Detection of late-onset adrenal hyperplasia in girls with peripubertal virilization

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Abstract. We investigated the value of serum levels of adrenal steroids (dehydroepiandrosterone sulphate, testosterone, 17-hydroxyprogesterone, cortisol) in the identification in peripubertal females with late-onset congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. Among 68 females (age 3–18 years) with virilization in childhood, peripubertally or postpubertally, we selected 21 girls for an ACTH test by measurement of basal blood-spot or serum 17-hydroxyprogesterone (17-OHP) levels. Eight of 21 patients had supranormal post-ACTH serum 17-OHP concentration (57–153 nmol/l) with low normal cortisol concentration. All of them had supranormal basal and post-ACTH 17-OHP to cortisol ratios. These data show a relatively high incidence (about 12%) of mild 21-hydroxylase deficiency among prepubertal and adolescent girls with virilization. It is concluded that the first step in the investigation of peripubertally virilized girls should be the determination of serum 17-OHP and cortisol. Patients with basal morning 17-OHP concentration and 17-OHP to cortisol ratio above reference range should be given an ACTH test.

An increasing number of females with virilization are referred for endocrinological consultation. The evaluation in these females centres on defining the source of the androgen excess in order to select the best therapy.

Androgen abnormalities revert by adrenal suppression in some hirsute patients (Abraham et al. 1981; Loughlin et al. 1986; Moore et al. 1983; Rittmaster et al. 1985; Sarris et al. 1978). Therefore therapy of hirsutism with menstrual disorders by glucocorticoids has become common. However, it is important to identify patients with adrenal hyperplasia, who do need long-term glucocorticoid therapy, and to avoid harming by such a therapy patients without enzyme deficiency.

We have evaluated the measurement of adrenal steroids and their ratios in detection of partial deficiency of 21-hydroxylase at peripubertal age.

Patients and Methods

Subjects
Our series consists of 68 females who were born with normal genitalia but referred for investigation at age 3–18 years for at least one of the following disorders: mild clitoral enlargement, precocious pubarche, progressive hirsutism, or severe acne. Measurement of 17-hydroxyprogesterone (17-OHP) in capillary blood spotted on filter paper or in serum obtained in the morning was used for screening for mild 21-hydroxylase deficiency.

Among the 68 patients, 21 had blood-spot 17-OHP levels above 50 nmol/l, and/or serum 17-OHP concentrations above 6 nmol/l. These 21 girls, 6 to 18 years of age, were selected for an ACTH test.
Venous blood for the measurement of serum basal hormone levels was drawn at 07.00–09.00 h. Menstruating patients were studied during the midfollicular phase (days 5–10). For the ACTH test we gave a single iv bolus of 0.25 mg (N = 15) or an im injection of 1.0 mg (N = 6) tetracosactide (Synacthen® Ciba). Blood was again drawn 60 min or 6 h, respectively, after the injection.

For comparison we investigated with the same procedure healthy females (18–24 years, N = 8), obligate heterozygotes for 21-hydroxylase deficiency (24–36 years, N = 13), and patients with classic 21-hydroxylase deficiency (1–14 years, N = 1).

Assays
The blood-spot 17-OHP (Sólyom et al. 1981), serum 17-OHP, testosterone and dehydroepiandrosterone sulphate (DHA-S) were measured by nonchromatographic radioimmunoassays (Sólyom 1984; Bodrogi & Fehér 1980; Fehér et al. 1985), cortisol by a nonchromatographic radiotriasinassay (Zoltán & Sólyom 1980), and 11-deoxycortisol after paper chromatographic separation by radioimmunoassay (Vecsei 1974, kindly performed by Dr E. Gláz, Budapest). In serum steroid measurements, the intra-assay and inter-assay coefficient of variation for low, medium, and high pools did not exceed 6% and 11%, respectively.

Our normal values for basal concentrations in girls are: 17-OHP, 1–10 nmol/l; testosterone, prepubertally 0.2–0.7 nmol/l and postpubertally 0.7–1.7 nmol/l; DHA-S, prepubertally 0.2–1.3 µmol/l and postpubertally 3–10 µmol/l; cortisol (at 07.00–09.00 h), 220–420 nmol/l.

For testing normality of the frequency distribution of serum 17-OHP/cortisol ratios we used the method of rankits (Sokal & Rohlf 1969).

Results
Eleven of 21 patients had supranormal basal serum 17-OHP concentration. However, the values of the 21 patients showed a continuous distribution. Post-ACTH serum 17-OHP levels distinguished two subgroups. Thirteen patients responded normally (post-ACTH levels < 30 nmol/l). Eight patients gave supranormal response (57–153 nmol/l), and they all had supranormal basal levels, too (Fig. 1).

The cortisol response to ACTH was lower (P < 0.01) in the eight patients with supranormal post-ACTH 17-OHP levels (basal 235 ± 78 nmol/l, mean ± SD, post-ACTH 378 ± 84 nmol/l) than in the 13 patients with normal post-ACTH 17-OHP concentrations (280 ± 137 and 664 ± 227 nmol/l, respectively).

The eight patients with supranormal 17-OHP and low cortisol response were considered to have late-onset adrenal hyperplasia (LO-CAH) owing to 21-hydroxylase deficiency. 11-beta-hydroxylase deficiency was excluded by normal serum levels of 11-deoxycortisol.

The clinical data of the 21 patients studied are presented in Table 1.

Basal (+) and post-ACTH (+) serum 17-OHP concentrations in different groups of subjects: C, controls; HET, obligate heterozygotes for 21-hydroxylase deficiency; HIRS, peripubertally virilized females without 21-hydroxylase deficiency; LO-CAH, late-onset 21-hydroxylase deficiency; CAH, classic 21-hydroxylase deficiency.
Table 1.
Clinical data in female patients virilized during the peripubertal period.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age at investigation (years)</th>
<th>Age at menarche (years)</th>
<th>Presenting symptoms</th>
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Patients without 21-hydroxylase deficiency

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Patients with the late-onset type of 21-hydroxylase deficiency

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Testosterone (nmol/l)  
DHA-S (μmol/l)

Fig. 2.
Serum testosterone and DHA-S concentrations in peripubertally virilized female patients with late-onset 21-hydroxylase deficiency (▲, basal; Δ, post-ACTH) and without (●, basal; ○, post-ACTH). Shaded area represents age-related normal basal values.
There are few estimates of the incidence of LO-CAH among such girls. Our 21 patients were selected from 68 female patients on the basis of slightly supranormal basal blood-spot or serum 17-OHP levels. Our data suggest a higher incidence of mild 21-hydroxylase deficiency among peripubertally virilized female patients (approximately 12%) than among nonselected adult hirsute women (Chetkowski et al. 1984; Chrousos et al. 1982; Conway et al. 1983; Kuttenn et al. 1985; Lobo & Goebelsmann 1980; Lucky et al. 1986; Moore et al. 1983).

The basal serum and blood-spot 17-OHP levels, as well as basal and post-ACTH testosterone and DHA-S levels in patients with mild 21-hydroxylase deficiency overlap those in other virilized females, which is in contrast to classic congenital adrenal hyperplasia (Bouchard et al. 1981; Kuttenn et al. 1985; Lobo & Goebelsmann 1980; New et al. 1983; Scaroni et al. 1986).

Two of our patients with abnormally high serum DHA-S levels should be investigated for a mild form of 3-beta-hydroxysteroid-dehydrogenase deficiency (Pang et al. 1985). Five of our eight LO-CAH patients had supranormal basal serum levels of testosterone, explaining the virilization.

Our observation confirms previous reports (Gourmelen et al. 1979; New et al. 1979; Rosenwaks et al. 1979) of post-ACTH serum 17-OHP level as the most sensitive indicator of 21-hydroxylase deficiency. However, mild virilization of females is so common that we recommend the ACTH test only in patients with progressive virilization and supranormal basal serum 17-OHP levels. This is in agreement with the proposal of Dewailly et al. (1986). Supranormal post-ACTH serum 17-OHP levels confirm the diagnosis of late-onset steroid-21-hydroxylase deficiency.

Owing to diurnal rhythm, blood 17-OHP levels can be misleadingly low in the afternoon both in late-onset and classic 21-hydroxylase deficiency (Chetkowski et al. 1986; Sólyom 1984). However, our observation that all patients with a 21-hydroxylase deficiency had abnormally high 17-OHP to cortisol ratios also in base-line conditions can be of help for selecting virilized female patients for ACTH stimulation test.

We believe that this approach for screening peripubertally virilized female patients for late-onset congenital adrenal hyperplasia owing to 21-hydroxylase deficiency should identify all patients with this disorder.
Acknowledgments

The antisera were donated by Dr T. Fehér and Dr L. Bodrogi, Budapest, and this is gratefully acknowledged. We thank Dr J. Egyed, Dr G. Károlyi and Dr R. Koós for referring some of the patients, and Mrs G. Kirchknopf for excellent technical assistance.

References


Received November 21st, 1986.
Accepted March 20th, 1987.

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