New reliable biochemical marker for screening 21α-hydroxylase deficiency without index person among hirsute women in agreement with HLA-haplotypeing

A. Chryssikopoulos, I. Phocas, A. Sarandakou, E. Trakakis and D. Rizos
2nd Department of Obstetrics and Gynecology, University of Athens, Areteion Hospital, Athens, Greece

ABSTRACT. Late onset congenital adrenal hyperplasia due to 21α-hydroxylase deficiency (LO21OH def), as many other diseases, is the cause of hirsutism, menstrual disorders, infertility (PCO-like symptoms). We evaluated the reliability of a new biochemical marker for screening LO-21OH def in 47 women with PCO-like symptoms and 11 men, members of their families, comparing the results of separation using this new marker with those of HLA-haplotypeing in 21 members of the patient population. All subjects were stimulated with 0.25 mg synthetic ACTH iv. Serum progesterone (P), 17-hydroxyprogesterone (17-OHP) and cortisol (F) at 0, 15, 30, 45 and 60 min following ACTH administration were determined and the new marker, namely the difference between 60min and 0min of the ratio F/17-0HP [L1 F/17-0HP (60 min -0 min)] was calculated. According to the established biochemical criteria for the detection of LO-21OH def cases, (Gutai 30 min ≥12 ng/dl/min and 17-OHP 60 min ≥12 ng/ml for severe 21-OH def and Gutai 30 min<6.5 ng/dl/min and 17-OHP 60 min<5 ng/ml for “healthy” individuals regarding 21-OH def) two groups, A and B respectively, were separated from the patient population. In group A (n=8), with LO-21OH def, the new marker showed negative values in all cases, while in group B (n=9), without LO-21OH def, this marker was positive. The remaining subjects, depending on the results of the new marker were separated in 2 subgroups, C_neg (n=28), with negative values, composed, consequently, of members with 21-OH def and C_pos (n=13), with positive values, composed, consequently, of subjects with absence of LO-21OH def. HLA-typing was in agreement with the results of screening by the new marker, in 20 out of 21 cases, while there was only one false negative result. In conclusion, the proposed biochemical marker ΔF/17-OHP (60 min-0 min) seems to be a reliable parameter for the LO-21OH def detection among young women with PCO-like symptoms as well as males suspected for congenital adrenal hyperplasia.

INTRODUCTION

Impairment of the 21-hydroxylation (21α-hydroxylase deficiency, 21-OH def) is the most common autosomal, recessive, inherited, enzymatic deficiency (1), resulting in the syndrome of congenital adrenal hyperplasia (CAH) which may be presented either in the classical form for the newborns or the nonclassical form in older individuals of both sexes (late-onset CAH, LO-21OH def). The clinical phenotype of the females with LO-21OH def (hirsutism, menstrual irregularities and sterility-infertility) does not differ from that of patients with polycystic ovarian syndrome (PCO) (2, 4), idiopathic hirsutism (3, 4) or hyperinsulinemia (5). Additionally, the manifestation of clinical symptoms in all 4 cases usually at puberty makes the diagnosis even more complicated.

The incidence of 21-OH def among the hirsute women varies between 1.2% and 20.0% (1, 2, 4, 6-8). The disparity of these findings could be due to the variability of incidence of the 21-OH def-gene in different populations (9), the variability of protocols in relation to patient populations, and the lack of similarity of the patient populations in the different studies.

The accurate identification of patients with LO-21OH def is important for several reasons:

1. The treatment of LO-21OH def is specific: administration of corticosteroids. Oral contraceptives or cyproterone acetate (10) used for the treatment of hirsutism in patients with LO-21OH def are an inappropriate choice as primary therapy, since they...
do not remedy the source of the problem (decreased cortisol production).

2. The need of a life-time therapy in those patients presenting a blunt response of cortisol and in all cases in critical instances with increased stress (1).

3. The purpose of genetic counselling (11, 12), especially in populations with known high incidence of LO-21OH def such as the Askenase Jews (13), or Mediterranean people (14, 15) and "closed societies" (16).

4. The treatment of sterility presented in patients with LO-21OH def with PCO-like symptoms as well as of infertility (abortion rate about 30%) in LO-21OH def cases without menstrual irregularities and spontaneous conception (17).

Serum 17-hydroxyprogesterone (17-OHP) assay after ACTH administration has been considered by various researchers (3, 4, 18) to be the most sensitive biochemical diagnostic test for the identification of LO-21OH def cases. The abnormal levels of 17-OHP in response to ACTH among obligate CAH heterozygotes led several authors (1, 18-21) to the suggestion of numerous protocols for the detection of both severe form of LO-21 OH def and heterozygous carriers. In most protocols the biosynthetic precursors of cortisol (F) such as 17-OHP, alone or in combination with progesterone (P) have been used. In contrast, only scarcely the end product of adrenal steroid biosynthesis F (18, 22) or its relation to 17-OHP (17-OHP/F ratio) have also been included (23).

The aim of this study was to investigate the reliability of a new biochemical marker in which the relation of the end product F to the precursor 17-OHP during ACTH-test was estimated, namely the difference of the F to 17-OHP ratio between 60 min and 0 min following ACTH administration [Δ F/17-OHP (60 min-0 min)], for screening the females with PCO-like symptoms as well as the males suspected for LO-21OH def.

MATERIALS AND METHODS

Subjects

Fifty-eight persons (47 females and 11 males) were studied. Most of the women were presented to the Reproductive Endocrinology Outpatient Clinic complaining for hirsutism, acne, obesity, menstrual irregularities and unexplained sterility and/or infertility. Some men showed low body height, obesity, and libido disturbances. All females and males participating in this study were subjected to physical examination and endocrinological testing. In the case of women, gynecological examination and pelvic ultrasonography were also included in the protocol. Patients with Cushing syndrome, virilizing ovarian or adrenal tumors and hyperinsulinemia were excluded from this study.

The 37 women (78.7%), who were hirsute, had progressive hair growth on the face, chest, abdomen, buttocks or limbs. The severity of their hirsutism according to the method of Ferriman and Gallwey (24) scored between 7 and 25. The 10 women (21.3%) with acne had inflammatory papular or pustular lesions of the pilo-sebaceous unit. Eight women (17%) and 2 men (18.2%) were obese, weighing 110% or more of their ideal body weight. The 31 women (65.9%) with menstrual irregularities had had oligomenorrhea or secondary amenorrhea for at least 6 months. In 5 women (10.6%), the only clinical problem was unexplained infertility. Thirteen women and 8 men, members of families with an index person for LO-21OH def had been already subjected to HLA-typing and the results were presented in an earlier study (16).

Protocol

All female and male individuals of this study were subjected to adrenal stimulation with iv bolus administration of 0.25 mg of synthetic α1-24 ACTH (Synacten, Ciba-Geigy, Switzerland) at 9.00 am. We determined serum P, 17-OHP and F at 0, 15, 30, 45, and 60 min following ACTH administration. In addition, basal serum FSH, LH, PRL, T, Δ4-androstenedione (Δ4-A), dehydroepiandrosterone sulfate (DHEA-S), SHBG and insulin (I) were measured. In women, ACTH stimulation test was done in the early follicular phase, while in the cases with secondary amenorrhea menstrual bleeding was the result of interruption of a progestogen administered for 10 days.

The marker proposed by Gutai (19) at 30 min, 17-OHP (30 min-0 min)+P (30 min-0 min)/30 min, the ratio F to 17-OHP (F/17-OHP) at 0, 15, 30, 45, 60 min and the new marker under evaluation, namely the difference of this ratio F/17-OHP between 60 min and 0 min [Δ F/17-OHP (60 min-0 min)] were calculated. According to the established biochemical criteria for the detection a) of severe form of LO-21OH def [Gutai 30 min>12.0 mg/dl/min (19) and 17-OHP 60 min>12.0 ng/ml (20, 25)] and b) of "healthy for 21-OH def" individuals [Gutai 30 min<6.5 mg/dl/min (19) and 17-OHP 60 min<5 ng/ml (20)], the study population was separated in 3 groups: A (n=8), with both criteria for severe LO-21OH def positive; B (n=9), with both criteria for absence of LO-21OH def positive and C with the remaining 41 individuals. The two biochemical criteria for grouping the