Pelvic and breast ultrasound abnormalities and associated metabolic disturbances in girls with premature pubarche due to adrenarche

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Abstract

Objective: Premature adrenarche (PA) has been suggested as a risk factor for future health problems, such as metabolic syndrome and early menarche. However, not all girls with PA have these features and it is not certain who will develop them. We propose that these abnormalities might be identified earlier, even before they are visible.

Design: Case-control study.

Setting: Tertiary care hospital.

Participants: Forty-eight girls with premature pubarche due to PA and age (mean age 7.6 ± 1.0 years), weight, body mass index (BMI), birth weight and gestational age-matched 49 girls with no palpable breast tissue.

Measurements: Early pubertal pelvic and breast ultrasonographic changes and their associations with obesity and metabolic parameters were evaluated. Blood samples were collected, breast and pelvic ultrasound examinations were performed and bone ages were assessed.

Results: Girls with PA were taller and their bone ages were higher (p = .049 and p = .005). Fasting blood glucose, insulin, triglycerides, high-density lipoprotein and low-density lipoprotein cholesterol were not different between the groups. Luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estradiol were not different either. Ultrasonography revealed breast gland tissue in 30% of girls with PA and 5% of controls (p = .006). Uterine volume and endometrial thickness were higher in girls with PA (p = .03 and p = .04). Endometrial thickness was positively associated with serum insulin levels in the whole study group and after adjusting for age, diagnosis, BMI, mean ovarian volume and LH, FSH, estradiol levels, this association remained with a borderline p-value (R² = 0.486, p = .050).

Conclusions: We found early changes in uterus and breast glands of girls with PA and endometrial thickness was positively associated with insulin levels.

KEYWORDS
breast ultrasonography, metabolic syndrome, obesity, pelvic ultrasonography, premature adrenarche

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1 | INTRODUCTION

Premature adrenarche (PA) is defined by early maturation of the adrenal zona reticularis resulting in increased secretion of the adrenal androgen precursors before the age of 8 years in girls and 9 years in boys. Increase in androgen production manifests with pubic and axillary hair growth, adult-type body odour, oily skin, acne and comedones. The prevalence of PA in girls varies in different populations, with reported rates between 4.5% and 17.7%.2-5

PA has been considered as an extreme of normal variation with no need for special follow-up or treatment.6 However, especially after the late 1990s, connections between PA and metabolic syndrome features, including central obesity, high blood pressure, decreased serum high-density lipoprotein (HDL) cholesterol, elevated serum triglycerides and impaired fasting glucose levels, were widely reported.7-12 Additionally, many studies showed earlier menarchal age in girls with PA13-15 and significant bone age advancement was reported in both genders.16 Moreover, in a prospective cohort study, Remer et al.17 showed that higher urinary androgen levels in prepubertal years were correlated with an earlier onset of breast development and a shorter duration of the pubertal growth spurt. However, not all girls with PA show these abnormalities and it is not certain who will proceed with them. We propose that these abnormalities might be interrelated and possibly identifiable earlier, even before they are visible. In a previous article with a different group of girls with PA, we showed that high body mass index (BMI) at presentation might be a triggering factor for future metabolic abnormalities.7

In this study, we aimed to evaluate early pubertal changes and potential metabolic disturbances in girls with PA. Additionally, we wanted to investigate probable associations between these abnormalities. To this end, we conducted a case-control study on a group of 48 girls with premature pubarche (PP) due to PA and compared it with age, weight, BMI, as well as birth weight (BW) and gestational age matched 49 girls having no pubertal signs. In these girls, we evaluated early pubertal pelvic and breast ultrasonography (USG) changes and their associations with obesity and metabolic parameters.

Obesity is a common finding in children with PA and it is not easy to differentiate the direction of the association between PA and obesity.6 Additionally, being born small for gestational age (SGA) is associated with insulin resistance, metabolic syndrome and increased androgen secretion.11,12,18-20 Therefore, in this study setting, we matched our study groups for BMI and BW.

2 | MATERIALS AND METHODS

2.1 | Subjects

Subjects with PP due to PA were recruited from the Pediatric Endocrinology Clinic of Istanbul University Faculty of Medicine. The control group was recruited either from the Well Child Clinic of Istanbul University Faculty of Medicine, on whom detailed information has been published previously,21 or from the Obesity Clinic of Istanbul University Faculty of Medicine. The diagnosis of PP due to PA was based on the development of pubic or axillary hair without palpable breast glands, before 8 years of age with a serum dehydroepiandrosterone sulphate (DHEAS) level higher than 1 μmol/L (≥40 μg/dl).6

In girls with PP, the mean DHEAS was 2.42 ± 1.46 μmol/L (range: 1.09-7.76 μmol/L). Thyroid dysfunction was ruled out in all subjects. Since non-classical congenital adrenal hyperplasia is common in Turkey, it was ruled out with adrenocorticotrophic hormone stimulation test; adrenal malignancies were ruled out with adrenal USG in patients with PA. Additionally, if there was a clinical suspicion, Cushing’s syndrome was investigated and ruled out. Subjects with other chronic diseases or medication use were not included in this study.

This study was conducted in Istanbul, the largest city in Turkey with all the ethnic groups and people from every socioeconomic status. Additionally, in Turkey, there is universal healthcare coverage for all children, promoting equal access to care.

The Ethical Committee of Istanbul University Faculty of Medicine approved the study. Written consent was obtained from the parents.

Anthropometric measurements and a physical examination were performed in the morning after an overnight fast. Heights were measured by using a Harpenden stadiometer (Holtain, United Kingdom), in the standing position with bare feet. Weight measurements were conducted in the children wearing minimal underclothes, using an electronic scale sensitive to 0.1 kg (Seca, Germany). Waist circumferences (WCs) were measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, using a plastic non-stretch measuring tape (Seca measuring tape). Hip circumferences (HCs) were measured at the largest circumference of the buttocks, using the same measuring tape. Children were evaluated for puberty according to Tanner classification;22 breast development was assessed by both inspection and palpation to avoid misclassification of fat tissue as breast gland.

2.2 | Laboratory evaluation

Fasting blood samples were collected for glucose, insulin, total cholesterol, HDL cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estradiol. Samples were immediately processed and sera were stored at −80°C for subsequent analysis.

Glucose was measured by the hexokinase method (Roche Diagnostics using Cobas Integra kits). Serum total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride levels were measured by absorbance photometry (Roche Diagnostics, Cobas Integra 800 Device). Insulin levels were measured by immunoradiometric assay (IRMA) (DIAsource ImmunoAssays S.A. Nivelles). Intra-assay cutoff values (CV) were 1.5%-2.1%, inter-assay CV 6.1%-6.5% and the lowest measured value was 6 pmol/L. LH levels were measured by DSL-4600 Active® LH coated tube IRMA (Diagnostic Systems Laboratories, Inc.), the limit of detection was 0.12 IU/L with an intra- and inter-assay (CV) of 4.8%-8.9% and 6.8%-8.9%, respectively. FSH levels were measured by DSL-4700 Active® FSH coated tube IRMA.
(IRMA, Diagnostic Systems Laboratories, Inc.) with an intra- and inter-assay CV of 1.6%–3.6% and 5.6%–7.7%, respectively, and the limit of detection was 0.11 IU/L. Estradiol levels were measured by electrochemiluminescence immunoassay (ECLIA, Cobas e 601 analyser, Roche Diagnostics) kits with intra- and inter-assay CV of 1.3%–6.1% and 1.9%–7.0%, respectively, and the functional sensitivity was 44 pmol/L.

Bone ages were assessed by a single observer (BKA), using Greulich and Pyle atlas for the determination of skeletal age.

Since obesity is not rare in girls with PA and it is hard to evaluate breast tissue by palpation in girls with obesity, we assessed pubertal development by using breast and pelvic USG. Pelvic and breast USG were performed by the same radiologist (AK), who was blinded to the clinical and laboratory findings using a Siemens S2000 scanner (Siemens Medical Solutions USA, Inc.), with a 6 MHz probe for pelvic USG and an 18 MHz probe for breast USG. Retromamillary fluid retention, development of tiny ducts, increase in subcutaneous adipose tissue, centrifugal lengthening of the ducts and thickening of their roots, glandular tissue and peri glandular stromal tissue development were evaluated using breast USG. Since breast development can be asymmetric, each gland was examined separately. The presence of the breast gland was determined by visualisation of the retromamillary fluid and/or the lactiferous ducts and/or the fibroglandular tissue (Figure 1). Right and left ovarian volumes, uterine length, uterine volume, endometrial thickness and fundus/cervix ratio were measured with pelvic USG.

### 2.3 Calculations and definitions

Data regarding BW, birth length, gestational age, mother’s and father’s height were collected from chart review and if missing in the files, by additional interviews. Ponderal index was calculated as BW (kg)/[birth length (m)]³. BMI was computed as weight (kg)/[height (m)]². Weight, height and BMI standard deviation scores (SDS) were calculated according to national standards. WC (cm)/height (cm) and WC (cm)/HC (cm) ratios were also calculated. Target height was determined using the mother’s and father’s height and target height SDS was calculated. Target height SDS and height SDS difference was also calculated.

Overweight was defined as a BMI SDS ≥ 1, and obesity was defined as ≥ 2 SDS. National standards for gestational age were used for the calculation of BW z-scores. SGA was defined as BW below the 10th percentile for the gestational age, whereas large for gestational age (LGA) was defined as having a BW that lies above the 90th percentile for that gestational age. Appropriate for gestational age (AGA) was defined as BW between 10th and 90th percentile. Homoeostatic model assessment for insulin resistance (HOMA-IR) was calculated as [fasting glucose (mmol/L) x fasting insulin (pmol/L)]/135.

### 2.4 Data analyses

We used the Shapiro–Wilk test to determine if variables were normally distributed. Comparisons were made by using Independent samples t-test, Mann–Whitney U-test and X²-test, where appropriate. We used Pearson or Spearman analyses to determine correlations between the clinical parameters. We created linear regression models to examine the associations between uterine and ovarian measurements and metabolic parameters. We adjusted the models for age, presence/absence of PA, BMI SDS, LH, FSH and estradiol levels. Data were presented as the mean ± SD. All statistical analyses were conducted with SPSS version 15.0 (SPSS Inc.). Statistical significance was defined as p < .05.

### 3 RESULTS

Table 1 presents the comparison of the clinical characteristics of girls with and without PA. The average age of the subjects was 7.6 ± 1.0 years and it was not different between the groups. Girls with PA were taller than their peers with no pubertal signs. Other clinical characteristics were not significantly different between the study groups.

Table 2 shows the comparison of the biochemical and imaging results of girls with and without PA. Bone age was significantly higher in girls with PA. Bone age–chronological age difference was also
higher in these girls. Although there was no breast enlargement detectable by palpation in any of the study subjects, USG revealed breast gland tissue in 30% of girls with PA and 5% of healthy controls. Uterine volume and endometrial thickness were also higher in girls with PA.

Table 3 displays the correlations among ultrasonographic findings and anthropometric measurements, bone ages and serum insulin levels in girls with and without PA. There were positive correlations between uterine length and weight SDS, height SDS, WC/HC ratio in the whole study group as well as bone age in girls with PA. Additionally, endometrial thickness was positively correlated with bone age and serum insulin levels in the whole study group.

Endometrial thickness was positively associated with serum insulin levels and this association remained with a borderline p-value after adjusting for age, presence/absence of PA, BMI SDS, mean ovarian volume and LH, FSH, estradiol levels ($R^2 = 0.486, p = .050$).

We did not find any significant association between ovarian volumes and measured metabolic parameters (data not shown).

### DISCUSSION

In this study, we showed that there are early pelvic and breast USG changes in girls with PA when compared to their age, weight, BMI, BW and gestational age matched healthy peers. There was no breast enlargement detectable by palpation in any of our study subjects but USG revealed breast gland tissue in 30% of girls with PA and 5% of girls in the control group. Additionally, uterine volume and endometrial thickness were higher in girls with PA. Although these pubertal changes are heavily under the influence of sex hormones, gonadotropin and estradiol levels were not different between the groups in our study.

Gonadotropins are secreted in a pulsatile pattern and prepubertal girls have irregular gonadotropin pulses with low amplitudes and low frequency, especially during the daytime. Since it is not feasible to do 24 h gonadotropin monitoring, evaluation with breast and pelvic USG would be a viable option for future studies on prepubertal girls.

In another study from our group, Ucar et al. reported that AGA born girls with PA had greater uterine length than their BMI-matched healthy peers. Although mean ovarian volumes were not significantly different between the groups in that study, decreasing insulin sensitivity was strongly associated with an increase in mean ovarian volume. We could not find any significant association between metabolic parameters and ovarian volumes in this study but, we found a positive association between insulin levels and endometrial thickness. Additionally, uterine length was positively correlated with weight SDS and WC/HC ratio. Increased insulin may lead to decreased sex hormone-binding globulin levels and eventually increased sex steroid bioavailability in prepubertal years. Since girls with PA have already increased androgen levels, with the help of aromatase activity they would have more estrogens available compared to their peers without PA and this might explain the differences between breast and pelvic USG findings in these girls.
### TABLE 2
Comparison of the biochemical and imaging results of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Girls with no pubertal signs (n = 49)</th>
<th>Girls with premature adrenarche (n = 48)</th>
<th>p(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone age (years)</td>
<td>7.45 ± 1.4</td>
<td>8.2 ± 1.0</td>
<td>0.005</td>
</tr>
<tr>
<td>Bone age–chronological age difference</td>
<td>−0.16 ± 1.0</td>
<td>0.49 ± 1.0</td>
<td>0.005</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.2</td>
<td>0.73</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>1.5 ± 0.7</td>
<td>1.7 ± 1.0</td>
<td>0.49</td>
</tr>
<tr>
<td>Estradiol (pmol/L)</td>
<td>44.8 ± 46.6</td>
<td>46.6 ± 51.0</td>
<td>0.87</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.72 ± 0.4</td>
<td>4.71 ± 0.6</td>
<td>0.77</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>48.0 ± 27.6</td>
<td>49.2 ± 33.0</td>
<td>0.99</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.99 ± 1.3</td>
<td>1.58 ± 0.9</td>
<td>0.50</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.79 ± 0.28</td>
<td>0.82 ± 0.31</td>
<td>0.82</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.39 ± 0.83</td>
<td>4.31 ± 0.83</td>
<td>0.73</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.47 ± 0.39</td>
<td>1.59 ± 0.39</td>
<td>0.28</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.51 ± 0.74</td>
<td>2.42 ± 0.73</td>
<td>0.62</td>
</tr>
<tr>
<td>Presence of breast tissue on ultrasonography</td>
<td>5%</td>
<td>30%</td>
<td>0.006</td>
</tr>
<tr>
<td>Mean ovarian volume (mm(^3))</td>
<td>1.2 ± 0.5</td>
<td>1.2 ± 0.7</td>
<td>0.90</td>
</tr>
<tr>
<td>Uterine length (mm)</td>
<td>31.7 ± 4.9</td>
<td>31.9 ± 4.8</td>
<td>0.81</td>
</tr>
<tr>
<td>Uterine volume (mm(^3))</td>
<td>1.3 ± 0.6</td>
<td>1.7 ± 0.9</td>
<td>0.03</td>
</tr>
<tr>
<td>Fundus/cervix ratio</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.27</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>1.5 ± 0.6</td>
<td>1.8 ± 0.7</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Note: All values are means ± SDs, except if otherwise stated.
Abbreviations: FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; HOMA-IR, homoeostasis model assessment for insulin resistance; LDL, low-density lipoprotein; LH, luteinizing hormone.

\(^a\)Bold text indicates a statistically significant difference with a p-value < 0.05.

### TABLE 3
Correlations among ultrasonographic findings and anthropometric measurements, bone ages and serum insulin levels in girls with and without premature adrenarche

<table>
<thead>
<tr>
<th></th>
<th>Girls without premature adrenarche (n = 49)</th>
<th>Girls with premature adrenarche (n = 48)</th>
<th>All subjects (n = 97)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uterine length (mm)</td>
<td>Endometrial thickness (mm)</td>
<td>Uterine length (mm)</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>r: 0.263</td>
<td>p:0.11</td>
<td>r:0.265</td>
</tr>
<tr>
<td></td>
<td>p:0.12</td>
<td></td>
<td>p:0.55</td>
</tr>
<tr>
<td>Height SDS</td>
<td>r:0.259</td>
<td>p:0.12</td>
<td>r:0.221</td>
</tr>
<tr>
<td></td>
<td>p:0.16</td>
<td></td>
<td>p:0.25</td>
</tr>
<tr>
<td>WC/HC</td>
<td>r:0.533</td>
<td>p:0.004</td>
<td>r:−0.222</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>r:−0.104</td>
</tr>
<tr>
<td>Bone age (years)</td>
<td>r:−0.057</td>
<td>p:0.75</td>
<td>r:0.353</td>
</tr>
<tr>
<td></td>
<td>p:0.03</td>
<td></td>
<td>r:0.115</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>r:0.112</td>
<td>p:0.59</td>
<td>r:0.271</td>
</tr>
<tr>
<td></td>
<td>p:0.14</td>
<td></td>
<td>r:0.200</td>
</tr>
</tbody>
</table>

Note: Bold text indicates a statistically significant difference with a p-value < 0.05.
Abbreviations: p, p-value for the correlation; r, correlation coefficient; SDS, standard deviation score; WC/HC, waist circumference/hip circumference ratio.
Children with PA are often heavier and taller than their peers. Moreover, the prevalence of PA is higher in girls than boys and this phenomenon is usually explained by higher female adiposity and peripheral DHEAS conversion to active androgens. The rise in adrenal androgens can result in transient growth acceleration and contributes to earlier bone maturation in children with PA. Similarly, girls with PA were taller than their age, weight and BMI matched peers. Additionally, their bone ages and bone age chronological age differences were higher.

It is well known that children with PA are more likely to have metabolic syndrome features. However, in our study, we did not find a significant difference in fasting blood glucose, insulin and lipid parameters between the girls with PA and their age, weight, BMI and BW matched peers. Many previous studies reporting unfavourable metabolic profiles in PA used healthy lean children as the control group. Similar to our results, Utriainen et al. reported that weight-for-height adjusted blood pressure, lipid or glucose levels did not differ between the girls with PA and the control group. They concluded that prepubertal Northern European PA girls have an increased prevalence of hyperinsulinemia and metabolic syndrome mainly due to being overweight. In a previous study, our group showed that girls with PA had a better lipid profile (higher serum HDL cholesterol, lower triglycerides and LDL cholesterol) than birth weight, BMI and WC-matched control girls. Additionally, in another more recent study, we reported that BMI at adrenarche is the most important parameter to predict insulin resistance in puberty. Although the exact mechanisms of PA are still not completely understood, the adipose tissue seems to play an important role in its multifactorial aetiology. Increased serum DHEAS levels and advanced bone maturation are well-known concomitants of obesity. However, not all peripubertal girls with obesity have elevated androgens and also not all children diagnosed with PA are obese or overweight, suggesting that obesity per se is not sufficient to produce PA. There are other preceding factors, including genetic background, ethnicity, as well as alterations in BW and infancy weight gain.

Our two groups were comparable for BW SDS, ponderal index, gestational age and SGA/AGA/LGA ratios. Low BW is shown to be associated with insulin resistance and metabolic syndrome. Especially, studies in Catalanian girls reported that premature androgen excess, hyperinsulinemia and dyslipidemia are associated with low BW. It was suggested that low BW followed by an excessive catch-up weight gain may initiate a pattern of events leading to the manifestation of PA and the metabolic syndrome.

To the best of our knowledge, this is the first study that has evaluated breast USG in girls with PA, and we observed breast gland tissue in 30% of these girls. Uterine volume and endometrial thickness were also higher in girls with PA than the controls. We could not find any significant association between metabolic parameters and ovarian volumes in this study, but we found a positive association between insulin levels and endometrial thickness. Future studies, with a larger cohort and a longitudinal design, would be helpful to further investigate and understand these associations.

5 CONCLUSIONS

Although there was no breast enlargement detectable by palpation in any of our study subjects, breast USG revealed breast gland tissue in 30% of girls with PA and 5% of girls in the control group. Uterine volume and endometrial thickness were also higher in girls with PA than the controls. We could not find any significant association between metabolic parameters and ovarian volumes in this study, but we found a positive association between insulin levels and endometrial thickness. Future studies, with a larger cohort and a longitudinal design, would be helpful to further investigate and understand these associations.

ACKNOWLEDGEMENTS

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

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

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