The value of serum levels of dehydroepiandrosterone sulfate as a screening test for late-onset congenital adrenal hyperplasia

O valor do sulfato de desidroepiandrosterona sérico como teste de triagem das formas de início tardio da hiperplasia congênita da supra-renal

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ABSTRACT

Objective: To evaluate the use of serum level of dehydroepiandrosterone sulfate as a screening test for late-onset congenital adrenal hyperplasia. Methods: Fourteen hirsute women with elevated serum levels of dehydroepiandrosterone sulfate, 17 hirsute women with normal serum levels of dehydroepiandrosterone sulfate, and 12 non-hirsute ovulatory women were selected. Blood samples were collected before and 1 hour after the adrenocorticotropic hormone stimulation test. Serum levels of dehydroepiandrosterone sulfate were measured before the adrenocorticotropic hormone stimulation test. Serum levels of 17-hydroxyprogesterone, compound S, 17-hydroxypregnenolone, dehydroepiandrosterone, androstenedione, and cortisol were measured 1 hour after the adrenocorticotropic hormone stimulation test. Results: Two hirsute women with elevated serum levels of dehydroepiandrosterone sulfate satisfied the criterion for late-onset congenital adrenal hyperplasia due to 21-hydroxylase deficiency, i.e., a serum level of 17-hydroxyprogesterone after the adrenocorticotropic hormone stimulation test >1000 ng/dL. No hirsute woman had 11b-hydroxylase or 3b-hydroxysteroid dehydrogenase deficiency. Conclusions: Serum level of dehydroepiandrosterone sulfate is useful as a screening test for late-onset congenital adrenal hyperplasia. An elevated serum level of dehydroepiandrosterone sulfate does not necessarily mean an adrenal enzymatic defect, whereas a normal value excludes the possibility of late-onset congenital adrenal hyperplasia.

Keywords: Adrenal hyperplasia, congenital; Dehydroepiandrosterone; Hirsutism; Steroid 21-hydroxylase; Steroid 11-beta-hydroxylase; 3-Hydroxysteroid dehydrogenases

RESUMO

INTRODUCTION

A relative deficiency in cortisol (F) production secondary to a congenital enzymatic deficiency is common to all forms of congenital adrenal hyperplasia (CAH). This deficiency leads to an increased adrenocorticotropic hormone (ACTH) release. The serum levels of the precursor hormones, which are substrates of the deficient enzymes, are elevated. The location of the block determines which precursor is elevated, whereas the intensity of the enzymatic deficiency determines the extent to which the individual is affected.

The adrenal enzymatic deficiencies that cause hyperandrogenism are 21-hydroxylase (21OH), 11β-hydroxylase (11βOH), and 3β-hydroxysteroid dehydrogenase (3βHSD) deficiencies. All are autosomal recessive disorders.

The late-onset CAH (LOCAH) due to 21OH deficiency is an allelic variant of the classical form. The signs and symptoms in women appear after puberty causing different degrees of hirsutism and acne. Additionally, they can present irregular menses simulating the polycystic ovary syndrome (POS). It is questionable whether the LOCAH due to 11βOH and 3βHSD deficiencies exists. Diagnosis of the enzymatic deficiency is made by means of serum quantification of the precursor hormone after the ACTH stimulation test.

Dehydroepiandrosterone sulfate (DHEAS) is the most abundant adrenal androgen in blood, and the fact of being an androgen secreted mostly by the adrenal cortex has made its serum measurement very important. Another important characteristic is the fact of being secreted in its conjugated form. It has been demonstrated that the serum concentration of DHEAS, different from dehydroepiandrosterone (DHEA) or F, does not vary during the day or during menstrual cycle phases, and does not correlate with body surface area or body mass index (BMI). Dehydroepiandrosterone sulfate has a long half-life and its metabolic clearance rate is merely 8 to 16 liters per day. An elevated serum level of DHEAS in the virilizing adrenal syndromes indicates that DHEAS is secreted in excess together with other adrenal androgens with biological potency. Thus, it could be useful in the differential diagnosis of hyperandrogenic conditions, even without knowing the biological role of DHEAS.

OBJECTIVE

To evaluate whether the serum level of DHEAS in hirsute women could be useful as a screening test for LOCAH and whether an elevated serum level of DHEAS in hirsute women would mean an intrinsic adrenal disorder.

METHODS

Subjects. Group A consisted of 14 hirsute women with serum levels of DHEAS >400 µg/dL, aged 17 to 44 years (mean, 24.6 years) with regular cycles or oligomenorrhea seen at the Division of Gynecological Endocrinology, Department of Gynecology, Escola Paulista de Medicina – Universidade Federal de São Paulo. The degree of hirsutism was evaluated by the semi-quantitative score of Ferriman and Gallwey. The women were considered hirsute when they presented a score >8. The scores for group A ranged from 8 to 17 (mean 10.6). Body mass index ranged from 17.9 to 35.7 (mean 26.0). Group B consisted of 17 hirsute women with serum levels of DHEAS <400 µg/dL, aged 18 to 49 years (mean 26.9 years), with regular cycles or oligomenorrhea, with a hirsutism score ranging from 8 to 23 (mean 14.2) and BMI ranging from 19.2 to 33.3 (mean 23.6).

All hirsute women presented normal pelvic examinations. Pelvic ultrasonography was normal or presented enlarged and micropolycystic ovaries. A control group (group C) consisted of 12 ovulatory (luteal phase serum progesterone [P] >4 ng/mL) non-hirsute women aged 22 to 29 years (mean 25.8 years) with regular cycles or oligomenorrhea. None of the women studied had been taking any hormonal medication up to 6 months before the investigation.

The Ethical Committee in Research of Universidade Federal de São Paulo/Hospital São Paulo analyzed and approved the study project.

ACTH stimulation test and hormone assays. Venus blood was collected during the early follicular phase (between the 1st and 5th day). On the day before the test, each subject orally took 1 mg of dexamethasone at 10:00 PM. After an overnight fast, 0.25 mg of ACTH® (Cortrosyn®, Organon, São Paulo, SP) was injected as intravenous bolus between 8:00 and 10:00 A.M. Blood samples were obtained just before (T0) and 1 hour (T1) after ACTH injection. After centrifugation, serum was stored at -20°C until assayed.

Serum DHEAS was measured in T0. Serum 17α-hydroxyprogesterone (17OHP), 11-deoxycortisol or compound S (S), 17α-hydroxyprogrenolone (17Preg), DHEA, androstenedione (A) and F were measured in T1. Serum P was measured in control group during the
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...luteal phase in order to confirm the ovulatory cycle (P > 4 ng/mL).

The hormone assays were performed by radioimmunoassay using a commercial kit, Immuno Chem™, acquired from ICN Pharmaceuticals, Inc. (Costa Mesa, CA). Serum 17Preg measurement was taken after extraction in organic solvent and chromatography in a celite column, as recommended by the manufacturer. The interassay coefficients of variation for DHEAS, 17OHP, S, 17Preg, DHEA, A and F were 9.5%, 11.2%, 13.7%, 13.5%, 7.8%, 6.0%, and 8.2%, respectively.

The biochemical criterion adopted for the diagnosis of LOCAH due to 21OH deficiency was a serum level of 17OHP after the ACTH stimulation test >1000 ng/dL(14-15) and for the diagnosis of LOCAH due to 11bOH deficiency it was a serum level of S after the ACTH stimulation test of more than threefold the 95th percentile of control group(15-16). For the diagnosis of LOCAH due to 3bHSD deficiency, the hirsute women had to fulfill all the following criteria after the ACTH stimulation test, namely, serum levels of 17Preg and DHEA, ratios of 17Preg to 17OHP, DHEA to A and 17Preg to F more than 2 standard deviations above the mean of control group(6,17).

Sensitivity, specificity, positive predictive value, and negative predictive value of DHEAS were then calculated. Sensitivity is the proportion of true positives (women with LOCAH) that are correctly identified by the test (elevated serum level of DHEAS). Specificity is the proportion of true negatives (women without LOCAH) that are correctly identified by the test (normal serum level of DHEAS). Positive predictive value is the proportion of patients with positive test results (women with elevated serum level of DHEAS) who are correctly diagnosed (women with LOCAH). Negative predictive value is the proportion of patients with negative test results (women with normal serum level of DHEAS) who are correctly diagnosed (women without LOCAH).

RESULTS

Hormonal data after the ACTH stimulation test in normal group (group C) and hirsute women (group A and B) are depicted in table 1 and figure 1, respectively. Figure 2 shows the relationship between serum level of DHEAS and LOCAH in hirsute women.

Table 1. Mean serum levels of 17a-hydroxyprogesterone (17OHP) (ng/dL), compound S (S) (ng/dL), 17a-hydroxypregnenolone (17Preg) (ng/dL), dehydroepiandrosterone (DHEA) (ng/dL) and mean 17Preg:17OHP, DHEA:androstenedione (A), 17Preg:cortisol (F) ratios after ACTH stimulation test in normal women.

<table>
<thead>
<tr>
<th></th>
<th>17OHP</th>
<th>S</th>
<th>17Preg</th>
<th>DHEA</th>
<th>17Preg:17OHP</th>
<th>DHEA:A</th>
<th>17Preg:F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>144</td>
<td>334</td>
<td>700</td>
<td>1208</td>
<td>5.2</td>
<td>3.5</td>
<td>35.8</td>
</tr>
<tr>
<td>SD</td>
<td>37</td>
<td>129</td>
<td>355</td>
<td>443</td>
<td>3.2</td>
<td>1.5</td>
<td>20.9</td>
</tr>
</tbody>
</table>

Figure 1. Serum levels of 17a-hydroxyprogesterone (17OHP) (ng/dL), compound S (S) (ng/dL), 17a-hydroxypregnenolone (17Preg) (ng/dL), dehydroepiandrosterone (DHEA) (ng/dL) and 17Preg:17OHP, DHEA:androstenedione (A), 17Preg:cortisol (F) ratios after ACTH stimulation test in hirsute women with elevated serum level of dehydroepiandrosterone sulfate (DHEAS) (group A) and hirsute women with normal serum level of DHEAS (group B). The horizontal lines indicate the upper limits of normality.

Two women of group A presented 21OH deficiency. The serum level of 17OHP after the ACTH stimulation test was 4940 ng/dL in one woman and 3932 ng/dL in another. None of the patients presented 11OH deficiency, i.e., serum level of S after the ACTH stimulation test >1776 ng/dL. None of the patients fulfilled all the biochemical criteria for 3bHSD deficiency after the ACTH stimulation test, i.e., a serum level of 17Preg >1410 ng/dL, serum level of DHEA >2094 ng/dL, ratio of 17Preg to 17OHP >11.6, ratio of DHEA to A >6.5, and ratio of 17Preg to F >77.6.

The sensitivity of DHEAS serum level to detect LOCAH was 100%. The specificity of the test was 54%. The positive predictive value was 14%, the negative predictive value was 100%, and the ratio of positive predictive value to prevalence (pre-test probability) was 2.2 (figure 2).
DISCUSSION

Congenital adrenal hyperplasia constitutes an important cause of hyperandrogenism, and LOCAH due to 21OH deficiency is probably the most common autosomal recessive disorder in humans\(^{(18)}\).

Despite the advances in genetic studies, serum level determination of the substrates of the deficient enzymes after the ACTH stimulation test is still the main diagnostic means of the various forms of congenital adrenal hyperplasia\(^{(19)}\). However, the use of the ACTH stimulation test for every hirsute subject would be expensive and labored, especially to investigate the main causes of LOCAH. Oral dexamethasone suppression the night before the test causes a greater hormonal increment, although it does not alter the final result\(^{(20)}\). Its utilization, however, is controversial. We utilize it in order to have a greater uniformity in the results.

In our study, none of the 17 hirsute women with normal serum levels of DHEAS had LOCAH (negative predictive value of 100%). On the other hand, two of the 14 hirsute women with elevated serum levels of DHEAS had LOCAH (positive predictive value of 14%). Normal serum levels of DHEAS excluded the possibility of LOCAH. The ratio of positive predictive value to prevalence (pre-test probability) was 2.2. That means that if a hirsute woman has an elevated serum level of DHEAS, the probability that she has LOCAH will be 2.2-fold that of the general hirsute women population. The test is suitable to identify women who do not have LOCAH. On the other hand, the test fails to detect women who do have LOCAH as it is a fact that positive predictive value is low. Despite the limited application, these results show that the serum level of DHEAS is useful as a screening test because it excludes women who do not have LOCAH.

We did not find literature that showed positive or negative predictive values of DHEAS in order to detect the presence of LOCAH.

In our study, we did not find LOCAH deficiencies due to 11bOH or 3bHSD, maybe because they are very rare. Medical literature is very controversial on the existence of these disorders.

Some authors, using strict diagnostic criteria, excluded LOCAH due to a 3bHSD deficiency in hirsute women\(^{(21-22)}\). Other authors\(^{(23)}\) evaluated women with LOCAH due to a 3bHSD deficiency. They did not confirm the presence of a mutation in the type II 3bHSD gene encoding adrenal and gonadal 3bHSD enzyme. This finding strongly suggests that, unlike the classical form, LOCAH due to a 3bHSD deficiency would not be attributable to a genetic mutation of 3bHSD. Thus, it cannot be discarded that some cases of LOCAH due to a 3bHSD deficiency would be secondary to endogenous or environmental factors.

Marui et al.\(^{(24)}\), studying 14 hirsute women with elevated serum levels of 17Preg after the ACTH stimulation test, observed a greater hormonal increment. However, they did not confirm the presence of a mutation in the type II 3bHSD gene encoding adrenal and gonadal 3bHSD enzyme. This finding strongly suggests that, unlike the classical form, LOCAH due to a 3bHSD deficiency would not be attributable to a genetic mutation of 3bHSD. Thus, it cannot be discarded that some cases of LOCAH due to a 3bHSD deficiency would be secondary to endogenous or environmental factors.

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stimulation test, found mutation in the homozygous 3bHSD gene in only one virilized women who also had elevated serum level of DHEAS. They suggested that these women had the classical form of CAH, whose diagnosis was only made later.

Sakkal-Alkaddour et al.\(^{(17)}\) suggested that the hormonal criterion for LOCAH due to a 3bHSD deficiency might be a serum level of 17Preg after the ACTH stimulation test higher than 10 standard deviations above the normal mean value. Other authors\(^{(25)}\) suggested that this value might be higher than 21 standard deviations above the normal mean level.

It is accepted that a large proportion of hirsute women present an exaggerated S production after adrenal stimulation, probably due to adrenocortical hyperactivity and not an 11bOH deficiency\(^{(7)}\). When it is adopted as diagnostic criterion for 11bOH deficiency, the serum level of S after the ACTH stimulation test higher than threefold the 95th percentile of normal women, the prevalence of this disorder among hirsute women ranges from 0.8% to 6.5%\(^{(7,16)}\).

Another interesting datum in our casuistic was the absence of CAH in 86% of hirsute women who presented elevated serum level of DHEAS. Some authors try to explain this fact. The cause of adrenal abnormality in the absence of CAH is still controversial. One hypothesis would be the fact that elevated serum levels of estrogens and androgens modify the adrenal enzyme function, diminishing 21OH and 11bOH activities\(^{(26)}\). Besides that, 21OH deficiency produces an inhibition in 11bOH activity probably due to an elevated androgen production\(^{(25)}\). In our casuistic, we also found elevated serum levels of S after the ACTH stimulation test in one woman with LOCAH due to 21OH deficiency.

It’s clearly established that adrenal androgen production is abnormal in some patients with POS, although it is uncertain if this abnormality is involved in the development of the syndrome\(^{(28)}\). There is doubt as to whether a higher adrenal androgen production in women with POS could be due to an excessive adrenal stimulation by a supposed cortical androgen-stimulating hormone, which along with ACTH would probably coordinate adrenal androgen steroidogenesis, or whether this exaggerated production would occur as a consequence of increased cortisol clearance with compensatory hypersecretion of ACTH\(^{(28)}\). Another proposal to explain the adrenal androgen excess in women with POS is 17,20-lyase hyperactivity caused by ovarian steroids\(^{(29)}\).

Some authors observed a decrease in serum level of DHEAS after gonadotropin releasing hormone agonist administration. Causes mentioned for this reduction include an intrinsic abnormality of adrenal steroidogenesis, decreased availability of ovarian precursors, and changes in adrenal or hepatic sulfation\(^{(30)}\).

Siegel et al.\(^{(31)}\) tried to correlate serum level of DHEAS and adrenal response to the ACTH stimulation test in hirsute women. The authors concluded that the serum level of DHEAS was not useful to discriminate the different causes of increased androgen production, as it was clear that only 5 out of 19 hirsute women with presumed LOCAH had elevated serum level of DHEAS. However, the only woman with a supposed 11bOH deficiency had a serum level of S after the ACTH stimulation test equal to 336 ng/dL, which is incompatible with the disorder. The 5 women with a presumed 21OH deficiency had, in average, a serum level of 17OHP after the ACTH stimulation test equal to 381 ng/dL, which is also incompatible with LOCAH due to 21OH deficiency. The 13 women with supposed 3bHSD deficiencies did not satisfy the diagnostic criteria either.

CONCLUSIONS

In our study, we could exclude LOCAH when the serum level of DHEAS was normal. Although the positive predictive value of DHEAS is low and therefore a limiting factor, we recommend the use of serum measurements of DHEAS as a routine method for all hirsute women in order to identify the adrenal role in androgenic production.

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