Pediatric RESEARCH

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CLINICAL RESEARCH ARTICLE

Basal levels of 17-hydroxyprogesterone can distinguish children with isolated precocious pubarche

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BACKGROUND: Basal levels of androgens, in particular 17-hydroxyprogesterone (17OHP), are widely debated as predictors of non-classical congenital adrenal hyperplasia (NCCAH) among patients with precocious pubarche (PP). Many authors have recommended the use of adrenocorticotropic hormone (ACTH) stimulation test in children with PP. The aim of our study was to identify clinical and biochemical predictors of NCCAH in children with PP.

METHODS: We conducted a prospective study of 92 patients with PP undergoing an ACTH stimulation test. We tested the association of basal clinical and biochemical parameters with NCCAH diagnosis. Patients were suspected to have NCCAH if their stimulated 17OHP plasma levels were >10 ng/mL. In these patients, the diagnosis was confirmed by genetic test.

RESULTS: Seven (7.6%) patients resulted having NCCAH. The best basal biochemical predictor for NCCAH was 17OHP level >2 ng/mL. In fact, a basal 17OHP level >2 ng/mL had 100% (95% confidence interval (CI), 59.04–100) sensitivity and 93% (95% CI, 85.3–97.37) specificity. The area under the receiver-operating characteristic curve for 17OHP was 0.99 (95% CI, 0.98–1.007).

CONCLUSIONS: Basal 17OHP cut-off of 2 ng/mL was very effective in predicting NCCAH among our patients with PP. Assay-specific cut-off would probably be the best strategy to avoid unnecessary ACTH test.

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INTRODUCTION

Premature pubarche (PP) is defined by the appearance of pubic hair before the age of 8 years in girls and 9 years in boys, without other signs of puberty or virilization[1]. Generally, it has been considered an almost physiological event due to the early maturation of the zona reticularis, which leads to an increase in adrenal androgens to levels normally observed in early puberty[2].

However, in a variable proportion of cases with PP, ranging from 5 to 43%, the cause is non-classical congenital adrenal hyperplasia (NCCAH) due to 21-hydroxylase (21-OH) deficiency[3]. NCCAH diagnosis is based on elevated baseline and adrenocorticotropic hormone (ACTH)-stimulated 17-hydroxyprogesterone (17OHP) levels. In particular, ACTH-stimulated 17OHP values >10 ng/mL (>30 nmol/L) are considered diagnostic[4, 5]. To date, no clinical feature has been considered sufficiently effective in identifying NCCAH in patients with PP. Whereas basal androgens levels, in particular 17OHP, are widely discussed as predictors of this condition. Therefore, many authors suggested ACTH stimulation test execution in all children with PP[6, 7].

However, ACTH stimulation test may be expensive and uncomfortable for some children. For many children with PP, the results of ACTH stimulation testing are negative for NCCAH. A more discriminating approach would be preferable.

The aim of our study was to prospectively evaluate patients with PP referred to a single hospital to identify the clinical and biochemical predictors of NCCAH.

PATIENTS AND METHODS

An observational prospective study was conducted. Ninety-two children consecutively referred for PP to the Outpatient Pediatric Endocrine Clinic of Università degli Studi della Campania "Luigi Vanvitelli" were enrolled. The study began on 1 January 2012 and ended on 31 December 2016. The protocol was approved by the Ethical Committees of the Università degli Studi della Campania "Luigi Vanvitelli," Naples. Written informed consent was obtained from all patients and/or their parents.

PP was defined as early growth of pubic or axillary hair (before 8 years in girls and 9 years in boys). Patients with concurrent clinical signs of central precocious puberty (breast development before age 8 in girls or testicular development before age 9 in boys), rapidly progressive virilization, or Cushing syndrome (both endogenous abnormal cortisol secretion and chronic steroid therapy) were excluded. All subjects underwent a first evaluation including a detailed medical history, physical examination, and bone age determination. Clinical examination was performed in all children, including weight and height measurement, body mass index (BMI) *z*-score calculation using the LMS (lambda-mu-sigma) method, and staging of pubertal development according to Tanner's classification[8, 9]. Obesity was defined as BMI *z*-score ≥1.65[10, 11]. Bone age was evaluated by TW2 method. Bone age advance was defined as the difference between bone age and chronological age expressed in years. The ACTH test was performed as internationally recommended: a venous blood sample was collected at 08:00 hours, immediately followed by intravenous infusion of

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0.25 mg of tetracosactide acetate (Synacthen), and 60 min after the infusion. Cortisol and 17OHP were measured in basal and post-ACTH blood samples.

Patients were considered to have NCCAH if their stimulated 17OHP plasma level was ≥10 ng/mL; otherwise, patients were considered to have premature adrenarche (PA)[12] The diagnosis of NCCAH in all patients with a positive stimulation test was confirmed by mutational analysis of the *CYP21A2* gene[12]. The *CYP21A2* genotypes were obtained also for parents. Genetic analysis was also performed in a patient with an ACTH test-borderline result.

Total genomic DNA was isolated from peripheral blood leukocytes. The *CYP21A2* gene was analyzed in direct sequencing and multiplex ligation-dependent probe amplification analysis. NCCAH was diagnosed when both alleles of the gene were affected.

The basal blood sample included the baseline measurement of serum 17OHP, cortisol, delta-4 androstenedione, dehydroepiandrosterone (DHEAS), and testosterone. A single laboratory measured, by radioimmunological assays, 17OHP (OHP-CT: Cis Bio, Gif-sur-Yvette, France; intra-assay and inter-assay coefficient of variation (CV) 6% and 4.8%, respectively), cortisol (Cis Bio, Gif-sur-Yvette, France; intra-assay and inter-assay CV 3% and 9%, respectively), delta-4 androstenedione (DRG Diagnostics, Marburg, Germany; intra-assay and inter-assay CV 3.4% and 13.9%, respectively), DHEAS (DiaSorin, Saluggia, Italy; intra-assay and inter-assay CV 6% and 5.2%, respectively), and testosterone (Testo-CT2: Cis Bio, Gif-sur-Yvette, France; intra-assay and inter-assay CV 3.8% and 4.8%, respectively).

We described the general characteristics of the study population and used a non-parametric test (Mann–Whitney test) to compare the distribution of continuous variables in affected and not-affected patients. χ^2 test and Fisher's test, when appropriate, were used to compare categorical values. The discriminating power of the potential predictors was evaluated by comparing the areas under receiver-operating characteristic (ROC) curves (AUC) according to the standard method described by Hanley and McNeil[13]. The predictive value of each factor was assessed by calculating its sensitivity, specificity, and positive and negative predictive values. Logistic regression was used to perform a multiple variable analysis of predictors of NCCAH.

The Stat-Graph Centurion XVII software for Windows was used for all the statistical analyses, except ROC curve analysis that was performed with GraphPad Prism 7. Data were expressed as means ± SD for normally distributed variables and as medians (interquartile range) for non-parametric ones. $P < 0.05$ was considered statistically significant.

RESULTS

We analyzed 92 children (13 males, 14.1%). Overall, median age at pubic hair onset was 6.5 years (mean, 6.4 ± 1; range, 3–8.9). Among girls and boys, the mean age at pubic hair onset was 6.3 ± 1 and 7.5 ± 1 years, respectively.

Seven (7.6%) patients were diagnosed as NCCAH both by a positive ACTH test and *CYP21A2* genetic analysis (Table 1). Stimulated 17-OHP mean levels were 26.76 ± 9.2 ng/mL, while mean basal 17OHP levels were 8.27 ± 4.03 ng/mL. The remaining 85 patients had PA. In these patients, mean 17-OH basal levels were 0.88 ± 0.84 ng/mL, while stimulated 17OHP levels were 3.05 ± 1.52 ng/mL (medians and interquartile ranges are shown in Table 2). The maximum stimulated level in PA patients was 9.2 ng/mL in a girl whose genetic analysis showed a heterozygous state (Table 1).

No statistically significant differences were observed for the distributions of the following clinical and biochemical variables between patients with NCCAH and PA: age at pubic hair onset, age at first examination, height standard deviation score (SDS), BMI *z*-score, basal levels of DHEAS, and testosterone (Table 2). Bone age advance was statistically higher in patients with NCCAH ($p = 0.003$). However, as bone age is influenced by the presence of obesity, we compared the prevalence of obese patients among PA (23/85: 27%) and NCCAH (1/7: 14.3%) groups, but it was not significantly different ($p = 0.7$). It is noteworthy that among patients with PA, obese patients had a statistically significant higher bone age advance (3.7 ± 0.7 SDS vs. 1.7 ± 1.1 SDS; $p = 0.002$).

Table 1. Genetic analysis in patients showing positive/borderline ACTH test

ID patient	Peak 17OHP levels (ng/mL)	Gene	Protein	Homozygosis/heterozygosis
1	44	*844G>T*	V281L	Homozygosis
2	20	*844G>T*	V281L	Homozygosis
3	17.3	*655A/C>G* *841G>T*	I2splice V281L	Heterozygosis Heterozygosis
4	26	*841G>T*	V281L	Homozygosis
5	20	*841G>T* *512T>A*	V281L I171N	Heterozygosis Heterozygosis
6	32	*844G>T*	V281L	Homozygosis
7	28	*844G>T*	V281L	Homozygosis
8[a]	9.2	*814G>T* *952C>T* *WT*	V281L Q318X WT	Heterozygosis Heterozygosis Heterozygosis

[a]Patient 8 had a borderline ACTH test and resulted in a trimodular haplotype with a functional duplicated allele resulting in a non-affected heterozygous state
WT wild type

Table 2. Clinical and biochemical variables in patients with NCCAH and premature pubarche

Variables	NCCAH	Premature pubarche	*p*
n	7	85	
Clinical			
Sex male, *n* (%)	2 (28.5%)	11 (13%)	0.3
Age at pubic hair onset	6.3 ± 1	6.4 ± 1.1	0.5
Age at first examination	7.2 ± 1.1	7.5 ± 1.2	0.5
Bone age minus chronological age (years)	3.7 ± 0.7	1.9 ± 1.2	0.002
BMI *z*-score	1.26 (1.25–1.5)	1.02 (−0.1 to 1.7)	0.5
Height SDS	1.4 (1.6–2.7)	1.2 (0.1–1.8)	0.2
Biochemical			
Basal 17OHP (ng/mL)	8.5 (4.3–11.5)	0.9 (0.4–1.1)	0.0002
Peak 17OHP (ng/mL)	26.0 (20–32)	2.6 (2.1–3.4)	<0.0001
Basal cortisol (μg/dL)	6.9 (4.4–10.2)	9.9 (5.7–10.5)	0.42
Peak cortisol (μg/dL)	18.4 (17.8–23.1)	30.1 (26.8–34.4)	0.002
DHEAS (μg/mL)	0.95(0.8–3.1)	0.7(0.4–1.1)	0.2
Delta-4 androstenedione (ng/mL)	1.4 (1.1–2.3)	0.9 (0.7–1.1)	0.003
Testosterone (ng/dL)	15.5 (3.8–25)	7.5(4.5–15)	0.4

Data are expressed as medians (IQR) or mean ± SD

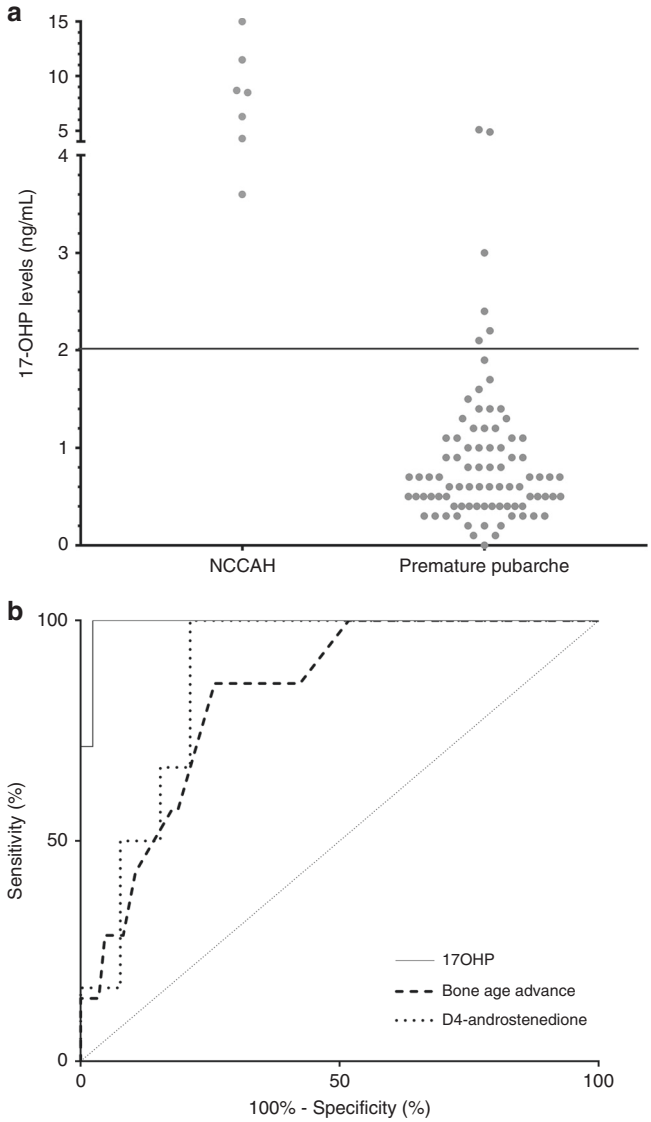

Fig. 1 **a** Basal 17OHP levels (ng/mL) in patients affected and not affected by non-classic congenital adrenal hyperplasia (NCCAH). **b** ROC curves for basal 17OHP, bone age advance, and D4-androstenedione. The AUC for 17OHP, bone age advance, and D4-androstenedione were 0.99 (95% CI, 0.98–1.007), 0.87 (95% CI, 0.78–0.97), and 0.92 (95% CI, 0.84–1.006), respectively. The AUC for 17OHP was significantly larger than the bone advance ($p = 0.04$). No differences between 17OHP and D4-androstenedione ($p = 0.11$), and between bone advance and D4-androstenedione were detected ($p = 0.85$)

Among biochemical parameters, delta-4 androstenedione was significantly higher ($p = 0.003$) in patients with NCCAH than in those with PA (Table 2), such as both basal (Fig. 1a) and peak plasma levels of 17OHP (Table 2).

The AUCs for 17OHP, bone age advance, and D4-androstenedione were 0.99 (95% CI, 0.98–1.007), 0.87 (95% CI, 0.78–0.97), and 0.92 (95% CI, 0.84–1.006), respectively (Fig. 1b). The AUC for 17OHP was significantly larger than the AUC for bone age advance ($p = 0.04$). No differences for the AUC between 17OHP and D4-androstenedione ($p = 0.11$), and between bone age advance and D4-androstenedione were detected ($p = 0.85$).

For 17OHP, we tested the historical cut-off level of 2 ng/mL[4] and we found that a basal 17OHP level >2 ng/mL had 100% (95% CI, 59.04–100) sensitivity and 93% (95% CI, 85.3–97.37) specificity for predicting NCCAH.

The positive and negative predictive values of this basal threshold were 53.8 and 100%, respectively. In our population, 17OHP threshold of 2 ng/mL corresponded to the 94th percentile of plasma levels in the group of children with PA. Among PA patients, 6 out of 85 patients (7%) showed basal 17OHP above 2 ng/mL (range 2.1–5.1 ng/mL). Based on the clinical features, these six patients could not be distinguished from those with NCCAH. Only bone age advance was greater in patients with NCCAH than in patients with PA and basal 17OHP above 2 ng/mL (3.7 ± 0.7 vs. 2.2 ± 1.2; *p*: 0.03). Patients showing a basal 17OHP above 2 ng/mL were genotyped and resulted wild type, except for patient no. 8 in Table 1 who resulted heterozygous, as reported above.

Neither D4-androstenedione nor bone age advance after dichotomization (≥0.95 ng/mL and >2 years, respectively) had a better predictive power than the basal 17OHP plasma level (Supplemental Table S1). Moreover, in a logistic regression analysis including, as categorical independent variables, the presence of obesity, 17OHP >2 ng/mL, bone age advance ≥2, and D4-androstenedione >0.95 ng/mL, only 17OHP remained a statistically significant predictor of NCCAH ($p = 0.003$; Model R2: 99.9%, $p = 0.001$).

No differences were found among basal cortisol levels of patients with NCCAH and those with PA, while stimulated peak cortisol were significantly lower in patients with NCCAH than in PA (Table 2). Within the NCCAH group, two patients had suboptimal stimulated peak cortisol (<18 µg/dL), but none of them had experienced signs or symptoms consistent with adrenal insufficiency. The finding of abnormal biochemical data in the absence of symptoms and or suggestive history in patients with NCCAH is difficult to interpret, and the optimal clinical management is still unknown. However, we suggested to our patients to carry a medical card indicating that stress-dose glucocorticoids were needed in case of major trauma or major surgery.

DISCUSSION

PP is a common reason of pediatric and endocrinological consultation, and it is often a benign condition. However, it is important to exclude NCCAH to warrant the correct treatment and to avoid related medical problems[3, 14]. In our cohort, prevalence of NCCAH was 7.6% (7/92), similar to that found in other cohorts with low prevalence of NCCAH[15, 16].

Data about clinical predictors of NCCAH among PP patients are contrasting, as in some studies patients with NCCAH were heavier, taller, and had accelerated bone age[17–19], while in other ones, no differences were found[16].

In our prospective study, among the clinical parameters, only accelerated bone age maturation was significantly different in patients with NCCAH, as previously reported[15]. However, obesity is frequently associated with bone age acceleration, and given the strong increase of prevalence of pediatric obesity and, in particular, its association with premature pubertal development, we do not think that this parameter could be useful in clinical practice[20]. In fact in our study, it lost statistical significance in the multivariable analysis adjusted for the presence of obesity. For biochemical parameters, once again data from literature are contrasting[16, 17, 21].

Our results are in accordance with the retrospective study by Armengaud et al[16]. In fact, basal 17OHP was a very good predictor of NCCAH in our cohort. In particular, the historical-proposed 17OHP cut-off of 2 ng/mL[4] had a 100% sensitivity. So a pediatrician could safely exclude NCCAH using this cut-off.

Among our patients, 79 unnecessary ACTH stimulation tests could be avoided using such threshold. However, this test was in any case necessary for seven patients with NCCAH and for six patients with PA showing basal 17OHP above 2 ng/mL.

Hormonal measurements are assay-specific and this could explain the differences between our results and results of Ghizzoni

et al.[21] reporting that the threshold of 2 ng/mL had only 92.7% sensitivity. In fact, in our cohort, as such, as in the cohort of Armegaud et al.[16]—using our same Radioimmunoassay (RIA) kit—the 17OHP threshold of 2 ng/mL corresponded to the 94th percentile of plasma levels in the group of children with PA, while this threshold value corresponded to the 80th percentile of unaffected children in the cohort of Ghizzoni et al.[21]. This difference could be attributed to the difference in the assays used, or to the selection of patients. In particular, different calibration curves used to build the two assays could explain the differences in the resulting hormonal values. We think that threshold percentile is important, as it allows comparison of studies using different assays [22].

A recent report questioned the utility of basal 17OHP to diagnose NCCAH[23]. However, clinical presentation of patients included in the study was not homogenous (including subjects with precocious puberty, precocious pubarche (PP), hirsutism in adolescence, clitoridomegaly, and short stature associated to advanced bone age), age range was very huge, and in a group of patients, diagnosis was not confirmed by genetic analysis, making false positive possible.

The strengths of the present study are the prospective design and the single-center laboratory analysis. Limitations of our study are the small number of patients with NCCAH and the lack of genotype of patients with negative ACTH test (making not possible excluding heterozygous carriers). Moreover, we used a RIA assay, while liquid chromatography coupled with tandem mass spectrometry is the preferable method to assess steroid levels. Anyway, this latter technique is not yet very diffused worldwide, and in several recent works on this topic, RIA or enzyme-linked immunosorbent assay have been used to assess 17OHP levels [23, 24].

In conclusion, in our study, basal level of 17OHP was a useful predictor of NCCAH in patients with PP. In particular, in our cohort, the cut-off of 2 ng/mL, corresponding to 94th percentile of unaffected children, had 100% sensitivity. Of course, the cut-off of 2 ng/mL for 17OHP could be not applicable to patients investigated by different hormonal assay.

We suggest a selection strategy based on the basal level of 17OHP, with a cut-off that can be different depending on the assay used, to decide in which patients with PP it is necessary to perform an ACTH test. However, in particular circumstances, performing ACTH test to exclude the diagnosis of NCCAH may be valuable to relieve the anxiety of parents, patient, and health care providers.

Summarizing that basal 17OHP values >2 ng/mL in PP patients warrant further investigation (i.e., ACTH test), while in patients with 17OHP <2 ng/mL ACTH test could be avoided. However, also in these latter subjects, given the assay dependence of 17OHP measurements and the contrasting literature (e.g., Ghizzoni et al.[21]), clinical judgment should guide diagnostic work-up, and in patients with excess signs of androgen, ACTH test could be considered to exclude NCCAH definitely.

In our opinion, a selective approach is cheaper and less distressing for children and their families, sparing several unnecessary ACTH tests.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41390-018-0096-7) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

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