Measurement of 11-Oxo-androgens, A Novel Biomarker, in Females with Clinical Signs of Premature Adrenarche

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What is already known on this topic?

Adrenarche is characterized by the activation of androgens precursors dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) which are released from the zona reticularis of the adrenal gland. Recent studies have demonstrated that adrenal production of 11-oxygenated steroids are also potent adrenal androgens that can bind to androgen receptors and therefore likely play a role in the clinical picture of adrenarche.

What this study adds?

Our study has indicated that these traditional markers like DHEA and DHEA-S are not sensitive enough. 11-oxo androgens could be used as novel biomarkers to evaluate premature adrenarche in female patients in whom previous biochemical evaluation yielded inconclusive results. Perhaps early identification of these patients will permit early therapy, in hopes to prevent metabolic syndrome, type II diabetes mellitus and polycystic ovarian syndrome associated with premature adrenarche.

ABSTRACT

Objective: Endocrine findings in premature adrenarche have been characterized by elevated dehydroepiandrosterone (DHEA) DHEA-sulfate (DHEA-S) levels in the past.

Methods: We reviewed female patients, aged 4 to 8 years, with premature adrenarche who were seen at a single center between 2019 and 2023. Data were collected on the traditional androgens (DHEA and DHEA-S) and novel 11-oxo-androgens, which were measured using liquid chromatography/tandem mass spectrometry assays in commercial laboratories (Lab Corp).

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Results: The study included 44 girls. The majority, 89% of patients from the youngest group (4-5 years olds), presented with apocrine odor as the only symptom of premature adrenarche. We have demonstrated that DHEA and DHEA-S levels were within the normal range in many girls with premature adrenarche, whereas 11-oxo-androgens, particularly 11-hydroxyandrostenedione and 11-beta-hydroxytestosterone, were elevated. Out of those with normal DHEA-S, 75% had elevated 11-hydroxyandrostenedione, and 77.8% of those patients with normal DHEA had the same elevated oxo-androgen. Moreover, advanced bone age greater than 1 year compared to chronological age was positively associated with 11-ketotestosterone [Spearman rho=0.32, 95% confidence interval (CI): 0.01-0.57, p=0.0429] and 11β-hydroxy testosterone (Spearman rho=0.32, 95% CI: 0.01-0.58, p=0.0395).

Conclusion: We propose that 11-oxo-androgens are a more sensitive steroid to be measured when premature adrenarche is suspected.

Keywords: Oxo-androgens, adrenarche, child, premature

Introduction

Adrenarche is characterized by the activation of androgen precursors, which are released from the zona reticularis of the adrenal gland. (1). Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEA-S) are both thought to be responsible for the clinical signs of adrenarche (2).

Pubarche is the appearance of pubic hair, which may also occur with the appearance of axillary hair and the presence of apocrine odor. This process is considered premature if it occurs before the age of 8 years in girls and before 9 years in boys (3,4,5,6,7).

The event of adrenarche occurs only in humans and higher primate species, such as chimpanzees, gorillas, and old world monkeys (3,4,5,6,7). It is important to differentiate premature adrenarche from precocious puberty by a lack of progressive breast development in girls or testicular enlargement in boys.

The absence of a relationship of testosterone levels to adrenarche, as in hirsutism, has raised the possibility of other bioactive androgens circulating in humans. Recent studies have demonstrated that adrenal production of 11-oxygenated steroids, such as 11-ketotestosterone (11KT) and 11-beta-hydroxytestosterone (11β-OHT) (11, are also potent adrenal androgens that can bind to androgen receptors and, therefore, likely play a role in the clinical picture of adrenarche (see Figure 1) (8).

Both DHEA and DHEA-S are typically elevated in premature adrenarche, though not in all patients. It has long been thought that these steroids may act as precursors for the increased production of testosterone in hair follicles and genital skin that exhibit the phenotypic effects associated with adrenarche (8,9,10,11,12,13).

One of the 11-oxo-androgens is 11-keto-testosterone, which has indeed been identified as the dominant bioactive androgen in children during adrenarche (11,12,13). Its androgenic capacity exceeds that of both DHEA and DHEA-S, which may well be at normal levels in premature adrenarche. Herein, we present data on 11-oxo-androgens, novel biomarker exclusively secreted by the adrenal gland with no admixture by the ovaries or testes.

Methods

We conducted a study and looked at laboratory and anthropometric data of female patients who presented to the Pediatric Endocrinology Outpatient Center at NYU Langone-Long Island from September 1, 2019, to April 15, 2023, with a history of clinical signs of premature adrenarche before 8 years of age. The study was approved by the NYU Winthrop/Long Island School of Medicine Institutional Review Board (approval no.: i20-01685, date: 05.11.2020), which granted us permission to look at the data retrospectively and also for current patients as they presented for evaluation to our center. Laboratory results and bone age results, as well as demographics, were obtained from the Epic Electronic Medical Record software program, made by Epic Systems Corporation, in Verona, Wisconsin, USA. NYU Langone-Long Island manages all of the aforementioned electronic medical records. These records were accessible to the study team members as part of their clinical responsibilities. The data collected was de-identified and stored in REDCap electronic data capture, according to NYU Langone Health's policy on data storage.

Inclusion Criteria

Female patients who are 10 years old or younger with a history and clinical signs of premature adrenarche and not showing signs of puberty (breast development).

Exclusion Criteria

1) Male patients, as there is no reference range available for male patients.

2) Patients with concomitant precocious puberty.

3) Patients with congenital adrenal hyperplasia.

4) Patients with an adrenal tumor.

The levels of 11-oxo-androgens were measured using liquid chromatography/tandem mass spectrometry (LC/MS-MS) assays in commercial laboratories (Lab Corp). DHEA-S and DHEA levels were also measured by LC/MS-MS assays. The reference values to evaluate normal levels of DHEA and DHEA-S were determined using reference ranges provided by the Esoterix manual of pediatric

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endocrinology (Lab Corp). Reference ranges for DHEA levels were <68 ng/dL for 1-5-year-olds, <111 ng/dL for 6-7-year-olds, and <186 ng/dL for 8-10-year-olds. Reference ranges for DHEA-S levels in prepubertal children were <57 ng/dL for 1-5 years old, <72 ng/dL for 6-7 years old, and <193 ng/dL for 8-10 years old (Lab Corp).

Currently, commercial laboratories do not provide a reference range for 11-oxo-androgens in the pediatric population by age. Rege et al. (8) measured levels of 11β-OHT, 11KT, and 11-hydroxyandrostenedione (11OHA) in pediatric female patients with normal and premature adrenarche. We have used the mean range described in their study for 11-oxo-androgens as the reference range for our patients, as described below:

11β-OHT (ng/dL): Ages 4-5 years, mean 3.0 (2.6-6.5); ages 6-8 years, mean 4.6 (3.2-6.7); and ages 9-10 years, mean 5.5 (4.1-6.5) (8).

11OHA (ng/dL): Ages 4-5 years, mean 17.6 (11.2-34.0); ages 6-8 years, mean 27.0 (20-39.7); and ages 9-10 years, mean 26.1 (17.4-44.8) (8).

11KT (ng/dL): Ages 4-5 years, mean 8.6 (7.3-10.9); ages 6-8 years, mean 13.4 (10.3-18.1); and ages 9-10 years, mean 17.6 (14.2-22.5) (8).

The recovery rate of the oxoandrogen assay is as follows: 11KT: 101.1-107.7%, 11OHA: 97.8-113.5%, and 11β-OHT: 101.1-110.9%. The laboratory does not routinely determine lower limits of detection values for these assays. The lower limit of quantification is 3 ng/dL for each analyte. This is the lowest value where precision is <20% and accuracy is within 20% of the target. The laboratory validated linearity with x2, x5 and x10 dilutions. Matrix effects are mitigated by the use of heavy isotope internal standards for each analyte. Specimen stability was determined to be at least 14 days at ambient and refrigerated conditions, and at least one year at frozen temperatures. Intra-assay coefficient of variability for 11β-OHT was from 2.5% to 10.9%, for 11OHA (2.9-7.8%), and 11KT (2.4-4.2%) (Esoterix/LabCorp, Calabasas Hills, CA, USA).

Statistical Analysis

Descriptive statistics (mean, standard deviation, median, 25th and 75th percentiles, minimum and maximum values for continuous variables; frequencies and percentages for categorical variables) were calculated separately by group (normal vs. elevated levels of DHEA and DHEA-S). The two groups were compared using the chi-square test or Fisher's exact test, as deemed appropriate, for categorical variables and the two-sample t-test or Mann-Whitney test for continuous data, as appropriate. Spearman correlation coefficients were used to assess the association between each of the biomarkers and advanced bone age. A result was considered statistically significant at the $p<0.05$ level of significance. All analyses were performed using SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

The cohort included 44 patients in total. The characteristics of the patients in our study are shown in Tables 1 and 2. Patients were divided into three different age groups. Interestingly, the majority, 89% (8 out of 9), of patients from the youngest group (4-5-year-olds) presented with apocrine odor as the symptom of premature adrenarche. Out of 44 patients, 25 had elevated body mass index (BMI) percentile (>85th), which is 57% of our patient cohort (Table 2).

The groups were further divided into patients with normal DHEA and DHEA-S values. We then determined the number of patients with normal DHEA and DHEA-S values and elevated 11-oxo-androgens. The median values, 25th, and 75th quartiles for 11-oxo-androgens (in ng/dL) for each age group are presented in Table 3.

Out of 44 patients, 36 (81.8%) had normal DHEAS levels. Two of those had elevated 11β-hydroxy testosterone (p=0.0349, Fisher's exact test). There was no relationship between the 11OHA values and increased BMI percentile. However, advanced bone age greater than 1 year compared to chronological age was positively associated with 11KT (Spearman correlation coefficient=0.32, 95% CI: 0.01-0.57, p=0.0429) and 11OHT (Spearman correlation coefficient=0.32 (95% CI: 0.01-0.58, p=0.0395). Out of 44 patients, 22 (50%) had bone age advancement of more than 1 year (Table 2). In addition, our data demonstrated that 77.8% (14/18) of those patients with normal DHEA had elevated 11-OHA. Out of those with normal DHEAS, 75% have elevated 11OHA. Figure 2 depicts the DHEAS values of the whole cohort plotted against 11OHA levels on the secondary y-axis on the left

Table 1. Patient demographics				
Age groups		**4-5 y.o.**	**6-7 y.o.**	**8 y.o.**
n value (% of the total group)		9 (21%)	23 (52%)	12 (27%)
Race	Caucasian/Non-hispanic	3 (33%)	12 (52%)	6 (50%)
	Hispanic	3 (33%)	5 (22%)	2 (17%)
	African American	2 (22%)	4 (18%)	0 (0%)
	Unknown	1 (11%)	2 (8%)	4 (33%)

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Table 2. Patient characteristics

Characteristics	Age groups		
	4-5 y.o.	6-7 y.o.	8 y.o.
BMI in the overweight range n value (% of the total group in that category)	1 (11%)	8 (35%)	1 (8%)
BMI in the these obese range	4 (44%)	7 (31%)	4 (33%)
Bone age advancement >1 year	4 (44%)	12 (52%)	6 (50%)
Presence of pubic hair	3 (33%)	13 (30)	8 (67%)
Presence of axillary hair	1 (11%)	10 (44%)	5 (42%)
Presence of body odor	8 (89%)	15 (65%)	5 (42%)
Presence of acne	3 (33%)	1 (4%)	2 (17%)
BMI: body mass index, y.o.: years old			

Table 3. Median oxo-androgen levels by age group in girls with premature adrenarche

	Age groups		
	4-5 years	6-7 years	8 years
11KT (ng/dL)	18.7 (16.0, 34.3)	20.1 (13.1, 27.3)	18.9 (16.9, 28.2)
11OH (ng/dL)	2.7 (1.5, 3.9)	3.0 (2.1, 3.6)	4.4 (2.6, 5.5)
11OHA (ng/dL)	75.0 (50.8, 84.6)	67.7 (35.4, 79.3)	63.8 (52.9, 82.4)
*Data presented as median (25th-75th percentiles)			
11KT: 11-ketotestosterone, 11OH: 11-hydroxytestosterone, 11OHA: 11-hydroxyandrostenedione			

side of the figure. The upper normal of 11OHA was indicated on the left, and the y-axis was arranged in multiples of that upper normal value (8). Our data shows that 69.6% (16/23) of those with normal levels of both DHEA and DHEAS had elevated 11OHA.

Discussion

We have demonstrated that DHEA and DHEA-S were in the normal range in many girls with premature adrenarche, while 11-oxo-androgens, particularly 11OHA and 11β-OHT, were elevated. That said, 48% of the patient population in our study were Caucasian girls from Long Island, NY, which could explain the finding and may not necessarily apply to the more diverse patient population of different ethnicities and races. This finding indicates that clinical symptoms of androgen excess were caused by 11-oxo-androgens as opposed to elevations of DHEA and DHEA-S, as previously thought. In addition, we did not see any correlation between BMI percentiles and 11-oxo-androgens. We would likely need a larger population size to confirm any correlation between BMI and 11-oxo-androgens, as it is often seen that children with premature adrenarche have increased BMI percentiles.

Rege et al. (8) identified 11KT as the dominant bioactive androgen during normal and premature adrenarche. Further prospective studies need to be conducted to determine if females with premature adrenarche with elevated oxo-androgens have a higher risk of developing polycystic ovarian syndrome (PCOS) in the future, as it has been previously shown that adolescent girls

with PCOS have higher 11-oxo-androgen values when compared to non-PCOS controls (14,15).

Conventional measurements of weaker androgens (DHEA, DHEA-S), which were previously used in patients with premature adrenarche, may not give clinicians relevant biological information about the extent of androgen excess (2,16,17).

In recent studies in patients with congenital adrenal hyperplasia, androstenedione was used as the key marker for adrenal-derived androgen excess, while it is well known that androstenedione has a mixed gonadal and adrenal origin (15,18). Therefore, when studying adrenally derived androgen excess, 11-oxo-androgens are the preferred method for clinical assessment (18), and as suggested in personal communication with R. Auchus, 2025.

Study Limitations

One of our main limitations was a small sample size for the study population. Further studies with a larger number of patients of diverse racial and ethnic backgrounds are needed to investigate if a correlation of other oxo-androgens can be found in females with premature adrenarche who have otherwise normal DHEA and DHEA-S.

Conclusion

Our data provides clinical data about 11-oxo-androgen levels in girls with premature adrenarche. Our results suggest the

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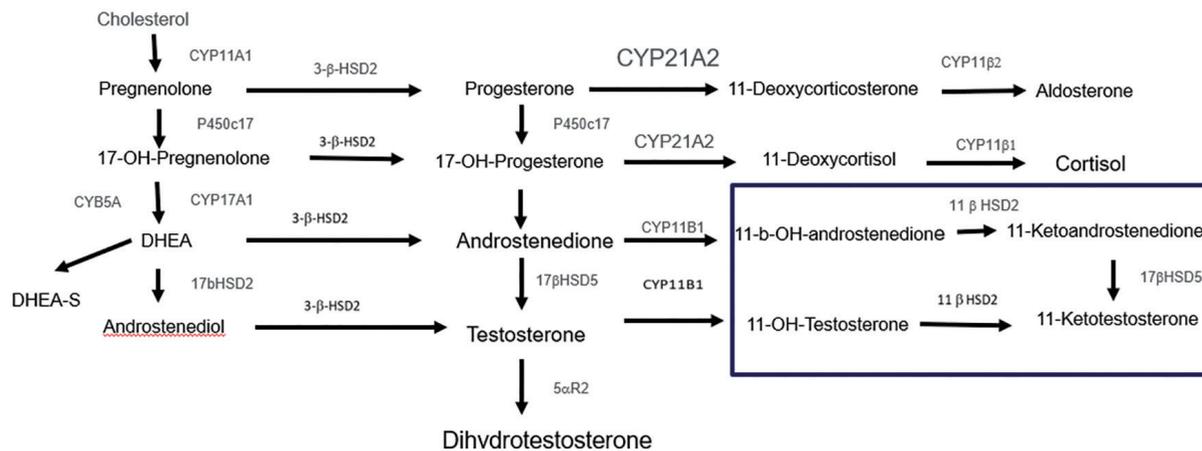
Figure 1. Steroidogenic pathway of adrenal gland [modified from Figure 3 Rosenfeld et al. (19)]
11-oxygenated androgens are enclosed in a box
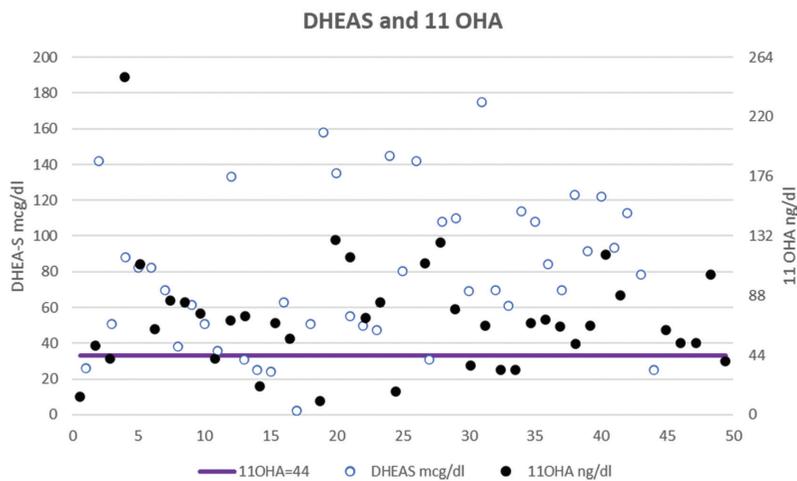
Figure 2. Relationship between 11-oxoandrogens and DHEAS levels
11OHA 44 ng/dL-upper normal value [Rege et al. (8)]

usefulness of measuring 11-oxo-androgens in a frequent clinical condition of androgen excess, in which conventional biochemical evaluation yielded inconclusive results. Perhaps early identification of these patients with premature adrenarche will permit early therapy, such as healthy lifestyle modifications, in hopes of preventing long-term complications such as metabolic syndrome, type 2 diabetes mellitus, and PCOS that are associated with premature adrenarche.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the NYU Winthrop Hospital/NYU Long Island School of Medicine Institutional Review Board (approval no.: i20-01685, date: 05.11.2020).

Informed Consent: We reviewed data retrospectively of our patients who were seen in our clinic and had this lab work done as part of their routine endocrine care.

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