yearly examination included measurement of height and weight, calculation of growth velocity, body mass index (BMI), parents' height, physical examination with pubertal staging, bone age, evaluation of basal and peak serum levels of gonadotropins after the GnRH stimulation test, E2 serum concentrations, uterine and ovarian measurements through pelvic ultrasound, according to Italian Society of Pediatric Endocrinology and Diabetology (SIEDP/ISPED) Guidelines (http://www.siedp.it/pagina/823/pdta+puberta%27+precoce+centrale)

Children with dimorphic syndromes, chromosome anomalies and/or endocrine or chronic diseases, neurological signs or CNS pathologies were excluded.

We analyzed data at diagnosis and after 18 and 30 months from starting therapy.

The study was approved by the "Comitato Etico Area di Pavia", the Ethics Committee of the Foundation IRCCS San Matteo Hospital, on May 17th, 2016 (reference number 20160005680) and was carried out according to the principles of the Declaration of Helsinki. Written assent was obtained from the children and written informed consent was obtained from their parents, in accordance with the study protocol.

Methods

Height, growth velocity and pubertal staging were recorded according to Tanner charts and staging classification, [9], The BMI score (SDS) was calculated according to Italian growth charts [10].

Bone age was using the Greulich and Pyle Atlas [11].

All subjects underwent the GnRH stimulation test (100 $\mu g/m^2$ GnRH administered via i.v. bolus). Serum LH and FSH levels were measured at 0, 15, 30 and 90 min after the injection. We considered peak LH levels of >5 IU/L as a diagnostic cutoff for CPP [12].

We assumed LH basal level <1 UI/mL as a predictor of the effectiveness of treatment when the corresponding peak concentration during GnRH stimulation test was inhibited (i.e. <3 IU/mL), this value being considered in literature as a reliable marker of HPG axis suppression during therapy [13].

LH serum and FSH values were measured by means of a chemiluminescent immunometric assay (Immulite 2000, Siemens HealthCare Diagnostics). The calibration range of the assay was up to 200 IU/L, with an analytical sensitivity of 0.05 IU/L and functional sensitivity of 0.1 IU/L, calculated as a coefficient of variation (CV) level of 20%. The intra-assay CV values were 13.1, 3.04, 3.71 and 3.6% at levels 0.15, 1.04, 1.89 and 8.7 IU/L, respectively. The corresponding interassay CV values were 23.9, 6.6, 6.2 and 6.7%. The cross-reactivity with human chorionic gonadotropin was 0.20%.

Serum concentration of E2 was measured using an automated immunochemistry analyzer (Siemens Health Care, Advia Centaur Enhanced Estradiol Assay, Tarrytown, NY, USA), with a detection limit of 11.8 pg/mL and functional sensitivity of 15 pg/mL). We considered E2 values of >20 pg/mL as suggestive of onset of puberty development.

The pelvic ultrasound was performed by a single experienced operator in order to avoid data heterogeneity. Uterine length and transverse diameter, ovarian volume (calculated using the ellipsoid formula V=D1×D2×D3×0.5233 where D1 is the largest longitudinal diameter, D2 the largest anteroposterior diameter and D3 the largest transverse diameter) and the presence of an endometrial echo were evaluated by means of thorough pelvic ultrasound echography (AlokaProsound SSD 5500 machine). In our study, we considered the following as indicators of estrogenic stimulation of the uterus: a uterine length of ≥3.5 cm, a fundus/cervix ratio of >1 and the presence of endometrial echo. An ovarian volume of >2 mL has also been considered indicative of a pubertal state [14, 15].

In all girls affected by CPP, magnetic resonance imaging (MRI) of the brain was performed at the time of diagnosis to determine whether lesions were present, in particular in the hypothalamic-pituitary region.

Statistical analyses

All statistical analyses were performed using the IBM SPSS Statistics software, version 23 (International Business Machines Corporation, Armonk, New York). Data were expressed as means±standard deviation. The relationship between data was tested by linear regression analysis. Differences from baseline were estimated using the Student's t-test or the Wilcoxon test for variables with normal or not-normal distribution, respectively. The distribution of parameters was assessed by Kolmogorov–Smirnov test. All reported P-values are two-sided, and the significant level was set at $p<0.05$.

Results

Clinical and auxological parameters

Table 1 shows the mean chronological age at diagnosis, and the parameters concerning height-SDS, bone age, BMI SDS, growth velocity of our patients at diagnosis and during follow-up.

Brain MRI showed a pineal cyst in 7 out of the 60 girls and nonspecific anomalies including Rathke cysts (n=3), pars intermedia cysts (n=3), and presence of microadenoma (n=2) in the remaining ones.

After confirmation of idiopathic CPP diagnosis, all the patients began treatment with an i.m. administration of GnRHa (triptorelin Gonapeptyl Depot 3.75 mg® Ferring which was provided by the Hospital Pharmacy Department) every 28 days (i.e. 1.875 mg up to 20 kg of body weight, 2.5 mg between 20 and 30 kg of body weight and 3.75 mg when body weight was >30 kg) and underwent a complete yearly assessment during follow up. Evaluations at 18 and 30 months were taken in account for the analysis, to evaluate long-term efficacy.

No significant adverse effects were recorded during follow-up.

Table 1: Clinical characteristics at diagnosis and during follow-up of girls diagnosed with precocious puberty.

	At diagnosis	18 months	30 months
Age at diagnosis (yrs)			
Mean±DS	7.8±1.35		
Min – max	1.5–9.71		
Height (SDS)			
Mean±SD	0.6±1.18	0.52±0.94°	0.35±0.93°
Min – max	−2.2 – +3.19	−2.0 – +3.19	−2.0 – +2.7
BMI (z-score)			
Mean±SD	0.03±0.99	0.22±0.93°	0.11±0.83°
Min – max	−2.76 – +1.77	−2.46 – +1.70	−2.09 – +1.67
Bone age (years)			
Mean±SD	8.8±1.96	9.24±2.34*	10.58±2.06*
Min – Max	2.0–12.0	2.34–12.75	3.5–13.75
Growth velocity (cm/y)			
Mean±DS	7.79±1.11	5.37±1.19*	5.39±0.95*
Min – max	5.30±10.40	3.20±7.90	3.80–7.10
Growth velocity (SDS)			
Mean±DS	2.28–1.39	−0.21±1.43*	0.40–1.53*
Min – max	−1.54–5.47	−2.57–3.60	−3.00–4.80
Stabilization or regression or breast development (n, %)		58/60 (96.6%)	56/60 (93.3 %)

Data are expressed as mean±SD. *p <0.01 vs value at the time of diagnosis. °p=NS vs value at the time of diagnosis.

Hormonal profile

The results of the hormonal measurements are shown in Table 2.

At the first evaluation, just before the beginning of GnRHa therapy, after GnRH i.v. injection, a peak of >5 IU/L

Table 2: Biochemical evaluation at diagnosis and during follow-up.

	At diagnosis	18 months	30 months
Basal FSH (UI/L)			
Mean±SD	2.95±1.65	1.28±0.7*	1.37±0.76*
Min – max	0.1–7.8	0.1–4.0	0.1–4.0
Peak FSH (UI/L)			
Mean±SD	12.29±6.6	1.56±0.94*	1.55±0.82*
Min – Max	1.5–30.6	0.1–5.1	0.1–4.8
Basal LH (UI/L)			
Mean±SD	0.98±1.19	0.44±0.29*	0.37±0.24*
Min – Max	0.1–5.4	0.1–1.3	0.1–1.4
Peak LH (UI/L)			
Mean±SD	16.63±14.39	0.85±0.65*	0.67±0.46*
Min – Max	5.0–74.4	0.2–3.4	0.1–2.2
Basal E2 (pmol/L)			
Mean±SD	24.54±13.79	18.74±8.72*	21.48±10.14°
Min – Max	10.0–85.2	10–50.7	9.0–48.3

*p=<0.01 vs value at the time of diagnosis. °p=NS vs value at the time of diagnosis.

in LH and FSH was observed in 60/60 girls (100%) and in 55/60 girls (91.6%) respectively, with a serum basal LH value of <1 IU/L in 42/60 girls (70%), a serum basal FSH level of <1 IU/L in 6/60 girls (10%), and serum E2 concentrations <20 pg/mL in 22/60 girls (36.6%) Figure 1.

In comparison, the data at the 18th month showed that a LH peak of <5 IU/L was recorded in all girls (100%, p<0.01) and a peak<3 IU/L in 57/60 (85%, p<0.01) and a FSH peak of <5 IU/L was found in 59/60 (98.3%, p<0.01). A serum basal LH value of <1 IU/L was detected in 57/60 of the patients (85%, p<0.01) and a basal FSH concentration ≤1 IU/L in 24/60 girls (40%, p<0.01). Serum E2 concentrations ≤20 pg/m were recorded in 37 out of the 60 patients (61.6%, p<0.01).

At the end of the follow-up (i.e. at 30 months), LH and FSH peaks of <3 IU/L were noted in all patients (100%, p<0.01) and in 59/60 (98.3%, p<0.01). We found serum basal LH concentrations of <1 IU/L in 60/60 girls (100%, p<0.01) and serum FSH basal values of <1 UI/mL in 23/60 girls (38.3%, p<0.01). Serum E2 concentrations ≤20 pg/mL were observed in 18 out of the 55 patients (32.72%, p=NS).

Pelvic ultrasound

Data on uterine length and mean ovarian volume are shown in Table 3.

At the first evaluation just before the beginning of GnRHa therapy, we observed a uterine length ≤3.5 cm in 12 out of 58 girls (20.68%) and a volume ≤2 cm^3 in 12/58 girls (20.7%). In comparison, the data at the 18th month showed that uterine length remained <3.5 cm in 8/57 girls (14%, p<0.01) and ovarian volume was <2 mL in 39 out of 60 girls (65%, p<0.01). At the end of the follow-up (i.e. at 30 months), uterine length was <3.5 cm in 11 out of 45 patients (24.4%, p=NS) and ovarian volume was <2 mL in 5 out of 45 girls (11.1%, p <0.01).

Table 3: Ultrasound evaluation at diagnosis and during follow-up.

	At diagnosis	18 months	30 months
Uterine length (cm)			
Mean±SD	4.31±1.06	4.00±0.70*	4.23±1.28°
Min – Max	0.21–0.73	0.21–0.62	0.24–0.82
Ovarian volume (cm^3)			
Mean±SD	2.75±1.44	2.0±1.03*	3.75±1.63*
Min – Max	0.6–7.6	0.6–4.85	1.2–8.5

*p=<0.01 vs value at the time of diagnosis. °p=NS vs value at the time of diagnosis.

Discussion

The primary aim of CPP treatment is the suppression of gonadotropin secretion by exposure to GnRHa, which acts via desensitization and down-regulation of pituitary GnRH receptors. Long-term therapy has been demonstrated to reverse or stabilize pubertal development without adversely affecting resumption of puberty after discontinuation of treatment.

The clinician generally uses the serum stimulated LH value in response to GnRH intravenous injection in order to assess effectiveness of GnRHa therapy and monitor gonadotropin suppression [16]. In clinical practice, FSH-stimulated peak level is not normally used to monitor the inhibitory effect of GnRHa therapy. In fact, GnRHa continuously stimulate the pituitary gonadotrope cells, leading to desensitization and a decrease in the release of LH and, to a lesser extent, FSH [17].

On the other hand, frequent GnRH tests during follow-up to confirm the suppression of basal or stimulated LH levels should be recommended for monitoring therapy. However, dynamic tests every month are inconvenient and impractical for both children and their families. Thus, investigators are looking for simpler and easier alternatives. Unfortunately, to date no long-term data have provided

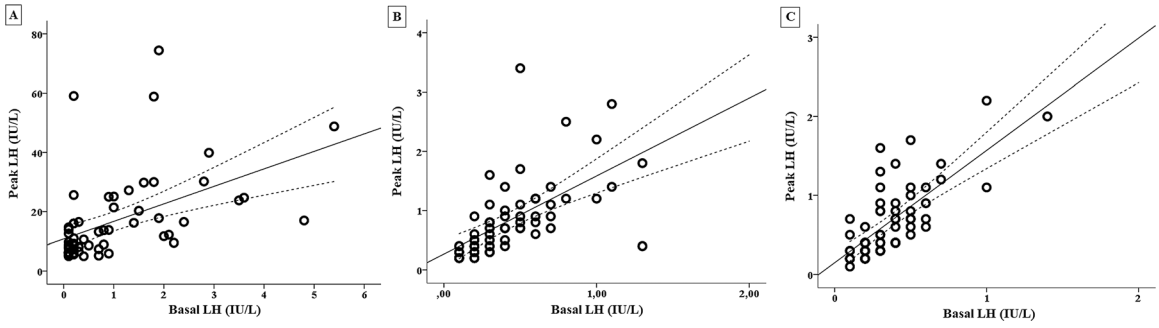

Figure 1: Relationship between basal LH and stimulated LH at baseline (A), after 18 months (B) and after 30 months (C) of treatment. A: r=0.043, p=<0.01. B: r=0.601, p=<0.01. C: r=0.737, p=0.01.

unanimous support for any specific short-term monitoring schema.

With the development of new and more sensitive immunoassays for measuring serum gonadotropins, a single basal serum LH value has been proposed to both diagnose CPP and monitor GnRHa therapy. However, some authors using different assays have demonstrated that the basal LH value for diagnosing CPP is variable [3, 7]. Indeed, basal ultrasensitive LH is often within the prepubertal range in early CPP and thus may be falsely reassuring [18, 19].

Our principal objective was to consider the possibility of replacing GnRH-stimulated gonadotropin peaks with basal values when monitoring GnRHa treatment. If confirmed, the use of baseline values would have a significant impact in terms of convenience for patients and their parents, as well as management costs for the National Health Service. In fact, while the stimulation test necessarily requires hospitalization, the test for basal dosage of gonadotropins can be easily planned on an outpatient basis.

The results of our study clearly demonstrate that the GnRH stimulation test is essential for the diagnosis of CPP, given that basal LH and FSH gonadotropin levels alone are unreliable. Our results do not match those of Lee et al. (*3*), who found that in most cases a single measurement was sufficient for the diagnosis of CPP. In fact, we observed that 42 out of 60 CPP girls showed serum basal LH values of <1 IU/mL while the GnRH test demonstrated a positive LH-stimulated level (i.e. >5 IU/mL) confirming the accuracy of the diagnosis. Moreover, Mogensen et al. reported that an elevated basal LH level was highly predictive of a pubertal GnRH test result, whereas a low level of LH did not exclude central pubertal activation [12]. On the other hand, both basal and stimulated FSH values have limited use in the diagnosis and follow up of our CPP girls. Our results are confirmed by Pasternak et al. [7], but are in contrast with those of other authors who used different cutoffs and laboratory methods [12]. Moreover, E2 levels are not useful in monitoring gonadotropin suppression, as confirmed by other authors [19]. Pelvic ultrasound echography proved to be a useful tool in the diagnostic procedure, particularly as regards uterine length and the ovarian volume. A uterine length of >3.5 cm has been reported as a useful indicator in distinguishing between central precocious puberty and premature thelarche [14]. In fact, in girls with premature isolated thelarche, estrogenic impregnation does not have time to occur and thus increase the longitudinal diameter and volume of the uterus. Despite its usefulness in diagnosis, the role of ultrasound is limited during follow up, particularly as regards uterine length, because of the physiological growth of the organ, as showed in our patients. In our cohort, we observed a decrease of ovarian volume after 18 months of treatment, with an increase thereafter. In effect, only 5 out of 45 girls (11.1%) had an ovarian volume <2 mL after 30 months (they were 65 % at 18 months). This phenomenon could be explained at least in part with physiological growth of gonads [16], that could explain a certain progression of ovarian volume, even in the absence of elements in favor of a recovery of HPG activation.

Other measurements, such Inhibin B, have been proposed for diagnosis and monitoring of precocious puberty in girls [20], but results are still controversial and thus not routinely used in clinical practice.

We are aware that our study has two major limitations. The number of patients is limited. Therefore, further research studies with larger samples are necessary to confirm the major role of baseline LH in evaluating treatment efficacy during follow up in girls treated with aGnRH. Additionally, as with most studies published so far, pelvic ultrasound data were restricted to the uterine length and ovarian volume although the shape and size of ovarian follicles could be useful in monitoring puberty evolution [15].

Finally, previous studies have suggested that basal LH value may express the inhibitory effect on the hypothalamic-pituitary-gonadal (HPG) axis in GnRHa-treated girls only in cases with pathological clinical and instrumental long-term parameters. To the best of our knowledge, our study is the first to demonstrate the LH inhibitory effect by simultaneous blunted response to GnRH test performed during follow up. This result indicates the usefulness of basal LH values in monitoring therapy.

Conclusion

This study demonstrates that after 30 months of treatment, a single basal LH measurement in all patients can be sufficient to verify the effectiveness of the GnRHa therapy, suggesting that the GnRH stimulation test is no longer necessary.

Our study also shows that the sole use of basal LH level evaluation is a distinct possibility for patient follow up, and would lead to better compliance from young girls and a significant reduction in healthcare expenditure.

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Author contribution: VC and GDF conceptualized and designed the study, drafted the initial manuscript and approved the final manuscript as submitted. RA performed biochemical analysis, carried out analysis and interpretation of data and approved the final manuscript as submitted. DR and BM carried out analysis and interpretation of data, revised the literature and approved the final manuscript as submitted. CMM gave technical assistance, revised the literature and approved the final manuscript as submitted. EB, AV and MB designed the study, revised the manuscript, gave conceptual advice and approved the final manuscript as submitted.

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