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A 4-hour Profile of 17-hydroxyprogesterone in Salt-wasting Congenital Adrenal Hyperplasia: Is the Serial Monitoring Strategy Worth the Effort?

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What is already known on this topic?

Clinicians need to consider various indicators, such as growth velocity, weight gain, and 17-hydroxyprogesterone (17-OHP) and androstenedione levels to avoid over- and under-treatment in children with congenital adrenal hyperplasia (CAH). However, no gold standard exists.

What this study adds?

A 4-hour 17-OHP profile is not useful in predicting hyperandrogenemia. Sex hormone-binding globulin can be considered as an indicator of hyperandrogenemia in CAH in pubertal children.

Abstract

Objective: Since there is no gold standard laboratory variable for adjustment of treatment in congenital adrenal hyperplasia (CAH), the aim was to assess the use of a 4-hour profile of serum 17-hydroxyprogesterone (17-OHP) to determine the most appropriate sample time and level of 17-OHP in predicting the metabolic control and evaluate the role of sex hormone-binding globulin (SHBG) in hyperandrogenemia.

Methods: This study included children with salt-wasting CAH. Measurements for 17-OHP and cortisol were made from samples obtained before and 1, 2, and 4 hours after the morning dose of hydrocortisone. Patients were designated to have poor metabolic control when androstenedione levels according to age and sex-specific reference intervals were high and annual height standard deviation score (SDS) changes were ≥0.5.

Results: The study cohort was 16 children (9 girls) with a median age of 7-years old. Premedication 17-OHP levels were strongly correlated with 17-OHP levels 1, 2, and 4 hours after the morning dose ($r_s = 0.929$, $p < 0.01$; $r_s = 0.943$, $p < 0.01$; $r_s = 0.835$, $p < 0.01$, respectively). 17-OHP profiles (0, 1, 2, 4 hours) of poor (n = 6) and good (n = 10) metabolically controlled cases were similar. Among the patients with poor metabolic control, two cases had 17-OHP levels < 2 ng/mL at all times. The remaining patients with poor metabolic control had median 17-OHP levels above 104 ng/mL, 82 ng/mL, 14 ng/mL, and 4 ng/mL, for baseline and 1, 2, and 4 hours, respectively. Differences between the poor and well-controlled group were androstenedione levels with respect to upper limit of normal [1.8 (1.5) and 0.5 (1.5) ng/mL, respectively p = 0.03], annual change in height SDS [0.7 (0.2) and -0.03 (0.8) SDS, respectively, p = 0.001], and daily hydrocortisone doses [7 (6) and 16 (8) mg/m^2/day, respectively, p = 0.02]. Androstenedione and SHBG levels were negatively correlated in the pubertal children ($r_s = -0.7$, $p = 0.04$).

Conclusion: We conclude that: (i) a 4-hour 17-OHP profile is not useful in predicting hyperandrogenemia; (ii) suppressed levels of 17-OHP do not always indicate overtreatment; (iii) reference intervals of 17-OHP for different time periods might be of importance; (iv) low hydrocortisone doses should be avoided; and (v) SHBG could be used in pubertal children as an indicator of hyperandrogenemia.

Keywords: CYP21A2, androgens, adrenocorticotropic hormone, steroid

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Introduction

Congenital adrenal hyperplasia (CAH) is an autosomal recessively inherited group of disorders characterized by cortisol deficiency (1,2,3,4). Neonatal screening programs report the incidence of CAH to be around 1:14,000 to 1:18,000 worldwide, and as of 2018, the approximate incidence is 1:15,000 in Turkey (3,5). CAH often develops due to pathogenic variants in the *CYP21A2* gene encoding the 21-hydroxylase enzyme. Impairment of cortisol synthesis, with or without aldosterone deficiency, causes increased secretion of adrenocorticotropic hormone with subsequent accumulation of androgen precursors (1,2).

Hydrocortisone, the mainstay of treatment, needs to be adjusted to suppress the accumulation of adrenal androgens while avoiding overtreatment and cushingoid side effects (6). 17-hydroxyprogesterone (17-OHP), androstenedione, 21-deoxycortisol, 11-oxysteroids, plasma renin activity, bone age, and height velocity are among the various indicators which are evaluated in disease control (2,3). Age and sex-specific reference ranges are accepted as targets for androstenedione (2). As for 17-OHP, another traditional marker for disease control, other than avoiding complete suppression, a consensus regarding the optimal sampling timing or reference ranges has not been made. In addition, factors limiting the accuracy of the test, such as prematurity, sickness, stress or methods of measurements also add ambiguity to the interpretation of results (2). Various strategies on sampling time including early in the morning or later in the evening, 3-4 hours after the morning dose, and even 24-hour monitoring have been suggested and assessed with other laboratory markers in the literature (6,7,8,9,10,11). Progesterone, sex hormone binding globulin (SHBG) and several other backdoor pathway metabolites measured in urine were also suggested as possible novel biomarkers (9,12). In the present study, we evaluated the efficacy of a laboratory marker, using a previously unassessed definition of metabolic control: a clinical indicator, change in height standard deviation score (SDS) over a year [annual delta height SDS (Δh SDS)] combined with a traditional laboratory marker (androstenedione). We aimed to assess the use of a 4-hour profile of serum 17-OHP to determine the most appropriate sample time and level of 17-OHP in predicting metabolic control, and evaluate the role of SHBG in response to hyperandrogenemia in terms of both clinical and laboratory parameters.

Methods

Patients

We designed a cross-sectional study with pediatric CAH patients who were followed in our pediatric endocrinology department between 2003-2021. Among 20 patients with classical CAH, aged between 2-17 years of age, one patient with inflammatory bowel disease, one receiving dexamethasone, and two patients with simple virilizing forms of CAH were excluded.

Methods

Clinical records of the patients over the last 1-year period were retrospectively collected. All patients with the salt-wasting form were diagnosed from the neonatal period, and regularly followed up in a single center. Patients had been evaluated every three months regularly, auxological measurements were obtained at each visit, and treatment adherence was encouraged by a phone call one month before the scheduled visit for the study. All patients received fludrocortisone replacement with a daily dose of 0.1 mg/d. None of the subjects had hypo- or hyperthyroidism, or hyperinsulinemia. The data collected regarding anthropometric characteristics included: age (years); sex; height [measured with a sensitivity of 0.1 cm, using a Harpenden stadiometer for those who could stand or crown-heel length was measured using a portable infantometer (Seca 417, Hamburg, Germany) for those who could not stand (cm)]; weight [measured using a scale with a sensitivity of 0.1 kg (Seca, Hamburg, Germany), (kg)]; body mass index (BMI) (kg/m^2); the respective SDSs [calculated with an online calculator for pediatric endocrinologists according to Turkish standards (13)]; height gain (Δh SDS), change in height SDS calculated by final height SDS minus height SDS measured 1-year earlier]; predicted adult height [SDS, calculated according to the Roche-Wainer-Thissen method (14)]; target height [SDS (mother's height + father's height)/2 ± 6.5]; and pubertal staging [evaluated according to Tanner and Whitehouse (15)]. Short stature was defined when height was < -2 SDS and obesity was defined if the BMI was ≥95th percentile for age and gender-specific reference ranges (13,16,17). Left hand and wrist radiographs of the patients were assessed using the Greulich-Pyle radiographic atlas and the SDSs were calculated using the tables of the atlas (18). Average hydrocortisone dose for the last six months, as well as current doses, were recorded.

Blood samples were obtained between 07.00 and 08.00 a.m., after an overnight fast, before morning doses of hydrocortisone and fludrocortisone. Serum levels of 17-OHP (ng/mL), cortisol (mcg/dL), androstenedione (ng/mL), sodium (mmol/L), potassium (mmol/L), glucose (mg/dL), SHBG (nmol/L), free T4 (ng/dL), TSH (mIU/L), and insulin (mU/L) were taken. Samples for 17-OHP and cortisol were also obtained 1, 2, and 4 hours after the morning dose. 17-OHP and cortisol were measured using

a commercial kit (catalog no LC72315, Euroka, Italy) with liquid chromatography-tandem/mass spectrometry (LC/MS) by an MS device (Shimadzu triple quadrupole, LC/MS-MS 8030, Japan). Androstenedione and SHBG were analyzed by Immulite 2000 systems (Siemens Inc, Germany). Age and sex-specific reference intervals (Immulite 2000 systems) of androstenedione (19,20) and SHBG (20) are provided in Supplementary Table 1.

Subjects were evaluated for hyperandrogenic state according to age- and sex-specific androstenedione levels (19) and annual change in height SDS (≥0.5 & <0.5) (21). Bone age was not considered as a criterion because advancements may be related to exposure to hyperandrogenic state at some point since the time of diagnosis.

Institutional approval was granted by the Ethics Committee of Dokuz Eylül University Faculty of Medicine (ethics approval number: 2021/16-25, date: 27.05.2021). When applicable, both patients and parents were required to sign the informed consent form to participate in the study.

Statistical Analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences application for Windows, version 24.0 (IBM Inc., Armonk, NY, USA). Data were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The data did not comply with normal distribution. Descriptive results were presented as median (interquartile range) (IQR) or as median (minimum-maximum) according to the distribution of the variables. Comparisons among groups of good and poor control were made using the Mann-Whitney U test for numeric variables and x^2-test or Fisher's exact test for categorical variables. Correlations were analyzed with Spearman correlation analysis. A p value of <0.05 was considered statistically significant.

Results

A total of 16 children [9 girls, 7 boys; median age 7 (7) years] with salt-wasting CAH due to 21-hydroxylase deficiency, all followed-up since the neonatal period, were included in the study. Median (IQR) values of chronological age, height, BMI, MPH, PAH SDSs were 7.1 (7), 0.3 (1.8), 1.1 (1.7), -0.6 (1.1), and -0.4 (1.7) respectively. Average hydrocortisone dose was 12 (11) mg/m^2/d. Clinical and laboratory data of each patient are presented in Table 1.

The serum levels of 17-OHP and cortisol of all patients over 4 hours are shown in Figure 1. Median (IQR) 17-OHP levels for baseline, 1, 2, and 4-hours after the medication were 22

Table 1. Auxological and laboratory data of the patients

Pt no.	Sex	Age, yrs	HC	Tanner stages	Height SDS	BMI SDS	MPH SDS	PAH SDS	Δh SDS	Bone age SDS	17-OHP, ng/mL				A4/ULN	SHBG/ ULN
											Baseline	1-hour	2-hour	4-hour		
1	M	16.9	19	5	-0.34	-0.19	-0.68	-1.10	-0.42	2.6	0.14	0.05	0.14	0.3	0.3	0.9
2	M	10.2	20	3	1.35	1.67	-0.36	0.37	0.17	3.9	0.54	0.87	0.22	8.96	0.3	0.3
3	M	3.3	24	1	-0.24	3.36	-0.90	-0.13	0.10	6.5	0.21	0.57	0.3	0.1	0.1	0.4
4	F	10.2	11	3	0.28	-0.02	-1.29	-0.87	-0.34	2	26.15	17.15	21.58	22.24	0.9	0.7
5	F	7.4	19	1	0.34	-0.58	-0.61	-0.21	0.40	3.1	6.7	1.61	1.09	0.61	0.1	0.5
6	M	6	16	1	1.13	1.01	0.21	1.47	0.45	6.8	41.43	30.2	7.14	3.35	0.7	0.6
7	M	3.4	6	1	-0.24	0.94	0.21	0.72	0.47	1	29.65	5.49	2.51	1.3	0.1	1.0
8	F	13.4	15	4	-2.15	2.96	-2.31	-3.28	-0.70	2.4	5.07	4.10	2.2	1.39	1.3	0.1
9	F	9.4	11	4	1.58	1.58	-2.06	-1.03	-0.31	3.4	38.86	23.76	9.03	18.31	2.6	0.2
10	F	10.4	15	4	1.80	1.20	-0.27	-0.56	0.8	3.4	134.56	91.30	92.26	77.35	2.8	0.02
11	F	13.6	12	5	-1.22	1.89	-1.04	-2.44	-0.16	1.8	18.4	0.27	1.16	1.38	2.3	0.3
12	F	6.9	11	2	1.20	-1.30	-0.60	0.40	0.78	3.2	0.35	0.46	0.69	0.39	1.9	0.01
13	M	2.3	6	1	-0.74	1.19	-1.98	-0.90	0.56	1.3	135.6	98.17	94.13	124.19	1.6	0.7
14	M	2.8	7	1	0.39	-0.02	-0.68	1.53	0.50	6.9	104.65	82.41	14.11	4.27	1.2	1
15	F	5.9	6	1	0.85	1.30	0.15	2.21	0.77	1.8	225.72	199.48	165.15	160.21	2.6	1.2
16	F	2.8	8	1	-2.24	0.68	-1.46	-2.06	0.70	0.8	0.83	1.05	0.31	0.49	1.1	1.2

Data are presented as numbers. Rows highlighted in grey indicate patients with poor control.
Pt no: patient number, F: female, M: male, yrs: years, SDS: standard deviation score, BMI: body mass index, PAH: predicted adult height, Δh SDS: annual change in height SDS (current height SDS-height SDS measured 1-year earlier). MPH: midparental height, HC: average hydrocortisone dose in the last 6-months (mg/m^2/day), SHBG: sex-hormone binding globulin. A4: androstenedione, 17-OHP: 17-hydroxyprogesterone, ULN: upper limit of normal.
Normal ranges for 17-OHP, <2 ng/mL

(88), 5 (69), 2 (19), and 2 (21) ng/mL, respectively. Median (IQR) cortisol levels for baseline, 1, 2, and 4-hours after the medication were 0.9 (1), 29 (16), 15 (25), and 9 (11) mcg/dL respectively. Median androstenedione level with respect to upper limits of normal was 1.1 (2).

Premedication 17-OHP levels were strongly correlated with levels measured 1, 2, and 4-hours after the morning dose ($r_s = 0.929$, $p < 0.01$; $r_s = 0.944$, $p < 0.01$; $r_s = 0.835$, $p < 0.01$, respectively). There was no correlation between the hydrocortisone dose and the reduction rate of 17-OHP ($r_s = -0.1$, $p = 0.5$).

With respect to indicators of hyperandrogenemia, nine patients had increased androstenedione levels, while growth was accelerated in six of them; and 17-OHP levels were over 10 ng/mL in seven, six, four, and four of these subjects at baseline, 1, 2, and 4 hours after, respectively. Among four patients with 17-OHP levels over 10 ng/mL at all consecutive measures, androstenedione levels were increased in three, and those who grew fast were also these three patients.

The groups of good and poor control based on the indicators of hyperandrogenemia are presented in Table 2. The 17-OHP profiles (0, 1, 2 and 4 hours) of good (n = 10) and poor (n = 6) metabolically controlled cases were similar ($p = 0.1$, $p = 0.08$, $p = 0.1$, and $p = 0.2$, respectively). Among the patients with poor metabolic control, two (33%) had 17-OHP levels < 2 ng/mL at all of the time points. When these two cases were excluded, those with 17-OHP levels measured above 104 ng/mL, 82 ng/mL, 14 ng/mL, and 4 ng/mL measured at 0, 1, 2, and 4 hours, respectively had poor metabolic control. In the poor control group, higher androstenedione levels [with respect to upper limit of normal, 1.8 (1.5) and 0.5 (1.5), respectively, $p = 0.03$], higher annual change in height SDS [0.7 (0.2) and -0.03 (0.8) SDS, respectively, $p = 0.001$], and lower daily hydrocortisone doses [7 (6) and 16 (8) mg/m^2/day, respectively, $p = 0.02$] were observed.

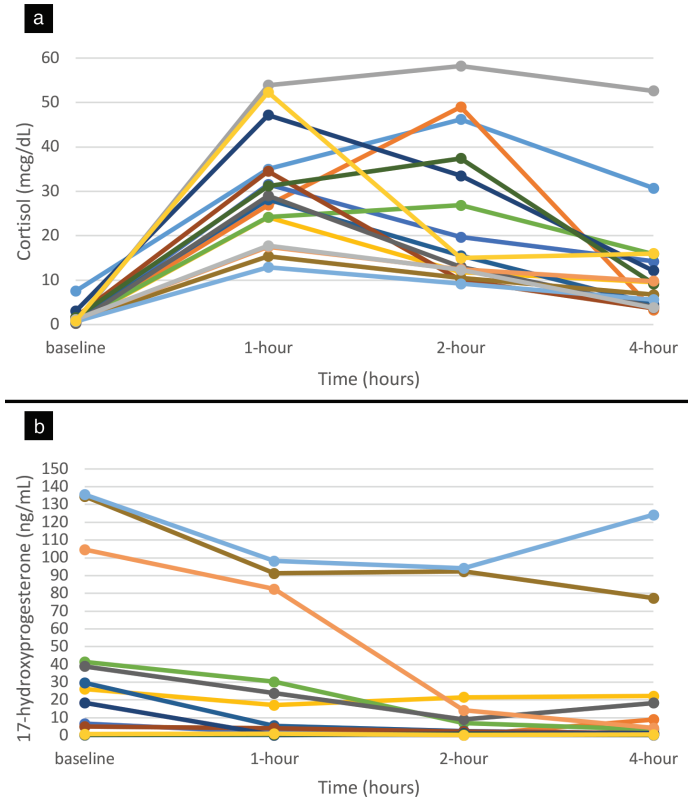

Figure 1. 17-hydroxyprogesterone (ng/mL) and cortisol (mcg/dL) concentrations after hydrocortisone dose in 16 patients over 4-hours are presented in (a) and (b)

When subjects were further divided according to their pubertal status and metabolic control was defined accordingly [poor vs good control, median (minimum-maximum)], annual change in height SDS was significantly higher in both prepubertal (n = 7) and pubertal (n = 8) children with poor control [prepubertal, 0.6 (0.5-0.8) vs 0.5 (0.1-0.5), $p = 0.03$; and pubertal 0.8 (0.78-0.8) vs -0.3 (-0.7-0.4), $p = 0.04$). Androstenedione levels with respect to upper limit of normal were increased only in the prepubertal group with poor control (0.1 (0.1-0.7) vs 1.4 (1.1-2.6), $p = 0.03$), whereas SHBG levels with respect to upper limit of normal was significantly decreased in the pubertal children with poor control (0.02 (0.01-0.02) vs 0.3 (0.1-0.9), $p = 0.04$). Androstenedione and SHBG levels with respect to upper limits of normal were negatively correlated in the pubertal children ($r_s = -0.7$, $p = 0.04$) but there was no correlation in the prepubertal group ($r_s = 0.5$, $p = 0.2$).

Discussion

This is the first study investigating the 4-hour serum profile of 17-OHP and the use of SHBG as a monitoring parameter in the context of changes in growth over a 1-year period in patients classical CAH. Several studies (7,9,22,23) evaluated the pharmacokinetic and pharmacodynamic actions of hydrocortisone. The utility of SHBG in CAH was explored with respect to other laboratory markers, such as 17-OHP which is already subject to debate in terms of reference ranges and sampling time (12). In contrast to these studies, we introduced another definition of metabolic control; in terms of a clinical indicator (annual change in height SDS) combined with a traditional laboratory marker (androstenedione) to evaluate other laboratory markers. There is a need for novel approaches since the interpretation of traditional disease markers can be challenging (2,7,9,24).

Previous studies suggested that single blood measurement of 17-OHP can be misleading, instead recommended serial samples, especially for cases of inadequate control (6,8).

Performing a 24-hour profile study for traditional markers, as recommended by Hindmarsh and Geertsma (11), would be ideal, but requires in-patient stay, increases infection risk, and may not be possible in most centers. Hence, we obtained a 4-hour profile and evaluated the laboratory results accordingly. In our study, median baseline 17-OHP concentrations were higher than the samples obtained after hydrocortisone administration. Even though serial monitored levels of 17-OHP were divergent for each patient, concentrations measured at different time points strongly correlated with the baseline values. Higher values obtained at any time point, most likely indicated that the other measurements were higher as well. On the other hand, treatment decisions based solely on the baseline measurements of 17-OHP would falsely result in overtreatment in more than half of the subjects, since either androstenedione and/or consecutive 17-OHP levels were in normal ranges for most of these patients. It is also noteworthy that Bizzarri et al (25) reported similar 17-OHP levels, mean height velocity, and androstenedione concentrations between groups of which adequacy of hydrocortisone therapy was adjusted according to samples taken prior to morning dose and 2-3 hours after the morning dose. Acknowledging these exceptions, excluding measurements taken before medication, irrespective of the sampling time, any single high value of 17-OHP may be

Table 2. Comparison of clinical characteristics and laboratory measurements between good and poor control groups

	Good control (n = 10)	Poor control* (n = 6)	p value
Age, years	9.8 [8]	4.3 [5.2]	0.06
Height SDS	0.02 [1.8]	0.6 [2.5]	0.6
BMI SDS	1.3 [2.2]	0.9 [1.6]	0.3
MPH SDS	-0.8 [1.3]	-0.6 [1.4]	0.8
Bone age SDS	2.9 [2.7]	2.5 [3.1]	0.5
Patients with advanced bone age, n (%)	8 (80%)	3 (50%)	0.3[i]
PAH SDS	-0.5 [1.9]	-0.08 [2.9]	0.3
Corrected height SDS	-0.5 [1.4]	-1 [2.5]	0.2
Annual delta height SDS	-0.03 [0.8]	0.7 [0.2]	0.001
Number of fast growing patients	0 (0%)	6 (100%)	< 0.001[i]
17-OHP (ng/mL)			
Patients with increased 17-OHP, n (%)	3 (30%)	6 (100%)	0.5[i]
Baseline	13 [31]	120 [157]	0.1
1-hour after morning dose	2.8 [18]	87 [123]	0.08
2-hours after morning dose	2 [7]	53 [111]	0.1
4-hours after morning dose	1.4 [11]	41 [133]	0.2
Cortisol (mcg/dL)			
Baseline	0.7 [1.7]	1 [0.5]	0.4
1-hour after the morning dose	30 [12]	18 [22]	0.08
2-hours after the morning dose	23 [34]	12 [10]	0.1
4-hours after the morning dose	11 [16]	8 [6]	0.7
A4 with respect to ULN	0.5 [1.5]	1.8 [1.5]	0.03
Number of patients with increased androstenedione	3 (30%)	6 (100%)	0.01[i]
SHBG with respect to ULN	0.5 [0.5]	0.9 [1.2]	0.4
Hydrocortisone dose (mg/m^2)			
6 months average of daily dose	16 [8]	7 [6]	0.02
Current daily dose	16.5 [8.4]	7 [6]	0.006
Current morning dose	6.6 [4.6]	3.8 [3.7]	0.05
Current afternoon dose	4.2 [2.5]	2 [1.7]	0.04
Current night dose	4.2 [2.4]	1.8 [1.1]	0.02

*Poor control defined when androstenedione is high and delta height SDS ≥0.5. Data are presented as median (interquartile range) or n (%).
[i]Difference between groups were calculated by Mann-Whitney U test; and [i]Fisher's exact test. All statistical assessments were considered significant at $p < 0.05$.
IQR: interquartile range, SDS: standard deviation score, BMI: body mass index, PAH: predicted adult height, Δh SDS: annual change in height SDS (current height SDS-height SDS measured 1-year earlier), MPH: midparental height, HC: average hydrocortisone dose in the last 6-months. SHBG: sex-hormone binding globulin, ULN: upper limit of normal

equally informative, only when it is evaluated with other indicators of hyperandrogenemia.

The guidelines (2) do not provide specific reference intervals for 17-OHP but complete normalization is related to overtreatment. In line with a previous report by Sarafoglou et al (7), suppressed values of 17-OHP, do not always show overtreatment as were demonstrated by patients #8, #11, #12, and #16 in our study. One alternative approach could be defining age and sex specific SDSs or time specific normal ranges for 17-OHP, as suggested by Clausen et al (26) and Neumann et al (27), but standard norms for SDSs has not been established yet.

As a monitoring parameter in disease control, we also investigated the role of SHBG, which is associated with several conditions, such as hyperinsulinemia, hyperandrogenism, and hyperthyroidism (28,29,30,31). To our knowledge, only a single report by Zamrazilová et al (12), explored the significance of SHBG in CAH in a retrospective study, irrespective of long term clinical control status, but found no relation with respect to 17-OHP levels, which are already changeable. Similarly, when we stratified groups according to indicators of hyperandrogenemia, we also found no difference in SHBG levels between the two control groups. However, SHBG levels depend on factors such as age, gender, puberty, obesity, and diet (20,32,33). When patients were further grouped according to their pubertal status, there was an inverse relationship between SHBG and androgens in pubertal children, while in pre-pubertal children, regardless of their androgen levels, SHBG levels were either at or above reference ranges. Due to the regulation of the hypothalamopituitary axis, neuroendocrine control, and the effect of insulin resistance, SHBG levels physiologically decline at puberty (30,33,34). This decline is more evident in pubertal children in the presence of hyperandrogenemia. However, high levels of SHBG seen in prepubertal children might be indicative of an age-specific protective mechanism, since SHBG determines the fraction of circulating testosterone by decreasing the metabolic clearance of testosterone, suppressing the conversion of testosterone to androstenedione, and reducing androgen availability to target cells by decreasing circulating testosterone (33,35). Further, as reported by Wallace et al (36), the administration of glucocorticoids lowers SHBG values too. This suggests that under the influence of both androgens and steroids, SHBG could be used in pubertal children as an indicator of hyperandrogenemia or a monitor of treatment adherence. However, the exact role of SHBG in prepubertal children is an area of research.

The use of the lowest effective hydrocortisone regimen in CAH is recommended for optimal long-term growth trajectory (25,37). Even though, the recommended daily dose range is 10-15 mg/m^2 in CAH patients, a recent retrospective multicenter study which included 11 countries demonstrated that up to 57% of cases used doses below 10 mg/m^2/day, whilst 75% of patient visits were above these ranges (38). Similarly, our patients received hydrocortisone doses between 6-24 mg/m^2/day. Although Pijnenburg-Kleizen et al (39) recommend higher hydrocortisone doses in early childhood, Bonfig et al (40) and Thilén et al (41) highlight the relative androgen insensitivity in growth patterns during the first 1.5 years of life. We also try to use the lowest possible hydrocortisone doses, and the growth patterns of children in our study were similar to the literature. In comparison to a meta-analysis (42) including 35 eligible studies, which showed that the corrected final height SDS of CAH patients to be 1.38 SDS lower than the population, the patients in our cohort were estimated to achieve a predicted adult height SDS of -0.4 (1.7). Although linear growth was not complete in our cohort, we believe that the clinical reasoning behind this better growth trajectory might be related to the use of lowest possible therapeutic doses, better nutritional status, and regular follow-up visits. However, in short term growth trajectory, the comparison between poor and good control groups indicates that doses lower than 7 mg/m^2/day should be avoided in CAH patients.

Study Limitations

Our study was weakened due to the retrospective nature of our data which consisted of only a small number of patients. Since our small cohort was heterogeneous with pre-, and post-pubertal children with varying ages, all factors affecting the levels of SHBG, age-defined reference ranges based on pubertal status for SHBG in CAH could not be concluded. However, interpretation of our results provides insights into SHBG in CAH but warrants further multicenter studies with a higher number of patients to unravel the underlying mechanisms. Our small numbered, salt wasting CAH cohort in a single center was also a strength, since we could evaluate the final follow-up year with standardized laboratory methods, and confounding factors, such as additional medications, concomitant diseases, or treatment adherence could be carefully monitored in a short, close period of time. As for changes in growth, to minimize the problems of pubertal height spurt, we also included a traditional laboratory marker (androstenedione) to define metabolic control status, and defined poor control when both indicators were elevated. Further, we used a change of 0.5 instead of 0.3 SDSs in height assessment, since both are recommended for monitoring growth velocity (43).

Conclusion

We conclude that: (i) the 4-hour 17-OHP profile is not useful in predicting hyperandrogenemia; (ii) normal reference intervals for different time periods should be developed for 17-OHP; (iii) contrary to the guidelines, a suppressed levels of 17-OHP does not always indicate overtreatment; (iv) low hydrocortisone doses should be avoided; and (v) SHBG can be considered as an indicator of hyperandrogenemia in pubertal children.

Ethics

Ethics Committee Approval: Institutional approval was granted by the Ethics Committee of Dokuz Eylül University Faculty of Medicine (ethics approval number: 2021/16-25, date: 27.05.2021).

Informed Consent: Retrospective study.

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Authorship Contributions

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