CLINICAL STUDY

Ultrasonographic and clinical parameters for early differentiation between precocious puberty and premature thelarche

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Abstract

Objective: To determine if uterine and ovarian measurements can significantly distinguish between precocious puberty (PP) and premature thelarche (PT) and whether ultrasound has any advantage over the gonadotropin-releasing hormone (GnRH) stimulation test.

Design: Prospective.

Methods: One hundred and three girls referred consecutively for evaluation of breast budding before age 8 years underwent physical examination, GnRH stimulation test, bone age assessment, and transabdominal pelvic ultrasound. The diagnosis of PP or PT was based on clinical judgment. The clinical, laboratory, and ultrasound data of the PP and PT groups were compared.

Results: Eighty-one girls were diagnosed with PP and 22 with PT. Significant differences in most of the uterine and ovarian measurements were found between the groups. On logistic regression analysis, bone age standard deviation score, uterine transverse diameter, and uterine volume were the most significant variables predicting PP. Comparison of 30 girls with PP and 21 with PT in whom peak luteinizing hormone was < 5 mIU/ml on the GnRH stimulation test, using analysis of variance, yielded significant differences in uterine width (P < 0.001), fundus diameter (P < 0.04), uterine volume (P = 0.006), and ovarian circumference (P < 0.02).

Conclusions: Increased uterine and ovarian measurements may be an early and sensitive sign of PP. Pelvic ultrasound, a noninvasive, inexpensive, and reliable tool, may give the clinician a complementary indication to the GnRH test in distinguishing isolated PT from early-stage PP in girls with early breast budding.

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Introduction

Precocious puberty (PP) in girls is defined as the appearance of secondary sex characteristics before the age of 8 years. In most cases, it is caused by premature activation of the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator (“central” PP) and is considered to be idiopathic. Central PP (CPP) may cause early epiphyseal maturation with compromised final height (1,2) as well as psychological stress (3,4). Thus, early initiation of treatment is important (2). However, CPP often resembles premature thelarche (PT), characterized by isolated early breast development, which is not associated with acceleration of growth or bone maturation and thus does not require therapy (5). The incidence of PT is highest in the first year of life, with a second peak after the fifth year (6). The latter increase may represent an “intermediate” entity between isolated PT and CPP (7), also called “thelarche variant”, “non-classical PT” and “atypical PT”. It is characterized by older age at onset and occasional progression to CPP (8).

The differentiation of CPP from PT is based on physical examination, bone age assessment, growth velocity, and the GnRH stimulation test. It is difficult to distinguish between early stages of CPP and PT. With the significant increase in obesity prevalence in recent years, the distinction between CPP and obesity with or without thelarche poses another diagnostic challenge. Bone age may be advanced in obese girls who present with pseudothelarche due to increased fat tissue (9,10), whereas bone age advancement and growth acceleration might not be observed in the early stages of CPP. The GnRH stimulation test is considered the gold standard for diagnosis. Yet, despite its high specificity,
its sensitivity is low (11–13), and researchers disagree as to which response to GnRH stimulation should be used for diagnosis of CPP. The aim of the present study was to identify clinical, biochemical and ultrasonographic parameters that can best differentiate CPP from PT, with emphasis on the contribution of pelvic ultrasound. In addition, we sought to determine if uterine and ovarian measurements by ultrasound have significant diagnostic value, especially when findings on the GnRH stimulation test have a prepubertal pattern. Although the clinical diagnosis of CPP was supported by ruling out other pathologies by further laboratory (adrenal androgens, prolactin, liver function, alpha-fetoprotein, beta-human chorionic gonadotropin (beta-HCG)) and imaging studies (MRI of CNS, abdominal ultrasound) according to clinical judgment. The diagnosis of PP after follow-up was based on progression of breast development accompanied by at least one of the following: growth acceleration, bone age acceleration, and appearance of pubic hair. All diagnoses were confirmed after at least 3 years of clinical and auxiological follow-up. Girls with chronic disease, bone dysplasia, organic brain disease, congenital adrenal hyperplasia, or other endocrinological abnormalities were excluded, as were girls after radiation therapy and/or chemotherapy. None of the patients had clinical features of McCune-Albright syndrome, and none had known exposure to exogenous androgens or estrogens. The clinical and laboratory data of the PP and PT groups were compared. To determine if uterine and ovarian measurements by ultrasound can significantly distinguish between girls with true PP and nonpubertal girls of the same age group, the findings were compared to findings in 83 otherwise healthy girls who were referred to the emergency department for intercurrent disease or to our institute for growth observation. One pediatric endocrinologist (L.D.) examined all control patients; none presented with PP or PT. The control findings were also analyzed against norms for healthy girls published in the literature (19). The ultrasound parameters were then compared between the girls with PT and PP in whom the GnRH stimulation test revealed a prepubertal pattern.

Written informed consent was obtained from all families. The institutional human research committee approved the study.

Materials and methods

Patients

Girls referred for evaluation because of the appearance of breast buds between ages 4 and 8 years were recruited consecutively. All patients underwent clinical, biochemical, and bone age evaluation on referral. Assessment of the bone age (BA) and calculation of the bone age standard deviation score (BA-SDS) were performed according to Greulich and Pyle (14). Pubertal stage was determined according to Marshall and Tanner (15). Only patients in Tanner stages 2 or 3 were included. The hormonal evaluation included basal and GnRH-stimulated levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) using a solid-phase, two-site chemiluminescent immunometric assay (Immulite 2000, DPC, Los Angeles, CA, USA). Serum basal blood levels of estradiol (Double Antibody Estradiol procedure, DPC), androstenedione (RIA, Diagnostic Systems Laboratory, Webster, TX, USA), and 17-hydroxyprogesterone (RIA, MP Biomedicals, Orangeburg, NY, USA) were determined. GnRH stimulation test was performed with 100 mcg GnRH, given as an i.v. bolus: serum LH and FSH concentrations were measured at 0, 30 and 60 min. Peak LH levels over 5 mL/mL were considered a “pubertal response”, as previously suggested for the immunochemiluminometric assay (16). Height was calculated as height-standard deviation score (Ht-SDS) for all girls and for both their parents, using the Centers for Disease Control growth charts (17). Body weight was expressed as body mass index (BMI = weight in kilograms/height in m²), and the BMI-SDS was calculated according to the method of Rosner et al. (18).

The diagnosis of PP was based on the appearance of breast buds before 8 years of age accompanied by the presence of one or more of the following findings: menses, pubic hair, accelerated growth velocity, or bone age greater than 2 SD above the chronological age. The diagnosis of PT was based on the presence of breast buds in the absence of bone or growth acceleration or pubic or axillary hair. Cases that were equivocal on referral were diagnosed after at least 6 months’ follow-up on the basis of clinical judgment by an experienced clinician, who was blinded to the ultrasound results. Other forms of precocious puberty were ruled out by performing further laboratory (adrenal androgens, prolactin, liver functions, alpha-fetoprotein, and beta-HCG) and imaging studies (MRI of CNS, abdominal ultrasound) according to clinical judgment. The diagnosis of PP after follow-up was based on progression of breast development accompanied by at least one of the following: growth acceleration, bone age acceleration, and appearance of pubic hair. All diagnoses were confirmed after at least 3 years of clinical and auxiological follow-up. Girls with chronic disease, bone dysplasia, organic brain disease, congenital adrenal hyperplasia, or other endocrinological abnormalities were excluded, as were girls after radiation therapy and/or chemotherapy. None of the patients had clinical features of McCune-Albright syndrome, and none had known exposure to exogenous androgens or estrogens.

Pelvic ultrasound

Transabdominal pelvic ultrasound scans were performed with a conventional full-bladder 5-MHz real-time sector scanner (Sonoline prima, Siemens) by the same investigator (L.D.). The following parameters were analyzed: (1) Uterus: Length, transverse diameter (width), endometrial thickness, fundal anteroposterior diameter, and cervical anteroposterior diameter. The ratio between the fundal and cervical diameters (FCR) was calculated, and the uterine length was multiplied by the fundal anteroposterior diameter to determine the uterine cross-sectional area (CSA). (2) Ovaries: Height,
width, length, number of follicles, and maximal diameter of largest follicle observed. Ovarian circumference was measured in the transverse position. Uterine and ovarian volumes were calculated according to the formula for ellipsoid bodies: \( V = \text{longitudinal diameter} \times \text{anteroposterior diameter} \times \text{transverse diameter} \times 0.5233 \).

To assess reproducibility, ultrasound examinations were performed twice on the same occasion in 15 girls by two experienced operators who were unaware of the other’s results. The correlation coefficients between two observers (Pearson correlation) for the 7 ultrasonographic parameters were 0.93–0.99 (\( P < 0.001 \) for all).

**Statistical analysis**

Statistical analyses were done with BMDP software (New System version, Statistical Solutions, Cork, Ireland). The results are given as mean ± s.d. ANOVA was used to compare the ultrasonographic parameters of the study and control groups and the published norms. Three separate analyses were performed to compare the ultrasonographic parameters between the girls with PT and PP who had a prepubertal response to the GnRH stimulation test, each using a different criterion for prepubertal pattern: peak LH ≤ 5 mIU/ml (20), peak LH ≤ 8 mIU/ml (21), and peak LH/peak FSH ratio ≤ 1 (11). The variables found to be significant were entered into a univariate model with a logistic regression to identify those that predict PP. Receiver operating characteristics (ROC) curves were used to select cutoff points for androstenedione level and ultrasonographic measurements. Sensitivity and specificity were calculated for each variable using the area under the ROC curve (AUC). A \( P \) value of ≤ 0.05 was considered significant.

**Results**

Eighty-one girls were diagnosed with PP and 22 with PT. The disposition of the girls is presented in Fig. 1.

Significant differences were found in uterine volume, length, and anteroposterior diameter and in ovarian volume between the control subjects and the PP group (Fig. 2), but not the PT group. There were no differences in any of these variables between the control group and published data for normal healthy girls (19).

The clinical and auxiological data are shown in Table 1. On referral, the girls with a final diagnosis of PP were slightly older than the girls with PT, but within the same age range (the youngest patient in each group was 5.7 and 4.4 years old respectively). Tanner stage 2 breast development was documented in 49 girls with PP and 20 with PT, and Tanner stage 3 in 32 girls with PP and 2 girls with PT. Pubarche at presentation in the PP group was stage P1 in 19 girls (23.5%), P2 in 43 girls (53%) and P3 in 19 girls (23.4%). None of the girls in the PT group presented with pubarche.

Bone age was more advanced in the PP group than the PT group, as expressed by both the bone...
There were no between-group differences in mean serum levels of estradiol, testosterone, dehydroepiandrosterone-sulfate, and basal gonadotropins. Compared to the PT group, the PP group had significantly higher mean levels of androstenedione, peak LH, and peak LH/peak FSH ratio. However, peak LH > 5, which is considered a pubertal response by many authors, was noted in only 51 girls (62%) in the PP group and 1 girl (4.5%) in the PT group, for a sensitivity of 62% and specificity of 93.7%. Peak LH/peak FSH ratio was greater than 1 in 29 girls (35.8%) with PP and in none of the girls with PT.

On ultrasound, a uterus was identified in all patients, and both ovaries were visualized in all patients but one, in whom only the left ovary was visible.

Most of the uterine and ovarian measurements were significantly different between the girls with PP and PT (Table 2). For the uterus, differences were noted in transverse diameter, length, fundal anteroposterior diameter, and volume. Endometrial echo was observed in 43 girls in the PP group (53%) and in none of the girls in the PT group. For the ovaries, differences were found in length, circumference, and mean (right and left) volume, but not in number of ovarian follicles or size of largest follicle. Continuous data for some of the crucial measures marking individual points as PT versus PP are shown in Fig. 3.

The variables found to be significant on univariate analysis were BA-SDS, peak LH, peak LH/peak FSH ratio, androstenedione, uterine width, volume, cross-section area, and length, FCR, mean (right and left) ovarian volume and ovarian circumference. We applied square root transformation to uterine volume which did not have a normal (Gaussian) distribution. The inclusion of these variables in the logistic regression model yielded uterine transverse diameter, BA-SDS, and uterine volume as the most significant predictors of PP (Table 3). The area under the ROC curve was 0.95. Peak LH was not as good a predictor of PP as the above variables. Cut-off points predictive for PP were as
Table 2 Ultrasound measurements in girls with precocious puberty (PP) or premature thelarche (PT).

<table>
<thead>
<tr>
<th>Variable</th>
<th>PP (n=81) (Mean±s.d.)</th>
<th>PT (n=22) (Mean±s.d.)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine transverse diameter (cm)</td>
<td>1.8±0.4</td>
<td>1.3±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uterine length (cm)</td>
<td>3.9±0.7</td>
<td>3.4±0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fundus (cm)</td>
<td>1.2±0.4</td>
<td>0.8±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fundus/cervical ratio</td>
<td>1.2±0.4</td>
<td>0.8±0.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Uterine volume (cm³)</td>
<td>4.5±3.4</td>
<td>1.8±0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Endometrial thickness (cm)</td>
<td>0.2±0.2</td>
<td>0.02±0.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Uterine cross-sectional area (cm²)</td>
<td>4.7±2.1</td>
<td>2.7±0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ovarian height (cm)</td>
<td>2.3±0.6</td>
<td>1.8±0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ovarian width (cm)</td>
<td>1.2±0.3</td>
<td>1.1±0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Ovarian length (cm)</td>
<td>1.8±0.5</td>
<td>1.7±0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Uterine transverse diameter (cm)</td>
<td>2.8±2.1</td>
<td>1.8±1.3</td>
<td>0.05</td>
</tr>
<tr>
<td>Ovarian circumference (cm)</td>
<td>5.0±1.2</td>
<td>4.0±0.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Number of follicles</td>
<td>4.0±1.9</td>
<td>3.4±1.6</td>
<td>0.2</td>
</tr>
</tbody>
</table>

follows: androstenedione > 1.0 nmol/l; uterine anteroposterior diameter > 8 mm; uterine transverse diameter > 1.5 cm; uterine length > 3.4 cm; uterine volume > 1.96 ml; ovarian circumference > 4.5 cm. The sensitivity and specificity of most of the significant variables are shown in Table 4.

Comparison of the 30 girls with PP and 21 with PT in whom peak LH was ≤ 5 mIU/ml on GnRH stimulation test yielded significant differences in uterine transverse diameter (P<0.001), fundal diameter (P=0.04), uterine volume (P=0.006), and left and right ovarian circumference (P<0.02 and P=0.03 respectively). Similar differences were noted on comparison of the 38 girls with PP and 20 with PT in whom peak LH was ≤ 8 mIU/ml, and the 48 girls with PP and 22 girls with PT in whom peak LH/peak FSH ratio was ≤ 1 (Table 5).

Ultrason had a good predictive power for PP in unequivocal cases. Specifically, the odds ratio for predicting peak LH > 8 mIU/ml was 1.3 (CI 1.12–1.5; P<0.001) for uterine transverse diameter (AUC=0.76) and 2.0 (CI 1.4–2.9; P<0.001) for uterine volume (AUC=0.85). The correlation between peak LH and uterine volume (Pearson correlation) by diagnosis was 0.47 for PP and 0.52 for PT.

Discussion

In the present study, pelvic ultrasound proved to be an efficient tool in differentiating PP from PT, especially when results of the GnRH stimulation test were equivocal.

Although previous studies have compared the ultrasonographic parameters of girls with PP and PT (22–25), only one examined the efficacy of ultrasound against GnRH stimulation test (11). The PT group in the latter study was significantly younger than the PP

Table 3 Results of logistic regression to predict precocious puberty.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio*</th>
<th>Lower</th>
<th>Upper</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine transverse diameter</td>
<td>1.87</td>
<td>1.1</td>
<td>3.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BA-SDS</td>
<td>2.93</td>
<td>1.0</td>
<td>8.53</td>
<td>0.006</td>
</tr>
<tr>
<td>Uterine volume</td>
<td>1.43</td>
<td>1.0</td>
<td>2.13</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Odds ratio for increased risk of precocious puberty; for every increase of 1 cm in uterine transverse diameter, of 1 standard deviation score in bone age, and of 0.1 cm³ in uterine volume.

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Ultrasound measurements in girls with precocious puberty (PP) or premature thelarche (PT) with a prepubertal response to GnRH stimulation test. Results for three analyses by three criteria of prepubertal response.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PP (n=30)</th>
<th>PT (n=21)</th>
<th>P value</th>
<th>PP (n=38)</th>
<th>PT (n=20)</th>
<th>P value</th>
<th>PP (n=48)</th>
<th>PT (n=22)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine transverse diameter (cm)</td>
<td>1.6±0.4</td>
<td>1.3±0.2</td>
<td>&lt;0.001</td>
<td>1.6±0.4</td>
<td>1.3±0.2</td>
<td>&lt;0.001</td>
<td>1.7±0.4</td>
<td>1.3±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uterine length (cm)</td>
<td>3.7±0.5</td>
<td>3.4±0.6</td>
<td>0.03</td>
<td>3.8±0.5</td>
<td>3.4±0.6</td>
<td>0.005</td>
<td>3.8±0.6</td>
<td>3.4±0.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Fundus (cm)</td>
<td>1.0±0.3</td>
<td>0.8±0.2</td>
<td>0.04</td>
<td>1.0±0.3</td>
<td>0.8±0.2</td>
<td>0.01</td>
<td>1.0±0.3</td>
<td>0.8±0.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Fundus/cervical ratio</td>
<td>1.2±0.3</td>
<td>1.1±0.3</td>
<td>0.19</td>
<td>1.2±0.3</td>
<td>1.1±0.4</td>
<td>0.31</td>
<td>1.2±0.3</td>
<td>1.1±0.4</td>
<td>0.20</td>
</tr>
<tr>
<td>Uterine volume (cm³)</td>
<td>3.1±1.9</td>
<td>1.8±0.7</td>
<td>0.006</td>
<td>3.2±1.7</td>
<td>1.8±0.8</td>
<td>0.01</td>
<td>3.5±1.8</td>
<td>1.8±0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uterine cross-section area (cm²)</td>
<td>3.7±1.6</td>
<td>2.7±0.8</td>
<td>0.01</td>
<td>3.8±1.4</td>
<td>2.7±0.9</td>
<td>&lt;0.001</td>
<td>3.9±1.6</td>
<td>2.8±1.4</td>
<td>0.009</td>
</tr>
<tr>
<td>Ovarian length (cm)</td>
<td>2.1±0.5</td>
<td>1.8±0.4</td>
<td>0.04</td>
<td>2.2±0.6</td>
<td>1.8±0.4</td>
<td>0.007</td>
<td>2.2±0.6</td>
<td>1.8±0.4</td>
<td>0.005</td>
</tr>
<tr>
<td>Ovarian circumference (cm)</td>
<td>4.8±1.2</td>
<td>4.0±0.4</td>
<td>0.02</td>
<td>4.9±1.2</td>
<td>4.0±0.5</td>
<td>0.01</td>
<td>4.9±1.2</td>
<td>4.0±0.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Ovarian volume (cm³)</td>
<td>2.3±1.4</td>
<td>1.7±1.2</td>
<td>0.14</td>
<td>2.4±1.5</td>
<td>1.8±1.2</td>
<td>0.10</td>
<td>2.5±1.6</td>
<td>1.8±1.2</td>
<td>0.06</td>
</tr>
</tbody>
</table>

LH = luteinizing hormone; FSH = follicle-stimulating hormone.
Previous studies report that at least one ovary is identified in about 90% of pediatric patients over 5 years of age, and both ovaries in up to 80% (31,32). Our high yield was probably attributable to our long-term experience.

Performing pelvic ultrasound in pediatric endocrinology institutes saves time and money, and provides the clinician with a broader clinical view, because the ultrasound data are obtained at the same time as the physical examination data. Furthermore, as the environment is already familiar to the patients, the test is less stressful.

At present, most of the girls have not yet reached final height, and some are still being treated. Therefore data on height outcome and age at menarche are sparse.

We found no increase in BMI-SDS in either group, thereby excluding obesity as a cause of PP or PT or “pseudo-precocious puberty”.

Overall, the girls with PT comprised 21% of our cohort, and the girls with PP, 79%. These rates are in accordance with the study of Battaglia et al. (33) wherein 33% of the girls had PT and 67% had CPP. Pescovitz et al. (12) reported rates of 26% PT, 38% CPP, and the rest intermediate forms of PP; the average chronological age of the girls with PT was 2.5 years. In another study, PT accounted for 18.5% of all cases of early breast budding (34). The relatively low ratio between the sizes of the PT and PP groups in the present study can be explained by our exclusion of girls younger than 4 years. The increased incidence of PT after age 4 years, following the progressive decrease up to age 4 years, may reflect “intermediate” or “atypical” forms of premature sexual maturation (6, 8, 35). Unfortunately there is no one parameter that is accurate and sensitive enough to distinguish among the different presentations of premature sexual development.

Interestingly, mean androstenedione level was significantly higher in the PP than the PT group. This most probably indicates true activation of the gonadotroph–ovarian axis. However, the androstenedione level by itself was not a sensitive parameter for differentiation of PP from PT. A similar observation was reported in one study comparing 3 girls with PT to 3 girls with CPP (23), but not in another study, which failed to find any such difference (33). No significant difference was found in mean estradiol level between the groups. Nevertheless, it should be noted that the sensitivity of the assay in our laboratory changed over the period of the study, from a lower limit of 20 pmol/l at onset to 2.3 pmol/l later.

Conclusions

An increase in uterine and ovarian measurements represents an early and sensitive sign of PP. Thus, pelvic ultrasound, a noninvasive, inexpensive, and reliable tool, may give the clinician a complementary indication to the GnRH test for the distinction between isolated PT and early-stage PP in girls with early breast budding. Therefore, we suggest that ultrasound scan should be included in the routine work-up of girls with PP.

Acknowledgements

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