

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

Gabriel L et al. Oxo-androgens and 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 Dehydroepiandrosterone- 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 DHEAS 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 DM and PCOS associated with premature adrenarche.

Abstract

Background: Endocrine findings in premature adrenarche have been characterized by elevated DHEAS levels in the past.

Methods: We reviewed 44 female patients, aged 4 to 8 years, with premature adrenarche who were seen at our center between 2019 and 2023. Data were collected on the traditional androgens (DHEA and DHEAS) and novel 11-oxo-androgens. 11-oxo-androgens, DHEAS, and DHEA levels were measured using Liquid chromatography/tandem mass spectrometry (LC/MS-MS) assays in commercial laboratories (Lab Corp).

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

Conclusion: We propose that 11-oxoandrogens are a more sensitive steroid to be measured in premature adrenarche.

Keywords: Oxo-androgens, adrenarche, child, premature

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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 (3-7).

The event of adrenarche occurs only in humans, higher primate species (chimpanzees, gorillas, and Old World monkeys) (3-7). 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 and 11-beta-hydroxytestosterone, are also potent adrenal androgens that can bind to androgen receptors and, therefore, likely play a role in the clinical picture of adrenarche (8). See Figure 1.

Both DHEA and DHEAS are typically elevated in premature adrenarche, though not in all patients. It has been thought for a considerable time 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 (8-13).

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

Materials and 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 local Institutional Review Board on November 5th, 2020, which granted us permission to look at the data retroactively and 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. 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

- 1) 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

11-oxo androgens were measured using Liquid chromatography/tandem mass spectrometry (LC/MS-MS) assays in commercial laboratories (Lab Corp). DHEAS and DHEA levels were also measured with Liquid chromatography/tandem mass spectrometry (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 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 prepubertal children's DHEAS levels 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. in their study measured levels of 11 β -hydroxytestosterone, 11-ketotestosterone, and 11-hydroxyandrostenedione in pediatric female patients with normal and premature adrenarche (8). We have used the mean range described in their study for 11-oxo androgens as the reference range for our patients, as described below.

11-hydroxytestosterone (ng/dl): ages 4-5 years mean 3.0 [2.6-6.5], ages 6-8 years mean 4.6 [3.2-6.7], ages 9-10 years mean 5.5 [4.1-6.5] (8).

11-hydroxyandrostenedione (ng/dl): ages 4-5 years mean 17.6 [11.2-34.0], ages 6-8 years mean 27.0 [20-39.7], ages 9-10 years mean 26.1 [17.4-44.8] (8).

11-Ketotestosterone (ng/dl): ages 4-5 years 8.6 mean [7.3-10.9], ages 6-8 years 13.4 mean [10.3-18.1], ages 9-10 years 17.6 mean [14.2-22.5] (8).

The recovery rate of the oxoandrogen assay is as follows: 11-Ketotestosterone: 101.1 – 107.7%, 11-hydroxyandrostenedione: 97.8 – 113.5%, and 11-hydroxytestosterone: 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 11-hydroxytestosterone was from 2.5% to 10.9%, for 11-hydroxyandrostenedione (2.9%-7.8%), and 11-ketotestosterone (2.4-4.2%) (Esoterix/LabCorp, Calabasas Hills, CA).

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. 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 have been 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 listed 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 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-oxoandrogens (in ng/dl) for each age group are presented in Table 3.

Out of 44 patients, 36 had normal DHEAS levels. Two of those had elevated 11 β -hydroxy testosterone (**P value 0.0349**, Fisher's exact test).

There was no relationship between the 11-oxo-androgen 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 demonstrates that 77.8% (14/18) of those patients with normal DHEA had elevated 11-hydroxyandrostenedione. Out of those with normal DHEAS, 75% have elevated 11 OHA. **Figure 2** depicts the entire cohort's DHEAS values plotted against 11OHA levels on the secondary y-axis on the left side of the figure. The upper normal of 11-hydroxyandrostenedione 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 DHEA sulfate had elevated 11OHA.

Discussion

We have demonstrated that DHEA and DHEAS were in the normal range in many girls with premature adrenarche, while 11-oxo-androgens, particularly 11-hydroxyandrostenedione and 11 β -hydroxytestosterone, 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 DHEAS as previously thought. In addition, we did not see the correlation between BMI percentiles and 11-oxo-androgens. We would likely need a larger population size to see a correlation between BMI and 11-oxo-androgens, as it is often seen that children with premature adrenarche have increased BMI percentiles.

Rege et al. have identified 11-keto-testosterone as the dominant bioactive androgen during normal and premature adrenarche (8). 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-oxoandrogen values when compared to non-PCOS controls (14, 15).

Conventional measurements of weak androgens (DHEA, DHEAS), 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 (CAH), 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-oxoandrogens are the preferred method for clinical assessment (18), and personal communication with R. Auchus, 2025.

Study Limitations

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

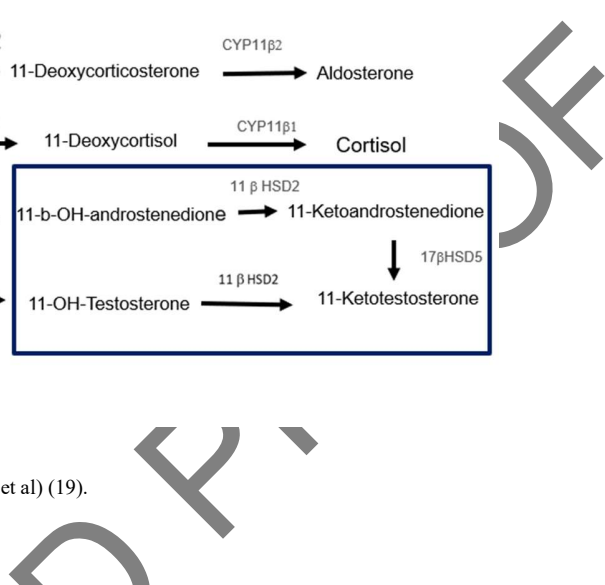
Conclusion

Our data provides clinical data of 11-oxo-androgen levels in girls with premature adrenarche and stresses the usefulness of measuring 11-oxo-androgens in a frequent clinical disease of androgen excess, in whom previous 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 Polycystic Ovarian Syndrome that are associated with premature adrenarche.

Authorship Contribution: All authors contributed equally to this work.

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et al) (19).

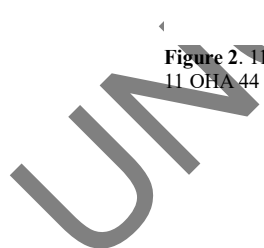


Figure 2. 11- hydroxyandrostenedione vs DHEAS levels
11 OHA 44 ng/dl – upper normal value (Rege et al.)(8).

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%)

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 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%)

Table 3. Oxo-androgen levels by Age Groups

	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)			