The frequency of late-onset 21-hydroxylase and 11β-hydroxylase deficiency in women with polycystic ovary syndrome

Yılmaz Şahin and Fahrettin Keleştimur
Departments of Obstetrics and Gynecology, and Endocrinology, Erciyes University, Faculty of Medicine, Kayseri, Turkey
(Correspondence should be addressed to Y Şahin, Department of Obstetrics and Gynecology, Erciyes University, Faculty of Medicine, 38039 Kayseri, Turkey)

Abstract
Objective: To determine the frequency of late-onset adrenal hyperplasia (LOCAH) due to 21-hydroxylase (21-OH) and 11β-hydroxylase (11β-OH) deficiency in women with clinical and biochemical features of polycystic ovary syndrome (PCOS).

Design: Eighty-three consecutively selected women with PCOS and eighteen normal women were included in the study.

Methods: Ultrasound, clinical and hormonal parameters were used to define PCOS. Basal FSH, LH, testosterone, free testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEA-S), sex hormone-binding globulin (SHBG) and cortisol levels were measured. Serum 17-hydroxyprogesterone (17-OHP) and 11-deoxycortisol (11-DOC) levels were also measured before, 30 and 60 min after a single bolus injection of 0.25 mg ACTH (1–24) at 0900 h during the mid-follicular phase of the cycle. ACTH-stimulated 17-OHP levels > 30 nmol/l were considered as the criteria of 21-OH deficiency. The diagnosis of 11β-OH deficiency was made if the adrenal 11-DOC response to ACTH stimulation exceeded threefold the 95th percentile of controls.

Results: Basal serum testosterone, free testosterone, androstenedione, DHEA-S, cortisol and 11-DOC levels were significantly higher in PCOS than in control subjects. ACTH-stimulated 17-OHP (P < 0.05) and 11-DOC (P < 0.0005) levels were found to be significantly higher in patients with PCOS than in controls.

Seven (8.4%) patients had an 11-DOC response to ACTH higher than threefold the 95th percentile of controls, while no patients showed evidence of 21-OH deficiency.

Conclusions: We have found that 8.4% of the women with clinical and biochemical features of PCOS could be presumed to have 11β-OH deficiency. No patients among the women with PCOS showed evidence of 21-OH deficiency. 11β-OH deficiency is unexpectedly more common than 21-OH deficiency in women with PCOS.

Introduction
Increased androgen production is the biochemical hallmark of polycystic ovary syndrome (PCOS), yet the relative contributions of the adrenal cortex and ovary to the over-production of androgen remain unclear. Hyperandrogenemia is central to the pathogenesis of the morphologic changes in the ovaries and the clinical syndrome. Any disorder causing very high intra-ovarian androgen levels will cause follicular maturation arrest, increased rates of follicular atresia, increased interstitial tissue in the stroma of the ovaries, and chronic anovulation. The expanded stroma produces more androgen even when the serum luteinizing hormone (LH) concentrations are normal (1–4). In approximately 10% of PCOS patients, PCOS may develop in association with conditions with clearly defined adrenal androgen overproduction (5), e.g. congenital adrenal hyperplasia (6), androgen-secreting adrenal tumors (7), Cushing’s syndrome (2), and insulin-resistant states (8) and acromegaly (9).

Recent studies suggest that up to 50% of women with PCOS show evidence of adrenal androgen overproduction (10–13). Late-onset (LO) 21-hydroxylase (21-OH) deficiency can present with clinical and biochemical features which are indistinguishable from PCOS, including raised LH levels and polycystic ovaries (14). Animals given adrenal androgens develop a disorder equivalent to PCOS (15). PCOS may develop as a consequence of excessive adrenal androgen secretion in at least some patients (16, 17). Polycystic ovaries have been noted on sonography moderately frequently in patients with LO 21-OH deficiency (6, 18), 11β-hydroxylase (11β-OH) deficiency (19) and 3β-hydroxysteroid dehydrogenase deficiency (20).

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Cobin et al. (21) reported that the frequency of 21-OH deficiency in 100 patients with clinical PCOS was 1%. Benjamin et al. (22) found the prevalence of the LO 21-OH deficiency to be 19% in 100 consecutive women presenting with the classic clinical features of PCOS. To our knowledge, the clinical prevalence of 11β-OH deficiency among PCOS women has not been reported. Therefore, we investigated the frequency of late-onset congenital adrenal hyperplasia (LOCAH) due to 21-OH and 11β-OH deficiency in a relatively large number of women with clinical and biochemical features of PCOS.

**Materials and methods**

The study protocol was approved by the Ethical Committee of Erciyes University Medical School, and all the women gave informed consent. One hundred and one women were enrolled in the study. Eighty-three women with PCOS (aged 16 to 36 years) were consecutively selected for study from our outpatient clinic population. The diagnosis of PCOS was made by the presence of polycystic ovaries on pelvic ultrasound examination combined with three or more of the following criteria: oligo/amenorrhea, hyperandrogenemia, hirsutism, and a serum luteinizing hormone (LH) : follicle-stimulating hormone (FSH) ratio >2. Of the patients, 30% were eumenorrheic (intermenstrual interval between 21 and 35 days), 65% were oligomenorrheic (intermenstrual interval >35 days) and 5% were amenorrheic (no menstrual period in more than 6 months). All patients had hyperandrogenemia (serum free testosterone >11.1 pmol/l, normal range 2.8 to 11.1 pmol/l). Seventy-six (92%) patients had hirsutism (modified Ferriman-Gallwey score ≥8) (23) and thirty-three (40%) women had an elevated ratio of serum LH to serum FSH (LH:FSH >2). Cushing’s syndrome and androgen secreting tumors were excluded by appropriate tests including dexamethasone suppression test. Pelvic ultrasound examinations were performed by the same investigator (Y S) using a 3.5 MHz abdominal transducer or a 6.5 MHz vaginal endoprobe (Hitachi, EUB 450, Japan). The ultrasound diagnosis of polycystic ovary was made by the presence of 10 or more cysts 2–10 mm in diameter, arranged around a dense stroma or scattered throughout an increased amount of stroma (24, 25). All sonograms were obtained early in the cycle.

The control group consisted of 18 normal women similar in age (aged 17 to 32 years) and body mass index (BMI: kg/m²) to the subjects with PCOS. All had regular menses every 26 to 32 days, no hyperandrogenemia, and no evidence of hirsutism. None had polycystic ovaries on ultrasound examination. None of the subjects in the PCOS or control groups had received any hormonal medication for at least 8 weeks before the study. A total of 101 women was studied, each in the mid-follicular phase (day 5 to 9) of her cycle in controls and in patients with either regular cycles or oligomenorrhea, or in the event that the patient was amenorrheic, when the serum progesterone level was less than 8.0 nmol/l (2.5 ng/ml).

Basal blood samples were obtained between 0800 and 0900 h. The following hormones were measured: FSH, LH, testosterone, free testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEA-S), sex hormone-binding globulin (SHBG) and cortisol. The adrenocorticotropic (ACTH) stimulation test was performed in PCOS and control women by administration of a single i.v. bolus of 0.25 mg synthetic ACTH-(1–24) (Synacthen, Ciba, Basel, Switzerland) at 0800 h. Venous blood was withdrawn through an indwelling catheter in the mid-follicular phase at 0, 30 and 60 min for determination of serum 17-hydroxyprogesterone (17-OHP) and 11-deoxycortisol (11-DOC). The serum samples were stored at −20°C until assayed. An ACTH-stimulated 17-OHP concentration >30 nmol/l was considered the criteria for LO 21-OH deficiency (26). The diagnosis of 11β-OH deficiency was made if the adrenal 11-DOC response to ACTH stimulation exceeded threefold the 95th percentile of controls (27).

Serum FSH, LH, testosterone, free testosterone, androstenedione, DHEA-S, 17-OHP (DPC, Los Angeles, CA, USA), cortisol and 11-DOC (ICN Biomedicals Inc. Costa Mesa, CA, USA) were measured by RIA, using commercial kits. SHBG (Orion Diagnostica, Espoo, Finland) was measured by immunoradiometric assay. The intra-assay and interassay precision coefficients of variation were, respectively, 3.2% and 8.4% for FSH; 6.8% and 7.9% for LH; 10% and 10.4% for testosterone; 4.3% and 5.5% for free testosterone; 8.3% and 9.2% for androstenedione; 3.9% and 7.0% for DHEA-S; 5.6% and 4.5% for 17-OHP; 5.8% and 6.5% for cortisol; 2.1% and 13.7% for 11-DOC; and 4.0% and 5.5% for SHBG. All samples from the same patients were assayed in the same assay. For statistical analysis Student’s t-test was used. A non parametric (Mann-Whitney U) test was also used for comparison of the Ferriman-Gallwey score between the patients and the controls. A P value of <0.05 was regarded as statistically significant.

**Results**

The clinical and basal hormonal characteristics of the patients and the control subjects are shown in Table 1. The women with PCOS and the control group did not differ in terms of age or BMI. The PCOS subjects had significantly higher hirsutism (Ferriman-Gallwey) scores (P<0.001), basal serum testosterone (P<0.0005), free testosterone (P<0.0005), androstenedione (P<0.0005), DHEA-S (P<0.005) and cortisol (P<0.025) concentrations than normal women. Basal serum SHBG concentration was significantly lower in the PCOS group compared with the controls (P<0.0005).

The mean 17-OHP responses to ACTH in the PCOS and control subjects are shown in Table 2. The mean
basal 17-OHP level in women with PCOS was slightly, but not significantly, higher than in the control group. 17-OHP responses to ACTH at 30 (P < 0.05) and 60 min (P < 0.02) were significantly higher in PCOS than in control women. The percentage increment in 17-OHP level has been found to be similar in PCOS and control women.

The mean 11-DOC responses to ACTH in PCOS and control subjects are shown in Table 3. The mean basal 11-DOC level and 11-DOC responses to ACTH at 30 and 60 min were significantly higher in PCOS than in control women (P < 0.0005). The percentage increment in 11-DOC level was significantly higher in PCOS women than in controls (P < 0.01). The 95th percentile for the 11-DOC response measured in the control women was 12.2 nmol/l. Seven patients (8.4%) had an 11-DOC response measurement greater than threefold the upper normal limit. The basal values of 11-DOC in 76 PCOS patients unaffected by 11 β-OH deficiency were significantly higher than those of normal subjects (12.74 ± 0.69 nmol/l vs 7.88 ± 0.38 nmol/l, P < 0.0005). The clinical findings of some patients have been reported previously (28).

**Discussion**

PCOS and non-classical forms of congenital adrenal hyperplasia may sometimes be indistinguishable by sonography and clinical presentation. There is also an overlap in the clinical presentation of LOCAH due to 21-OH deficiency, and of PCOS. Hirsutism, acne, menstrual abnormalities and infertility are the clinical manifestations of both disorders. In addition, sonographic abnormalities of the ovaries may be similar in women with PCOS and women with adrenal enzyme defects (29, 30). Although the classical laboratory findings in PCOS are an elevated level of serum LH and an elevated LH:FSH ratio, this can also be found in LOCAH (17, 31, 32) and approximately 60% of patients with PCOS do not have this inappropriate gonadotropin secretion (33, 34). Emans et al. (31) have reported a patient with LOCAH diagnosed by ACTH testing and HLA studies, an elevated LH level, and POC by laparoscopy. Dewailly et al. (17) have found an elevated LH: FSH ratio in 23% of patients with LO 21-OH deficiency. In many patients with PCOS, dexamethasone treatment is associated with a frequency of ovulation, and can reverse symptoms of hyperandrogenism (5, 30). The clinical feature suggesting PCOS was identified in 39% of females with diagnosis of LO 21-OH deficiency (17). Hague et al. (6) found polycystic ovaries in 83% of 36 adult patients with congenital adrenal hyperplasia due to 21-OH deficiency. This proportion is significantly higher than the 22% found in a population of normal women (35).

The prevalence of LO 21-OH deficiency largely depends on the ethnic composition of the studied

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**Table 1** Clinical and basal hormonal characteristics of PCOS and control subjects. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>PCOS group (n = 83)</th>
<th>Control group (n = 18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23.86 ± 0.67</td>
<td>26.11 ± 0.88</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.07 ± 0.80</td>
<td>24.15 ± 0.84</td>
<td>NS</td>
</tr>
<tr>
<td>Ferriman-Gallwey score</td>
<td>16.37 ± 0.74</td>
<td>2.95 ± 0.24</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>2.99 ± 0.12</td>
<td>1.00 ± 0.06</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>Free testosterone (pmol/l)</td>
<td>20.00 ± 0.87</td>
<td>7.84 ± 0.45</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)</td>
<td>15.68 ± 0.91</td>
<td>8.14 ± 0.42</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>DHEA-S (μmol/l)</td>
<td>7.50 ± 0.36</td>
<td>5.18 ± 0.39</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>33.35 ± 1.91</td>
<td>64.52 ± 4.03</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>590.62 ± 47.85</td>
<td>355.19 ± 21.75</td>
<td>&lt; 0.02</td>
</tr>
</tbody>
</table>

NS, not significant.

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**Table 2** 17-OHP (nmol/l) response 30 and 60 min after ACTH administration in PCOS and control subjects. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>PCOS group (n = 83)</th>
<th>Control group (n = 18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>5.55 ± 0.35</td>
<td>4.44 ± 0.41</td>
</tr>
<tr>
<td>30 min</td>
<td>9.41 ± 0.45</td>
<td>7.74 ± 0.54</td>
</tr>
<tr>
<td>60 min</td>
<td>10.20 ± 0.45</td>
<td>8.28 ± 0.53</td>
</tr>
<tr>
<td>Peak</td>
<td>10.71 ± 0.46</td>
<td>8.66 ± 0.51</td>
</tr>
<tr>
<td>Percent increment*</td>
<td>120.5 ± 11.06</td>
<td>133.34 ± 30.81</td>
</tr>
</tbody>
</table>

* Percent increment is percentage difference between the peak and the basal values.

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**Table 3** 11-DOC (nmol/l) response 30 and 60 min after ACTH administration in PCOS and control subjects. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>PCOS group (n = 83)</th>
<th>Control group (n = 18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>13.68 ± 0.80</td>
<td>7.88 ± 0.38</td>
</tr>
<tr>
<td>30 min</td>
<td>18.66 ± 0.84</td>
<td>9.75 ± 0.38</td>
</tr>
<tr>
<td>60 min</td>
<td>20.60 ± 0.99</td>
<td>9.84 ± 0.38</td>
</tr>
<tr>
<td>Peak</td>
<td>23.20 ± 0.96</td>
<td>10.48 ± 0.35</td>
</tr>
<tr>
<td>Percent increment*</td>
<td>97.48 ± 11.06</td>
<td>37.92 ± 5.52</td>
</tr>
</tbody>
</table>

* Percent increment is percentage difference between the peak and the basal values.
populations. Speiser et al. (36) found that the incidence of LO 21-OH deficiency ranges from 3.7% of Ashkenazy Jews to 1.9% of Hispanics to almost zero in most Caucasian populations. In a large study of a Mediterranean population Carmina (37) recently reported that 3.6% of 692 hirsute women had LO 21-OH deficiency. Although the frequency of 21-OH deficiency in patients with clinical PCOS has been reported in some studies (21, 22), the clinical frequency of 11β-OH deficiency among women with PCOS has not been reported. Very few data exist regarding prevalence of LO 11β-OH deficiency in the patients with hirsutism. Thus, we carried out the present study to look at the prevalence of 11β-OH deficiency among PCOS women. Using a stringent diagnostic criterion, we found that 8.4% of PCOS women had a presumed 11β-OH deficiency. As far as we know there is no generally accepted diagnostic criterion for diagnosing LOCAH due to 11β-OH deficiency. It would be necessary to genotype the patients with presumed LOCAH due to 11β-OH deficiency to be certain of the diagnosis of this disorder. But, at least to our knowledge, so far no mutation has been identified in the CYP11B1 gene of individuals with LOCAH due to 11β-OH deficiency. The prevalence of 11β-OH deficiency has been reported to be between 0.8 and 8% in different studies carried out in women with hirsutism or hyperandrogenism (38, 39). Carmina (37) recently found that only 0.6% of the hirsute women had LO 11β-OH deficiency. In our previous study the prevalence of LO 11β-OH deficiency was found to be 6.5% among hirsute patients (28). We suggest that different criteria used for the diagnosis of LOCAH due to 11β-OH deficiency and different ethnic populations in which the study was carried out might explain these discrepant results. So, we think that the prevalence of LO 11β-OH deficiency among Turkish PCOS women is relatively high.

We have also analyzed the data after excluding the 7 patients with 11β-OH deficiency and we have found that the levels of basal 11-DOC were still higher in the PCOS group compared with the controls. We have previously reported that there is adrenal hyperactivity among the patients with hyperandrogenemia when compared with normal women (28, 40). Increased adrenal androgen secretion is a common finding in hyperandrogenic women and it is more severe in women with PCOS (41). 17-Hydroxylase and 17, 20 lyase activities are common in the adrenal and ovary and they can be accounted for by cytochrome P450c17. Therefore, abnormal regulation of P450c17 activities in the adrenal gland accounts for most adrenal hyperandrogenism (42). Another cause of adrenal hyperandrogenism in PCOS is adrenal androgen hyper-responsiveness to ACTH, which may be ovarian induced (43). It has been reported that the association of 11-DOC and 17-OHP hyper-responses has raised the possibility that 11β-OH is inhibited secondarily by high intra-adrenal androgen concentrations arising from heterozygosity for 21-OH deficiency (42). Since we have not found a patient with 21-OH deficiency among the PCOS women, the inhibition of 11β-OH secondarily by high intra-adrenal androgen concentrations may not be valid. We think that PCOS is characterized by increased adrenal androgen secretion as well as ovarian hyperandrogenism and a general adrenocortical hyperactivity. Whether 11-DOC hyper-responsiveness to ACTH stimulation seen in the patients with PCOS reflects the ‘upper end’ of a distribution curve in a group with obvious adrenal hyperactivity or is really due to LO 11β-OH deficiency remains to be established.

In conclusion, the present study shows that LOCAH is not a common disorder among PCOS women at least in this population. Unexpectedly, we have found that 11β-OH deficiency is more common than 21-OH deficiency in PCOS.

References

12. Lobo RA. The role of the adrenal in polycystic ovary syndrome. Seminiers in Reproductive Endocrinology 1984 2 251–262.


