Polycystic ovaries in childhood: a common finding in daughters of PCOS patients. A pilot study

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BACKGROUND: Polycystic ovarian syndrome (PCOS) is a controversial endocrine pathology and, recently, it has been suggested that the condition is hereditary. The aim of this study was to prospectively determine in daughters of patients with PCOS, by ultrasonographic (US) and colour Doppler analyses, the incidence of polycystic ovaries and search any correlation with specific hormonal parameters. METHODS: Fifteen prepubertal offspring (Group I) of patients with PCOS and 10 normal control prepubertal girls (Group II) were submitted to clinical, auxological, and basal hormonal assay. In addition all patients were submitted to US and colour Doppler ovarian and uterine evaluation. RESULTS: Among Group I girls the prevalence of polycystic ovaries was 93%, whereas no subjects among Group II had polycystic ovaries. The ovarian volume (2.76 ± 1.21 ml versus 0.87 ± 0.46 ml; \( P < 0.001 \)) and the number of small sized follicles (5.36 ± 2.2 versus 0.75 ± 0.92; \( P < 0.001 \)) were significantly higher in Group I than Group II patients. In addition, a normal stromal score and an absent stromal vascularization was observed in the control group. The hormone levels did not differ between the two groups. CONCLUSION: In conclusion we speculate that polycystic ovaries in childhood may be considered a sign of genetic predisposition to PCOS and that environmental factors may express the adult clinical and hormonal presentation of the syndrome.

Key words: childhood/Doppler/echography/heredity/PCOS

Introduction

Polycystic ovarian syndrome (PCOS) is one of the most widely studied and controversial areas in gynaecological endocrinology. Minimal signs of hyperandrogenism in lean, normally menstruating women, and obesity, severe hirsutism and oligo- or amenorrhoea (as originally described by Stein and Leventhal, 1935) represent opposite poles of the clinical spectrum in this condition. The heterogeneity of clinical and endocrine features remains a confounding factor in the investigation of PCOS patients (Franks, 1989; Insler et al., 1993; Homburg, 1996). Despite the vast amount of clinical, laboratory and experimental data that have been accumulated, the aetio-pathogenesis of PCOS remains a subject of speculation.

Recently, it has been suggested that the condition is hereditary and that women with PCOS have additional metabolic disturbances, such as hyperinsulinaemia, increased insulin resistance, hypertriglyceridaemia and decreased high density lipoprotein cholesterol (O’Meara et al., 1993; Morales et al., 1996; Robinson et al., 1996; Rosenfield, 1996; Dunaif, 1997). Although some familial studies on PCOS have indicated both X-linked and autosomal dominancy, other segregation analyses have found an incidence of affected relatives exceeding autosomal dominance (Hague et al., 1990; Legro, 1995; Govind et al., 1999). The registered differences in trying to determine the familial contribution are, probably, correlated with the heterogeneity of the syndrome and with the diagnostic criteria used to identify probands with PCOS, as well as to characterize other affected family members.

The most widely accepted definition of PCOS results from the National Institutes of Health–National Institutes of Child Health and Human development (NIH–NICHD) conference on PCOS: hyperandrogenism, menstrual disturbances (oligo-amenorrhoea) and exclusion of other known disorders such as congenital adrenal hyperplasia, hyperprolactinaemia, or Cushing’s syndrome (Dunaif et al., 1992). However, the ultrasonographic (US) identification of polycystic ovaries (PCO), has called into question the validity of the above definition.

Assessment of ovarian morphology by means of ultrasound is currently employed as a substitute for histological examination in the diagnosis of polycystic ovaries. The US criteria for diagnosis of PCO has been established: enlarged ovary with multiple small follicles scattered around an echogenic stroma. However, the number of small follicles necessary to establish
the diagnosis of PCO has been reported to vary between ‘above five’ (Yeh et al., 1987), ‘more than 10’ (Adams et al., 1985) and ‘at least 15’ (Fox et al., 1991). Yoshino and co-workers (1992) showed that the number of microcysts in PCO is correlated with androstenedione, which in turn is correlated with LH and LH/FSH ratio. In addition, we recently demonstrated that as the number of ovarian microcysts increases and ovarian volume enlarges, endocrine and clinical abnormalities become more remarkable (Battaglia et al., 1999).

Colour Doppler facilitates the detection of small vessels in the utero-ovarian circulation and the measurement of impedance to flow in this vascular tree. A few years ago we showed (Battaglia et al., 1995), and it was successively confirmed (Zaidi, 1995a; Aleem and Predanic, 1996) that, in patients with PCOS, significant and typical vascular changes occur within the intraovarian vessels and that colour Doppler analysis has a high diagnostic value for PCOS.

We serendipitously and retrospectively observed (unpublished data) that girls with premature pubarche have, in about 60% of the cases, mothers with PCOS and/or fathers with premature baldness (significant female-pubic hair loss before the age of 30 years). The aim of the present study was to prospectively determine in daughters of PCOS patients, by US and colour Doppler analyses, the incidence of polycystic ovaries and search for any correlation with specific hormonal parameters.

Materials and methods

Study population

Nineteen Caucasian women with PCOS who previously attended the Gynaecological Endocrinology or Infertility Clinic and that delivered at term females of normal weight were asked to authorize us to study their daughters. The study protocol was in accordance with the Helsinki II declaration and was approved by the Hospital Research Review Committee. Girls participated in the study after informed consent from parents and assent from minors was obtained.

Fifteen women and their daughters agreed to participate in the study. In the adult patients PCOS had been previously diagnosed on the basis of the following criteria: hirsutism, menstrual disturbances (oligo- or amenorrhoea), infertility, increased plasma circulating androgens, LH/FSH ratio of >2.5 and typical US ovarian findings (>10 small sized 2–10 mm subcapsular follicles, ovarian volume >8 ml, and increased ovarian stroma echogenicity) (Battaglia et al., 1998). Girls (Group I, n = 15) were referred to the Paediatric Endocrine Clinic for history, physical, auxological and hormonal evaluation.

The pubertal development was staged by a single examiner (L.I.) according to the classification of Tanner (Tanner, 1962; Marshall and Tanner, 1969). Staging of the breast was as follows: stage I, preadolescent; stage II, elevation of breast and papilla as a small mound, enlargement of areola diameter; stage III, further enlargement of breast and areola, with no separations of contours; stage IV, projection of areola and papilla to form a secondary mound above the level of the breast; stage V, projection of mature papilla, areola part of general breast contour. Staging for pubic hair was as follows: stage I, preadolescent; stage II, sparse, slightly pigmented, straight; stage III, darker, coarser, beginning to curl, increased amount; stage IV, adult in type, but less area covered; stage V, adult in quantity and type, distributed as an inverse triangle, spread to medial surface of thighs. In the presence of pubic hair, a breast stage II development was taken as a definitive sign of gonadal puberty.

Standing height was measured using a Harpden stadiometer (Holtain Ltd., Crumry, UK) to the nearest 0.1 cm; weight was measured on a digital scale with a precision of 0.1 kg (SECA 707; HH, Modena, Italy). The mean body mass index (BMI; weight in kg/height in m2) was calculated. Skeletal maturation and hirsutism were staged according to established criteria (Greulich and Pyle, 1959; Ferriman-Gallwey, 1971) respectively.

Girls were further submitted to basal hormonal assay, US and colour Doppler ovarian and uterine evaluation.

Ten healthy girls (Group II), referred to the Paediatric Endocrine Clinic for Auxological evaluation and found to be normal on clinical examination and anthropometric assessment, served as control. The control group was selected among prepubertal girls whose parents presented with no PCOS or premature male baldness. Similarly to Group I, healthy girls were submitted to basal hormonal assay, US and colour Doppler ovarian and uterine evaluation.

None of the patients had received hormonal therapy before the study.

Ultrasound and Doppler examination

Uterine and ovarian US examinations were performed with the use of a 3.5 MHz. convex transducer (AU4 Idea; ESAOTE, Milan, Italy). The ultrasound scans were performed transabdominally when the participants had a full bladder, obtained by voluntary urine retention and oral administration of fluids. Uterine and ovarian volume, endometrial thickness, number, diameter and distribution of the follicles were recorded. Volumes were calculated by measuring length, width and depth assuming the forms to be ellipsoid, using the formula based on a prolate ellipsoid: \( V = \frac{A\pi D_3}{6} \), where \( D_1, D_2, \) and \( D_3 \) are the maximal longitudinal, antero-posterior and transverse diameters. The maximum diameter of each follicle was reported. Echogenicity of ovarian stroma (stromal score = SS) was subjectively scored as 0 (normal), 1 (moderately increased), or 2 (markedly increased) (Pache et al., 1992, 1993). No significant differences between left and right ovaries were observed, and therefore, the average value of both ovaries was used for statistical analysis. Midline endometrial echo was checked and endometrial thickness was measured as the distance between the two internal sides of the myometrium.

Doppler flow measurements of the uterine and intraovarian vessels were performed transabdominally with a 3.5 MHz colour Doppler system (AU4 Idea colour Doppler). All the patients were in recumbent position and were evaluated between 08.00 and 11.00 h to exclude the effects of circadian rhythmicity on utero-ovarian blood flow (Zaidi et al., 1995b). Furthermore, they rested in a waiting room for at least 15 min before being scanned in order to minimize external effects on pelvic blood flows. A 50 Hz filter was used to eliminate low-frequency signals originating from vessel wall movements. Colour signals were sought in the ovarian stroma at the maximum distance from the surface of the ovary. The ovarian stroma was considered to be ‘avascular’ if no blood vessels were demonstrated by colour Doppler imaging. When several blood vessels were detected inside the ovarian stroma, only the one with the lowest downstream impedance was selected for Doppler measurements. Colour flow images of the ascending branches of the uterine arteries were sampled laterally to the cervix in a longitudinal plane. The angle of insonation was always adjusted to obtain maximum colour intensity. When good signals were obtained, blood flow velocity waveforms were recorded by placing the sample volume across the vessel and activating the pulsed Doppler mode. The Pulsatility Index (PI), defined as the
difference between the peak systolic and end-diastolic flow divided by the mean maximum flow velocity, was calculated for the ovarian stromal and uterine arteries. For each examination, the mean value of three consecutive waveforms was obtained. No significant differences between the PIs of the left and right uterine arteries were observed, and therefore, the average value of both arteries was used. Similarly, the lowest PIs of the stromal arteries were not significantly different between the left and right ovaries and the mean value was utilised. The correlation between PI and heart rate was not tested (Battaglia et al., 1999). Ultrasound and colour Doppler analyses were performed by single examiner (C.B.). The hormonal status of the scanned patients was unknown. An indication of within-patients precision of the Doppler procedures was obtained by analysing the flow velocity waveforms recorded on three occasions from uterine and intraovarian arteries at 1 min intervals. An analysis of variance of the results from 10 patients gave a mean coefficient of variation of 5.8% for uterine and 6.3% for intraovarian arteries and showed no significant differences between the replicate analysis.

**Hormonal assay**

Peripheral blood was obtained from all patients between 08.00 and 11.00, after an overnight fast, on the same day that US and Doppler examinations took place, and different hormonal parameters were analysed. Plasma concentrations of LH, FSH, estradiol (E2), and testosterone were assayed as previously described (Battaglia et al., 1999). Dehydroepiandrosterone sulphate (DHEAS), and 17-hydroxy progesterone (17-OH-Pg) were determined by RIA using the Coat-A-Count kit (DPC; Los Angeles, USA). Androstenedione was measured by RIA with the Quantitative Measurement of Androstenedione in Serum and Plasma kit (DSL Inc.; Webster, USA). Furthermore, to exclude other endocrinopathies, thyroid hormones, prolactin, and adrenocorticotropic hormone (ACTH) were assayed.

To exclude a pubertal activation a GnRH stimulation test was performed in those patients who showed a baseline LH value >0.3 IU/l. The GnRH stimulation test was performed using a standard dose of 100 µg GnRH administered as an i.v. bolus. Serum LH and FSH concentrations were measured at 0, +30, +60, and +90 min. As criteria for defining a pubertal GnRH test a peak LH level >15 IU/l and a LH/FSH ratio >0.66 was used. All hormone analyses were performed in duplicate.

Results of hormonal values were converted to SI units using the following conversion factors: LH (IU/l) = mIU/ml × 1.0; FSH (IU/l) = mIU/ml × 1.0; E2 (pmol/l) = pg/ml × 3.761; testosterone (nmol/l) = ng/ml × 3.467; androstenedione (nmol/l) = ng/dl × 0.0349; 17-OH-Pg (nmol/l) = ng/dl × 0.03026; DHEAS (µmol/l) = µg/ml × 2.714.

**Statistical analysis**

A statistical analysis (SPSS software; SPSS Inc., Illinois, USA) was performed using the Mann–Whitney test and one-way analysis of variance. The relationship between the parameters analysed was assessed using the stepwise linear regression method. A probability of <0.05 was considered as statistically significant. Data are presented as mean ± standard deviation, unless otherwise indicated.

**Results**

On the basis of history, physical examination, basal ultrasonography and laboratory data the following conditions were excluded in all the participants: chronic diseases, Cushing’s syndrome, hyperprolactinaemia, hypo-hyperthyroidism, and sex steroid-secreting tumours. None of the studied girls had developed gonadal puberty, and, according to the Tanner scale, all the girls presented at breast stage I.

Among girls with PCOS affected mothers, the prevalence of US PCO-like ovaries (>5 small 2–10 mm in maximum diameter subcortical follicles, increased ovarian volume and increased ovarian stroma echogenicity) was of 14/15 (93%). In five cases (33%) a paternal premature baldness was also present. None of the control group presented polycystic ovaries.

The chronological age was similar in Group I (7.6 ± 0.6 years) and Group II (6.9 ± 0.6 years) patients. However, the bone age (8.9 ± 1.2 years vs 6.8 ± 0.8 years; P = 0.001), and the basal height (128.3 ± 3.3 vs 118.9 ± 1.6 cm; P = 0.021) was higher in Group I girls. The BMI was below the 75th centile of reference data for obesity (Must et al., 1991) and no significant differences were observed among the groups in this respect (Table I).

None of the subjects studied was hirsute (Ferriman–Gallwey score >8). However, in daughters of PCOS patients, 4/15 girls (26%) presented pubic hair limited to labia majora and in two of them axillary hair was associated. In addition, in almost half (7/15) of Group I girls, a slight increased hair distribution on the distal part of both arms and legs was observed.

The plasma levels of FSH, LH, E2, androstenedione, testosterone, 17-OH-Pg, DHEAS were in the normal range and did not differ among the groups (Table II). None of the girls with a baseline LH value >0.3 IU/l showed a pubertal response to the GnRH test (Table III).

The US assessments allowed us to evaluate the uterine size
and shape in 100% of the cases. The uterine volume was significantly higher in PCO-like than control patients (Table IV). The endometrial echo was never present.

In 23 (92%) out of 25 participants, both ovaries were visualized. The two cases in whom only one ovary was visualized were in the control group. In group II, the ovarian volume (0.87 ± 0.46 ml) was in the reference range of prepubertal stage (Bridges et al., 1993; Buzi et al., 1998), whereas in patients with PCO-like ovaries the volume was significantly higher (2.76 ± 1.21 ml; P < 0.001) and was similar to those of patients at Tanner stage B2. The number of small sized follicles was statistically different among Group I (6.36 ± 2.2) and controls (0.75 ± 0.92; P < 0.001). The ovarian stroma was scored as normal (SS = 0) in 100% of controls. In the PCO-like patients, the stromal score was normal in 1/15 (6%), moderately increased in 3/15 (20%) and markedly increased in 11/15 (73%) of the cases (Table III).

On Doppler analysis, elevated resistances within the uterine arteries were observed in all patients and the values were higher in Group II (PI = 4.23 ± 1.01) than in Group I (3.16 ± 0.74; P = 0.031). In the control group we failed, by colour flow mapping, to identify any ovarian stromal artery, whereas in Group I, the intraovarian arteries were identified, at least in one ovary, in 93% of the cases and a low downstream impedance to flow (PI = 1.62 ± 0.33) was shown (Table III).

In the whole studied population, bone age correlated with ovarian (r = 0.447; P = 0.048), and uterine volume (r = 0.543; P = 0.013).

**Discussion**

A small number of clinical studies on the PCOS familial clustering have been performed over the last two decades. These segregation analyses of PCOS, focused on probands and their relatives, showed a high incidence of affected relatives suggesting a genetic component to its aetiology (Cooper et al., 1968; Legro et al., 1995).

In the present study a strong association between maternal PCOS and US polycystic ovaries in prepubertal offspring has been evidenced supporting the hypothesis of a genetic transmission of the syndrome.

Assessment of ovarian morphology by means of ultrasound is currently employed as a substitute for histological examination in the diagnosis of PCO. It has been shown that anatomical structure of the pelvic organs could not be adequately assessed with a transabdominal approach in as many as 42% of cases (i.e. obesity, limited resolution of low-frequency transducers, troublesome full bladder technique and dilated bowel loops) (Hull, 1989; Pache et al., 1992) and that transvaginal US appears to provide a more accurate ovarian picture (Takahashi et al., 1993). Although we did not perform the transvaginal procedure in our girls, owing to their ages and virginal status, we visualised the uterine structure in 100%, both ovaries in 92% and at least one ovary in 100% of the cases. From this we derived that, by using the transabdominal approach, the percentage of visualization of prepubertal pelvic organs is basically dependent on the operator’s skill and the quality of the US machine.

In daughters of PCOS probands, the US polycystic ovary prevalence was of 93% and evidently exceeded either the 6% (Bridges et al., 1993) in 6 year old children found in 6 years and the 22–25% found in healthy adult women (Polson et al., 1988). In the present study we observed that patients of the control group presented a homogeneous echo-structure of the ovaries, an ovarian volume in the normal range of the prepubertal stage (Bridges et al., 1993; Buzi et al., 1998) and no stromal vascularization. On the other hand, girls with polycystic appearance of the ovaries were characterized by increased ovarian volumes (similar to those of patients at Tanner stage B2) (Bridges et al., 1993; Buzi et al., 1998), increased echogenic stroma, and a high number of small sized subcortical follicles. Furthermore, specific colour Doppler changes in ovarian vascularization (high percentage of intraovarian vessel visualization and low PI values) occurred at the level of the intraovarian arteries.

The above data were associated with circulating hormone levels in the normal range of prepubertal girls, an advanced bone age and a slightly increased hair distribution.

We previously reported that the typical vascular modification occurs in adult PCOS, that colour Doppler analysis has a high diagnostic value and that elevated plasma levels may be responsible for increased stromal vascularization (Battaglia et al., 1995). Our data, with the gonadotrophins in the prepubertal range, seem to not confirm, in childhood, the direct and prominent role of high LH plasma levels in the determination of those structural, vascular and hormonal modifications expressed in adult PCOS. In prepubertal girls alternative

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**Table III.** GnRH test in those PCOS offspring (Group I; n = 3) and controls (Group II; n = 4) who presented a basal LH value >0.3 IU/l

<table>
<thead>
<tr>
<th>Group I</th>
<th>Group II</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH peak (mUI/ml)</td>
<td>2.5 ± 1.7</td>
<td>2.3 ± 2.2</td>
</tr>
<tr>
<td>FSH peak (mUI/ml)</td>
<td>7.7 ± 2.3</td>
<td>8.2 ± 6.5</td>
</tr>
<tr>
<td>LH/FSH</td>
<td>0.37 ± 0.15</td>
<td>0.31 ± 0.26</td>
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</table>

NS = not significant.

**Table IV.** Main US and colour Doppler data in PCOS offspring (Group I; n = 15) and controls (Group II; n = 10)

<table>
<thead>
<tr>
<th>Group I</th>
<th>Group II</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine volume (ml)</td>
<td>3.65 ± 1.81</td>
<td>1.73 ± 1.1</td>
</tr>
<tr>
<td>Ovarian volume (ml)</td>
<td>2.76 ± 1.21</td>
<td>0.87 ± 0.46</td>
</tr>
<tr>
<td>Subcapsular follicles (n)</td>
<td>6.36 ± 2.2</td>
<td>0.75 ± 0.92</td>
</tr>
<tr>
<td>Stromal score (%)</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>–</td>
</tr>
<tr>
<td>Uterine artery (PI)</td>
<td>3.16 ± 0.74</td>
<td>4.23 ± 1.01</td>
</tr>
<tr>
<td>Ovarian stromal artery (PI)</td>
<td>1.62 ± 0.33</td>
<td>–</td>
</tr>
<tr>
<td>Visualization (%)</td>
<td>93</td>
<td>–</td>
</tr>
</tbody>
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NC = not calculated.
mechanisms must be sought to explain hyperandrogenism, morpho-structural ovarian modifications and increased ovarian vascularization.

In the present study, although none of the studied girls showed any evidence of hirsutism (Ferriman–Gallwey score >8), the offspring of patients with PCOS demonstrated a relatively high incidence of premature pubarche (26%) with pubic hair limited to labia majora and, in almost half (7/15), a slight increased hair distribution on the distal part of both arms and legs. The different hair distribution among PCO-like girls may be related to specific congenital sensitivity of the pilosebaceous unit to circulating androgens and/or to an enhanced peripheral conversion of testosterone to dihydrotestosterone by 5α-reductase (Ehrmann and Rosenfield, 1990).

The supposed increased sensitivity of target tissues to low levels of circulating androgens allows us to speculate that, in the absence of increased circulating estrogen concentrations, the growth spurt and skeletal maturation may be positively influenced, as demonstrated in PCOS offspring, by an increased height and a more advanced bone age than in matched controls (Ibanez et al., 1992).

On the basis of the data presented, we postulated that PCO in childhood may be considered a sign of a genetic predisposition to PCOS and that environmental factors (mainly nutritional) may express the adult clinical and biochemical predisposition to PCOS and that environmental factors (mainly nutritional) may express the adult clinical and biochemical manifestations of PCOS. In this case, in agreement with a previously published study (Homburg et al., 1996), we think that an ‘...early therapeutic intervention will not only temporarily alleviate the symptoms but will place the progress of the syndrome on hold. This would appear to be beneficial for future fertility prospects and possible delay or prevention of long-term sequelae...’.

References


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