Is basal serum 17-OH progesterone a reliable parameter to predict nonclassical congenital adrenal hyperplasia in premature adrenarche?

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SUMMARY: Gönç EN, Özön ZA, Alikaşifoğlu A, Engiz Ö, Bulum B, Kandemir N. Is basal serum 17-OH progesterone a reliable parameter to predict nonclassical congenital adrenal hyperplasia in premature adrenarche? Turk J Pediatr 53; 2011: 274-280.

To determine the critical features for the diagnosis of nonclassical 21 hydroxylase deficiency (NC21OHD) without performing adrenocorticotropic hormone (ACTH) test, we studied 186 cases with premature adrenarche. Clinical and laboratory features as well as basal 17-hydroxyprogesterone (17-OHP) were analyzed to determine factors important for differentiating NC21OHD.

Overall, 6 patients (3.2%) had ACTH-stimulated 17-OHP >10 ng/ml. A cutoff level of 2 ng/ml for basal 17-OHP was 66.7% sensitive and 78% specific for NC21OHD; however, a cutoff level of 1.55 ng/ml had higher sensitivity (83%) and specificity (70.6%). A cutoff of 1.55 ng/ml would lead to 31% of cases with premature adrenarche having to undergo ACTH test, and only one case would have been missed. That case had a bone age SDS >2. Three cases out of five with a basal 17-OHP >5 ng/ml had stimulated 17-OHP <10 ng/ml.

A cutoff of 1.55 ng/ml for basal 17-OHP together with bone SDS >2 in those with lower basal levels as a guide for carrying out an ACTH test may yield better results in the diagnosis of NC21OHD in the premature adrenarche population. A cutoff of 5 ng/ml for basal 17-OHP should not be used for diagnosis of NC21OHD.

Key words: premature adrenarche, adrenocorticotropic hormone (ACTH) stimulation test, nonclassical congenital adrenal hyperplasia, nonclassical 21 hydroxylase deficiency, 17-hydroxyprogesterone.

Nonclassical congenital adrenal hyperplasia (NCCAH) is an important diagnostic entity in the differential diagnosis of premature appearance of pubic or axillary hair in both boys and girls. Overall prevalence of NCCAH in patients with premature adrenarche (PA) is 4% in one of the largest series in the literature[1]. However, infrequent as it may be, an important complication of NCCAH is advancement of skeletal maturation (i.e. advancement of bone age) with an impact on adult height as a consequence: tall in childhood, short as an adult[2]. The gold standard for differentiating NCCAH from PA is a standard adrenocorticotropic hormone (ACTH) stimulation test. It is an invasive, expensive and time-consuming procedure. Some effort has been made to identify other specific diagnostic tools to avoid unnecessary ACTH stimulation tests.

In the current study, we retrospectively analyzed 186 cases with PA in order to determine the critical clinical and laboratory features for the diagnosis of nonclassical 21 hydroxylase deficiency (NC21OHD) without performing ACTH test and also to document the characteristics of patients with NC21OHD.

Material and Methods

Children admitted to the Division of Pediatric Endocrinology at Hacettepe University between

1999-2008 with premature pubic or axillary hair development were retrospectively analyzed. Patient records were checked for a presumptive diagnosis of PA and a standard ACTH stimulation test. Patients with a known chronic disease, positive family history for congenital adrenal hyperplasia, those using any medication as well as those with a basal 17- hydroxyprogesterone (17-OHP) >10 ng/ml were excluded from the analysis. One hundred and eighty-six subjects (158 girls, 28 boys) fulfilled the criteria, and were included in the analysis. All subjects were normotensive. Twenty-nine patients (15.6%) had consanguineous parents.

Chronological and skeletal ages, height and weight were noted from the hospital records. Body mass index (BMI) z-scores[3] and height standard deviation score (SDS) were calculated[4,5]. Bone age and bone age SDS for chronological age were determined using the method of Greulich and Pyle[6].

Basal serum testosterone, dehydroepiandrosterone-sulfate (DHEA-S), androstenedione, 17-OHP, 11-deoxycortisol (S), as well as the gonadotropin levels (and estradiol in the girls) were extracted from patient files. None of the girls had breast development or clitoromegaly, and none of the boys had penile or testicular enlargement. Basal gonadotropin levels were in the prepubertal range in all patients. Basal testosterone levels were <20 ng/ml in all patients, and DHEA-S levels were within the normal pubertal range.

Adrenocorticotropic hormone (ACTH) stimulation test was performed early in the morning after an overnight fast using 0.25 mg ACTH (Synacthen, Ciba-Geigy) injection intravenously. Blood samples were collected for serum 17-OHP and 11-deoxycortisol (S) levels both before and 60 minutes (min) after ACTH injection. None of the patients had elevated basal or stimulated 11-deoxycortisol (S) levels, excluding the diagnosis of 11-hydroxylase deficiency.

The patients were classified into three groups with respect to stimulated 17-OHP levels: Group 1: stimulated 17-OHP levels of 0 - 4.9 ng/ml; Group 2: 17-OHP: 5.0 – 9.9 ng/ml; and Group 3: 17- OHP >10 ng/ml.

Hormone Assays

Serum levels of androstenedione and 17-OHP were measured by radioimmunoassay (RIA) using DSL 3800 and 5000 coated tube kits (USA), respectively. Serum 11-deoxycortisol (S) level was also measured by RIA using coated tube kits of DIA source (Belgium). Serum total DHEA-S level was measured by solid-phase, competitive chemiluminescent enzyme immunoassay (Immulite 2000, USA).

Statistical Analysis

All data are presented as mean ± standard deviation. The following tests were used to determine the statistical significance: one way ANOVA, Pearson's correlation test and receiver operating characteristics (ROC) analysis. Statistical analysis was performed using SPSS version 16.0.

Results

Mean chronological age at the onset of symptoms was 6.6 ± 1.0 years (6.4 ± 1.0 years in girls and 7.4 ± 1.0 years in boys).

One hundred and forty-nine cases (80.1%) were in Group 1, 31 cases (16.7%) in Group 2 and 6 cases (3.2%) in Group 3, according to stimulated 17-OHP levels. Auxological findings in the groups are shown in Table I. Mean ages at the onset of symptoms were similar among the three groups (p=0.263). Mean height SDS was statistically similar as well (p=0.3). Although mean bone age in the third group was

Table I. Initial Clinical Findings of 186 Cases with PA

	Group 1 n: 149	Group 2 n: 31	Group 3 n: 6	p
Female/male	125/24	29/2	4/2	
Age	6.7±1.0	6.4±0.9	6.4±1.1	0.263
Height SDS	1.02±0.96	0.80±1.24	0.49±0.85	0.3
BMI z-score	1.0±1.1	1.5±0.9	2.0±0.9	0.014
Bone age SDS	1.4±1.2	1.3±1.3	1.8±1.7	0.674

PA: Premature adrenarche. SDS: Standard deviation score. BMI: Body mass index.

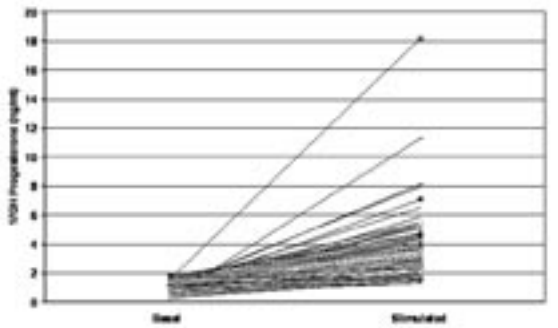


Fig. 1. Two of 139 patients (1.4%) whose basal 17-OHP levels were <2 ng/ml had stimulated 17-OHP level >10 ng/ml.

greater than in the first two groups, there was no statistically significant difference between groups (0.674). Mean BMI z-scores differed among the three groups (p=0.014). Stimulated 17-OHP levels were directly correlated with BMI z-scores (r: 0.237 p=0.001).

Laboratory findings are shown in Table II. Mean basal serum 11-deoxycortisol (S) and androstenedione levels were similar among the three groups. Group 3 had the highest mean basal DHEA-S level (p=0.035). Stimulated 11-deoxycortisol (S) levels were also high in Groups 2 and 3 (p=0.003). Mean stimulated basal 17-OHP level increased in parallel to increasing mean basal 17-OHP level. Stimulated 17-OHP levels were directly correlated to basal 17-OHP levels (r: 0.419, p<0.001).

One hundred and thirty-nine patients had basal 17-OHP levels <2 ng/ml. Two of these patients (1.4%) had stimulated 17-OHP >10 ng/ml (Fig. 1).

Forty-seven patients had basal 17-OHP levels >2 ng/ml. Four of these patients (8.1%) had stimulated 17-OHP >10 ng/ml (Fig. 2).

Table III shows the detailed clinical and laboratory characteristics of the 6 patients in Group 3. Two of these patients had a basal 17-OHP level <2 ng/ml. Both patients were girls and adrenarche was at the age of 6.2 years. Their heights were close to the mean according to age- and sex-related standards. One was obese (BMI z-score +2.83) with an advanced bone age (bone age SDS +3.24). Her 17-OHP level increased from 0.35 ng/ml to 11.28 ng/ml in the ACTH test. The other girl had normal weight; however, her bone age was advanced (bone age SDS +3.64). Her 17-OHP level increased from 1.59 ng/ml to 18.19 ng/ml in the ACTH test.

ROC analysis showed that basal 17-OHP level of 2 ng/ml, 1 ng/ml and 1.55 ng/ml had a sensitivity of 66.7%, 83% and 83%, and a specificity of 78%, 48% and 70.6%, respectively, for the diagnosis of NCCAH (Fig. 3). One hundred and twenty-eight of 186 cases had a basal 17-OHP level <1.55 ng/ml. Forty-six (35%) of these 128 patients had a bone age SDS >2 SDS, and in 20 patients, bone age was >3 SDS.

ROC analysis of basal 17-OHP was repeated after excluding the two patients mentioned above (basal 17-OHP <2 ng/ml and stimulated 17-OHP >10 ng/ml). A cutoff of 2 ng/ml for basal 17-OHP had 100% sensitivity and 80% specificity (Fig. 4). One hundred and thirty-eight patients (138 out of 186 patients, 74%) had basal 17-OHP <2 ng/ml. Forty-eight (34%) of these patients had a bone age SDS >2 SDS and 14 of them were >3 SDS.

Basal 17-OHP levels were >3 ng/ml in 14 patients. Twelve of these had a basal 17-OHP level <10 ng/ml. The sensitivity of basal 17-OHP level of 3 ng/ml was 77% and the specificity was 94%.

Five patients had basal 17-OHP levels >5 ng/ml. Only two of them had stimulated 17-OHP levels >10 ng/ml. A patient whose basal 17-OHP level was 6.03 ng/ml had stimulated level of 2.38 ng/ml.

Discussion

Six out of 186 patients (3.2%) with typical PA were diagnosed as NC21OHD using the normograms published before[7]. Since

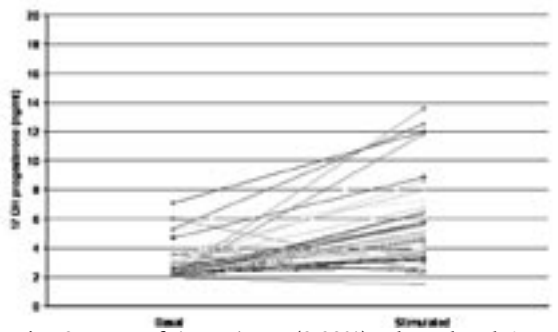


Fig. 2. Four of 47 patients (0.08%) whose basal 17-OHP levels were >2 ng/ml had stimulated 17-OHP levels >10 ng/ml.

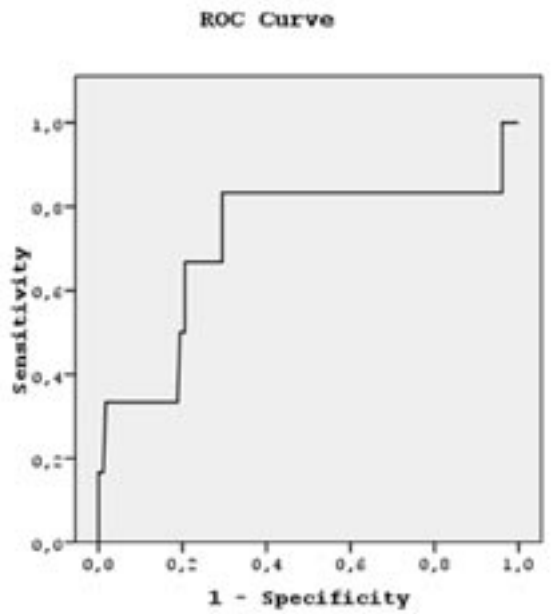
Table II. Laboratory Findings of Patients

	Group 1 n: 149	Group 2 n: 31	Group 3 n: 6	p
Basal 17-OHP (ng/ml)	1.2±0.8	2.0±1.3	3.0±2.5	0.002
Basal 11-deoxy cortisol (S) (ng/ml)	2.2±1.2	3.8±3.2	1.9±1.3	0.154
Basal DHEA-S (μg/dl)	83.9±51.2	91.0±38.9	138.4±80.7	0.035
Basal androstenedione (ng/ml)	0.9±0.4	0.9±0.4	0.9±0.5	0.842
Stimulated 17-OHP (ng/ml)	3.1±0.9	6.4±1.3	13.2±2.5	<0.001
Stimulated 11-deoxycortisol (S) (ng/ml)	3.8±1.6	5.1±1.7	5.9±2.3	0.003

17-OHP: 17-dehydroxyprogesterone. DHEA-S: Dehydroepiandrosterone-sulfate.

patients with a basal 17-OHP level >10 ng/ml were excluded from the cohort, this prevalence does not reflect the real frequency of NC21OHD among cases with PA in the Turkish population. Since 15.6% of the marriages are consanguineous in our cohort, a higher frequency would be expected. A number of studies showed variable prevalences for NCCAH in the respective study populations. Nonclassical forms of CAH other than 21OHD are quite rare. Prevalence of NC21OHD among cases with PA ranged from 0-30% in different studies[1,8-16]. Prevalence among females with hyperandrogenism varied from 0.02 to 2.2%[17-19].

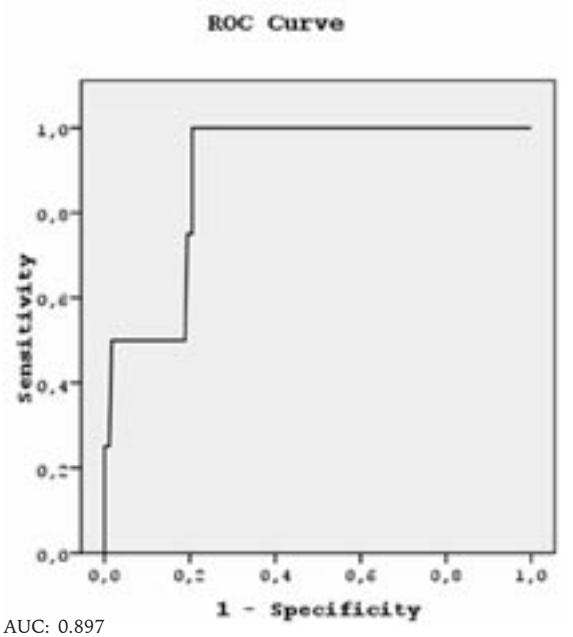
Diagnosis of NCCAH is based on ACTH testing. There are established normograms for the diagnosis of CAH due to 21OHD. The normograms use stimulated 17-OHP levels in response to ACTH to differentiate the cases into normal, heterozygous, nonclassical, or classical CAH[7]. ACTH is a strong tool in the diagnosis of NCCAH; however, it is costly and invasive. Clinical and laboratory clues for the diagnosis of NCCAH in an effort to escape performing the test have been analyzed by other researchers previously. Bone maturation and auxological parameters were reported to be similar between cases with PA and NCCAH[1,13,16]. However, Balducci et al.[9] showed advanced bone age in 80% of cases with NC21OHD. In our cohort, mean chronological



Area under the curve (AUC): 0.772

95% confidence interval (CI): 0.461-0.983

Fig. 3. ROC analysis of basal 17-OHP when all the patients are included.



AUC: 0.897

95%CI: 0.797-0.997

Fig. 4. ROC analysis of basal 17-OHP when 2 patients with basal 17-OHP <2 ng/ml and stimulated 17-OHP >10 ng/ml are excluded.

Table III. Clinical and Laboratory Findings of the Patients in Group 3

Patient	Sex	Age	Height SDS	BMI z-score	Bone age SDS	Androstenedione (ng/ml)	DHEA-S (μg/dl)	Stimulated 11-deoxy cortisol (S) (ng/ml)	Basal 17-OHP (ng/ml)	Stimulated 17-OHP (ng/ml)
1	F	6.3	-0.02	2.83	3.24	0.3	94.0	4.4	0.35	11.28
2	F	6.3	-0.07	0.67	3.64	1.6	129.0	4.5	1.59	18.19
3	M	6.7	0.73	3.19	1.52	0,9	89.1	4.2	2.09	11.85
4	M	7.0	2.06	2.24	3.1	0.8	137.0	10.1	2.14	13.59
5	F	4.5	0.49	1.39	-0.39	0.9	297.0	7.4	5.30	12.53
6	F	8.0	-0.25	1.9	0.09	0.8	84.3	5.1	7.10	12.00

17-OHP: 17-dehydroxyprogesterone. DHEA-S: Dehydroepiandrosterone-sulfate. SDS: Standard deviation score. BMI: Body mass index.

age and height did not differ among groups of cases. Bone age SDS for chronological age was also statistically comparable among groups. Bone ages overlapped among groups. However, three of our patients with NC21OHD had bone age SDS above, and the remaining below, 2 SDS.

Armengaud et al.[1] noticed increased BMI in patients with NCCAH. The mean BMI was also higher in our cases with NC21OHD. However, again, half of them had BMI z-score above 3 and the other half below 2 SDS. Thus, bone age and BMI do not seem to be reliable predictors for the diagnosis of NCCAH. Mean basal levels of DHEA-S and 11-deoxycortisol (S) were higher in our cases with NC21OHD. Unfortunately, there is no clear cutoff for these variables to differentiate NCCAH from normal. Leite et al.[8] also studied basal androgen levels and showed that there was overlap between PA and NCCAH. Armengaud et al.[1] reported increased androstenedione and testosterone levels in cases with NCCAH. We could not find any difference in androstenedione levels among the three groups, and testosterone levels were in the prepubertal range in all of our cases.

Many researchers are still trying to identify a cutoff level for basal 17-OHP that will exclude the diagnosis of NC21OHD and avoid unnecessary ACTH testing. Generally, a basal 17-OHP level of 2 ng/ml is accepted as a cutoff level to document the need for an ACTH test in the diagnosis of NC21OHD. Armengaud et al.[1] showed that 2 ng/ml for a basal level of 17-OHP is 100% sensitive and 99% specific for the diagnosis of NC21OHD among 238 cases with PA. Likitmaskul et al.[14] also reported that none of the 12 children with NCCAH had a basal 17-OHP level <2 ng/ml. A similar question is forwarded in adult females with hyperandrogenism. Azziz et al.[17,18] demonstrated that all NCCAH females investigated for hyperandrogenism had basal 17-OHP >2 ng/ml, and suggested 2 ng/ml as a cutoff level in female hyperandrogenism. Dewailly et al.[20] recommended an ACTH test when the basal 17-OHP level was >2 ng/ml, as NC21OHD was unlikely in the case with lower basal 17-OHP. Contrary to all these findings, there are several reports showing NC21OHD cases with lower basal 17-OHP levels. Bachega

et al.[21] reported that four of 58 patients with NCCAH had basal 17-OHP levels <2 ng/ml. Similarly 13-14% of patients with NC21OHD would have been missed if the basal 17-OHP level of 2 ng/ml had been used[11,22]. Escobar-Morreale et al.[19] proposed a cut-off level of 1.7 ng/ml with 100% sensitivity and 88.6% specificity.

In the current cohort, 33% of NC21OHD cases had a basal 17-OHP level of <2 ng/ml. Even a basal level of 0.35 ng/ml can end up with a stimulated level of >10 ng/ml in the ACTH test. A cutoff level of 2 ng/ml was 66.7% sensitive and 78% specific for NC21OHD. ROC analysis of our data showed that a cutoff level of 1.55 ng/ml had higher sensitivity (83%) and specificity (70.6%). If the cutoff level was taken as 2 ng/ml, 26% (48/186) of cases with PA would undergo ACTH test, and two patients (33%) would have been missed; whereas, if the cutoff level was taken as 1.55 ng/ml, 31% (58/186) of cases with PA would undergo ACTH test, and only one case would have been missed. Two NC21OHD patients whose basal 17-OHP levels were <2 ng/ml had bone ages above 3 SDS. Thus, it is possible to perform ACTH test in cases with PA whose basal 17-OHP is >1.55 ng/ml and in those with a 17-OHP level <1.55 ng/ml and advanced bone ages. Eighty-two cases out of 128 (64%) with basal 17-OHP level <1.55 ng/ml had bone age SDS <2 SDS, meaning that at least 82 of 186 cases (44%) with PA can be followed without performing ACTH test.

Is there a cutoff level for basal 17-OHP to diagnose NC21OHD without performing ACTH test? Leite et al.[8] showed that a basal level of 17-OHP >3 ng/ml was sufficient for the diagnosis of NC21OHD. The study group was small in that report; thus, it is hard to reach a conclusion. The most commonly used cutoff in this respect is 5 ng/ml[10,20,21,23]. We had five cases with PA whose basal 17-OHP levels were >5 ng/ml. Three (60%) of them had stimulated 17-OHP level <10 ng/ml. Thus, if a cutoff level of 5 ng/ml is accepted, 60% of cases were misdiagnosed as NC21OHD. One patient whose basal 17-OHP level was 6 ng/ml had lower stimulated level than basal. This might be explained by the fact that, at times, the stress of blood sampling may be a stronger stimulus than the ACTH itself. Thus, individual factors that may alter basal levels of steroids should be considered when diagnosing NC21OHD.

In conclusion, the overall prevalence of NC21OHD among cases with PA whose basal 17-OHP levels were <10 ng/ml was only 3.2%. Use of a cutoff of 2 ng/ml for basal 17-OHP for carrying out an ACTH test may miss a diagnosis of NC21OHD in up to one-third of the cases. Overdiagnosis of NC21OHD can occur in 60% of cases if 5 ng/ml is accepted as a cutoff for basal 17-OHP.

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