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Salivary morning androstenedione and 17 α -OH progesterone levels in childhood and puberty in patients with classic congenital adrenal hyperplasia

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Abstract

Background: Treatment of congenital adrenal hyperplasia due to 21-hydroxylase deficiency can be monitored by salivary androstenedione (A-dione) and 17 α -hydroxyprogesterone (17OHP) levels. There are no objective criteria for setting relevant target values or data on changes of 17OHP and A-dione during monitoring.

Methods: We evaluated A-dione and 17OHP levels in nearly 2000 salivary samples collected during long-term treatment of 84 paediatric patients with classic 21-hydroxylase deficiency.

Results: A-dione and 17OHP levels and its ratio 17OHP/A-dione remained constant from 4 to 11 years with no sex-related differences. During puberty, A-dione and 17OHP levels both increased, starting at earlier age in girls than in boys. The ratio 17OHP/A-dione declined. Normalised A-dione concomitant with elevated 17OHP [1.43 nmol/L (0.46–4.41) during prepuberty; 2.36 nmol/L (0.63–8.89) for boys and 1.99 nmol/L (0.32–6.98) for girls during puberty] could be obtained with overall median glucocorticoid doses of 11–15 mg/m²/day. A-dione levels above the upper

reference limit (URL), suggesting undertreatment, coincided with 17OHP levels ≥ 10 times URL. The percentage of A-dione levels above URL was 16% at ages 4–8 years, but increased to 31% for girls at 16 years and 46% for boys at 17 years.

Conclusions: Normalised A-dione consistent with 17OHP three times URL during prepuberty and normalised A-dione consistent with 4–6 times URL during puberty could be obtained by moderate glucocorticoid dosages. A constant 17OHP/A-dione ratio during prepuberty suggested absence of adrenarche. During puberty, a higher percentage of samples met the criteria for undertreatment, especially of boys.

Keywords: congenital adrenal hyperplasia; 21-hydroxylase deficiency; monitoring; salivary androstenedione; salivary 17 α -OHprogesterone.

Introduction

Congenital adrenal hyperplasia (CAH) is a group of inherited disorders caused by deficiency of one of the enzymes involved in the biosynthesis of adrenal steroids. The most frequent cause of CAH is deficiency of the enzyme 21-hydroxylase due to a mutation in the CYP21A2 gene, which accounts for approximately 90%–95% of the cases of CAH.

21-Hydroxylase deficiency results in impairment of the production of cortisol and, depending on the residual enzymatic activity, also of aldosterone. Due to lack of a negative feedback of cortisol to the pituitary gland, adrenocorticotrophic hormone (ACTH) concentration increases. ACTH stimulates the adrenal gland to produce cortisol. However, because of the 21-hydroxylase block, conversion of 17 α -hydroxyprogesterone (17OHP) to 11-deoxycortisol is impaired. 17OHP accumulates and subsequently converts

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into androstenedione (A-dione) and testosterone. These androgens are responsible for virilisation of female external genitalia, precocious puberty in both sexes and irregular menstruation in adolescence and adulthood [1–4].

Variability in severity of the mutation results in various degrees of residual enzymatic activity of 21-hydroxylase and different clinical and biochemical presentations [1, 3, 4]. Therefore, 21-hydroxylase deficiency is divided into classic and non-classic forms. The classic forms are the salt wasting (SW) and non-salt wasting, simple virilising (SV) types. Residual enzymatic activity is 0% in SW and 1%–2% in SV. In both classic forms there is insufficient cortisol production and excess of androgen production. Additionally, aldosterone production is impaired in SW. In the non-classic, late onset form of 21-hydroxylase deficiency residual enzymatic activity is 30%–50%. Both cortisol and aldosterone production are generally sufficient, while adrenal androgen production is still slightly increased [1, 3, 4].

The purpose of treatment of classic CAH is to overcome glucocorticoid and mineralocorticoid deficiency and suppress elevated levels of adrenal androgens. Glucocorticoid substitution results in lowering of ACTH secretion by restoring the negative feedback to the pituitary, thereby also lowering adrenal androgen production. Unfortunately, often supraphysiological doses of glucocorticoids are necessary to suppress adrenal androgen production with the potential risk of poor growth, hypertension and cushingoid features. In contrast, undertreatment might lead to persistently elevated androgens with premature epiphyseal maturation and loss of growth potential. Therefore, monitoring of glucocorticoid treatment is important [2, 5–8].

Monitoring of glucocorticoid therapy may be accomplished by measuring early morning serum 17OHP and A-dione concentrations [2, 5, 6, 9]. However, a venipuncture is inconvenient for children. Venipuncture causes stress, which may activate the ACTH-adrenal axis and influence the actual levels of steroids. Saliva has been found an excellent alternative and is almost stress free [10–12]. Collection can be done at home before the morning glucocorticoid dose, reflecting the endogenous production of androgens and the ACTH pressure on the adrenal gland. Salivary 17OHP and A-dione levels have been found to correlate well with serum values [10–12].

There are no objective criteria for setting relevant target values for A-dione and 17OHP in CAH patients. According to clinical guidelines A-dione levels should be normalised, but 17OHP levels within the normal range indicate overtreatment [5, 6]. Furthermore, no data on changes of A-dione and 17OHP levels during long-term

treatment are available. Charmandari et al. [13] indicated that puberty imposes increased difficulty in attaining adrenocortical suppression despite optimal substitution therapy and adherence to medical treatment.

The objective of the present study was to retrospectively evaluate the morning levels of A-dione and 17OHP measured in nearly 2000 salivary samples during long-term moderate glucocorticoid treatment of a large cohort of paediatric patients with 21-hydroxylase deficiency. We determined androgen changes during childhood and puberty and estimated the extent of androgen control.

Materials and methods

Patients

The data of 84 patients with classic 21-hydroxylase deficiency, treated by paediatric endocrinologists at the Radboud University Medical Centre Nijmegen in the period 1981–2010, were collected in a local database and analysed anonymously. All collected data were part of the routine follow-up within patient care. The retrospective analysis was approved by the local Ethical Committee. No informed consent was asked because of the historical and anonymous evaluation of the data.

Sixty-five patients were classified as having the SW form (32 females, 33 males) and 19 patients as having the SV form (9 females, 10 males) of CAH. Classifications were based on DNA mutation analysis, clinical presentation (genital ambiguity in females, SW crisis) and/or biochemical measurements (serum/plasma ACTH, cortisol, androgens, renin and aldosterone at diagnosis).

Therapeutic follow-up within our centre was started at 0.3 years of age (median; 75% of patients <4.0 years, 25% between 4.0 and 16.2 years) in SW and 4.0 years of age (median; 75% <5.2 years, 25% between 5.2 and 10.2 years) in SV patients. Some patients were referred to our clinic after initial treatment in another centre. Median duration of follow-up was 12.8 years (range 3.2–19.0) in SW and 11.9 years (range 6.1–18.6) in SV patients.

Patients were treated with hydrocortisone, divided into three daily doses or with dexamethasone in one or two daily doses in some postpubertal patients. In case of mineralocorticoid requirement, fludrocortisone acetate was given. Glucocorticoid doses were related to the body surface area, expressed in milligrams per square meter. For comparability, all glucocorticoid formulations were transformed into equivalent doses of hydrocortisone using dosage equivalents based on growth retarding and androgen suppressing effects, i.e., 10 mg hydrocortisone=0.125 mg dexamethasone [14, 15]. The surface area (SA) of the total body was calculated as: $\ln(SA) = -3.751 + 0.422 \cdot \ln(H) + 0.515 \cdot \ln(W)$, in which $\ln$ is the natural logarithm, SA is surface area in square meters, H is height in centimetres, and W is weight in kilograms [16].

During follow-up, the therapy was monitored as described earlier [15] every 3–6 months by evaluating clinical symptoms, length and weight, bone age once a year and biochemical monitoring (see below). Patient numbers, duration of follow-up and medication are summarised in Table 1.

Table 1 Patient numbers, follow-up and medication.

	Total CAH	Salt wasting CAH	Simple virilising CAH
Number of patients	84	65	19
Male	43	33	10
Female	41	32	9
Start follow-up, years	1.2 (0.0–16.2)	0.3 (0.0–16.2)	4.0 (0.0–10.2)
Duration of follow-up, years	12.8 (3.2–19.0)	12.8 (3.2–19.0)	11.9 (6.1–18.6)
Glucocorticoid dose, mg/m ² /day			
4–11 years	12.2 (4.6–22.8)	11.7 (4.6–21.1) ^b	14.2 (4.9–23.5)
Male	12.5 (4.6–23.3)	11.9 (4.6–20.4) ^b	14.8 (6.1–24.1)
Female	11.9 (4.8–22.2) ^c	11.5 (4.8–21.7) ^b	12.8 (4.9–21.1) ^c
12–19 years	12.6 (4.2–23.0)	11.6 (4.2–22.2) ^b	14.6 (8.0–23.8) ^a
Male	13.3 (6.6–23.1) ^a	12.3 (6.6–22.2) ^{a,b}	14.9 (8.0–27.1)
Female	11.9 (4.2–22.9) ^c	10.9 (4.2–22.2) ^{a,b,c}	13.7 (8.1–21.1) ^{a,c}

All data are given as medians and ranges. CAH, congenital adrenal hyperplasia. Glucocorticoid dose expressed as hydrocortisone equivalents [14–16]. ^aSignificant difference in glucocorticoid dose between 4–11 vs. 12–19 years of age; $p < 0.05$; ^bSignificant difference in glucocorticoid dose between SW and SV patients; $p < 0.05$; ^cSignificant difference in glucocorticoid dose between male and female; $p < 0.05$.

Biochemical monitoring

Saliva was obtained from children older than 3 years of age (for younger children salivary collection is hard to apply). Samples were collected four times a year before routine visits to the outpatient clinic of our hospital. Saliva samples were taken before administration of the glucocorticoid dose between 07.00 and 09.00 a.m. by asking the patient to salivate in a polypropylene tube (Greiner Bio-one). Clear instruction was given to prevent blood contamination in the samples. After collection, samples were sent to the laboratory and frozen at -20°C until analysis.

Three millilitres of saliva was extracted by diethyl ether and purified by paperchromatography as previously described [10, 17, 18]. A-dione and 17OHP levels in the elutes were measured by in-house radioactive immunoassays [10]. In the immunoassays antibodies raised in sheep against androstenedione-19-carboxymethylether and against 11-deoxycortisol-21-hemisuccinate, conjugated to BSA (ICN Biomedicals Inc.) were used. In the time period that is covered by this study (almost 30 years), this laboratory method remained unchanged. Long-term quality was assessed by control material, consisting of serum and saliva pools. The limit of detection of both A-dione and 17OHP in saliva was 0.02 nmol/L. Intra- and inter-assay coefficients of variation were $<8\%$ for A-dione and $<9\%$ for 17OHP.

Upper reference limits (URL) of morning A-dione and 17OHP levels are 0.24 and 0.45 nmol/L, respectively, for children ≤ 11 years of age, independent of sex [10]. URL of morning A-dione and 17OHP levels are 1.10 and 0.32 nmol/L, respectively for female adolescents/adults and 0.96 and 0.61 nmol/L, respectively for male adolescents/adults (URL was determined by logarithmic transformation of the data of previous studies [10, 19]) and subsequently, calculation of the 95% reference interval).

Data analysis

For descriptive and statistical analyses the data were categorised in intervals of years or by pubertal stage of the patients. Data collected

between 3 and 19 years of age were analysed. Data obtained between ages 3 and 4 were categorised as 4 years, between ages 4 and 5 as 5 years and so on. Pubertal stage was categorised as prepubertal or pubertal. Prepubertal stage was defined as Tanner stage < 2 . Pubertal stage was defined as Tanner stage ≥ 2 . Tanner stage 2 was reached at breast stage M2 for girls and at a testicular volume ≥ 4 mL for boys [20].

As the distribution of steroid concentrations was skewed in several groups, results are expressed as medians and ranges. Ranges are defined as minimal to maximal value, when outliers are excluded. Outliers are defined as values > 1.5 times the interquartile range from the edge of the box in the box plot (Tukey's Hinges) as calculated from SPSS. One way Kruskal-Wallis analysis was used to analyse overall differences between groups. Mann-Whitney U-test was used as a post hoc test to consider differences between two groups. Overall, p -values < 0.05 were considered statistically significant. When post hoc tests (for various age differences) were performed, a p -value < 0.01 was considered significant because of repeated testing. The ratio 17OHP/A-dione was calculated for A-dione levels > 0.05 nmol/L as very small A-dione values may influence the ratio disproportionally. SPSS version 20 was used to perform statistical analysis.

Results

Glucocorticoid medication

Glucocorticoid doses are shown in Table 1. Median glucocorticoid doses were between 11 and 15 mg/m²/day for the various subgroups. Glucocorticoid doses were significantly higher in SV than in SW patients in all subgroups and higher in boys than in girls (except for SW patients of 4–11 years). In the subgroups SW male and SV female slightly higher doses were given at 12–19 than 4–11 years,

while in the subgroup SW female lower doses were given at 12–19 than 4–11 years. The wide range of doses was due to incidentally adjustment of individual daily dosages using hormonal and linear growth data.

Salivary A-dione and 17OHP levels during glucocorticoid treatment

Figure 1 and Table 2 present morning salivary A-dione and 17OHP levels and the ratio 17OHP/A-dione in CAH patients treated with glucocorticoids. In Figure 1 the levels are plotted against age and in Table 2 the data are categorised by pubertal stage.

The total number of results was 1892 for A-dione, 1913 for 17OHP and 1462 for the ratios of 17OHP/A-dione. The number of results categorised by age varied from 18 to 169 and by pubertal stage from 311 to 500.

Prepuberty

Median A-dione levels remained constant from 4 years (0.10 nmol/L, range <0.02–0.28) to 11 years (0.11 nmol/L, range <0.02–0.64). Median 17OHP levels also remained constant during the age period of 4–11 years (1.06 nmol/L, range <0.02–6.83). The median 17OHP/A-dione ratio was about 11 (range 0.17–33.21) during this period.

Puberty

In the overall group, median A-dione concentration significantly increased at the age of 13 years (0.29 nmol/L; range <0.02–1.21; $p < 0.01$) when compared to prepubertal years. The maximal value was reached at the age of 16 years (median 0.72 nmol/L; range <0.02–2.71) and

gradually, but not significantly, declined to 0.45 nmol/L (range 0.07–1.30) at 19 years of age (Figure 1).

Similar to A-dione, 17OHP levels started to increase significantly at the age of 13 years (median 1.79 nmol/L; range <0.02–10.81; $p < 0.01$). A maximum of 17OHP was reached at 15 years (median 3.40 nmol/L;

Figure 1 Morning salivary A-dione (A) and 17OHP (B) levels (nmol/L) and the ratio 17OHP/A-dione (C) of CAH patients, categorised by age in years. Patients were treated with glucocorticoids. Results are expressed as medians and ranges (logarithmic scale). A-dione, androstenedione; 17OHP, 17 α -OH progesterone. Points which extend more than 1.5 times the interquartile length from the edge of the box are illustrated by 'o' and more than 3.0 times the interquartile length from the edge of the box are illustrated by *. The number of results per age group (age: n): (A) 4: 26; 5: 79; 6: 128; 7: 167; 8: 163; 9: 153; 10: 155; 11: 153; 12: 150; 13: 144; 14: 134; 15: 137; 16: 111; 17: 89; 18: 61; 19: 42. (B) 4: 27; 5: 81; 6: 132; 7: 169; 8: 164; 9: 154; 10: 156; 11: 155; 12: 151; 13: 144; 14: 135; 15: 137; 16: 112; 17: 91; 18: 63; 19: 42. (C) 4: 18; 5: 47; 6: 77; 7: 97; 8: 108; 9: 110; 10: 106; 11: 102; 12: 118; 13: 126; 14: 126; 15: 133; 16: 107; 17: 85; 18: 60; 19: 42.

Table 2 Morning salivary A-dione and 17OHP levels (nmol/L) and the ratio 17OHP/A-dione at prepuberty (Tanner stage <2) and puberty (Tanner stage ≥2) of patients with classic 21-hydroxylase deficiency, who are treated with glucocorticoids.

	n	Median	Range
A-dione			
Prepuberty			
Male	500	0.09	<0.02–0.52
Female	311	0.09	<0.02–0.45
Total	811	0.09	<0.02–0.51
Puberty			
Male	465	0.41 ^b	<0.02–2.12
Female	470	0.42 ^b	<0.02–1.65
Total	935	0.42 ^b	<0.02–1.80
17OHP			
Prepuberty			
Male	508	0.96	<0.02–6.91
Female	314	1.00	<0.02–6.12
Total	822	0.99	<0.02–6.60
Puberty			
Male	470	2.31 ^{a,b}	<0.02–14.47
Female	473	1.72 ^b	<0.02–8.89
Total	943	1.93 ^b	<0.02–11.14
17OHP/A-dione			
Prepuberty			
Male	319	10.47	0.17–31.96
Female	199	11.01	0.20–35.29
Total	518	10.81	0.17–34.12
Puberty			
Male	424	5.40 ^{a,b}	0.03–17.18
Female	417	4.02 ^b	0.03–12.73
Total	841	4.76 ^b	0.03–15.00

All data are given as medians and ranges. A-dione, androstenedione; 17OHP, 17 α -OH progesterone. ^aSignificant difference between male and female; $p < 0.05$; ^bSignificant difference between prepubertal and pubertal; $p < 0.05$.

range <0.02–11.70), maintained at this level until 17 years and slightly, but not significantly, decreased at 18 and 19 years of age. The ratio 17OHP/A-dione was 7.09 (range 0.20–22.67) at 12 years of age and significantly ($p < 0.01$) decreased to 2.81 at 19 years of age (range 0.28–7.10).

When patients were categorised by Tanner stage (Table 2), the median salivary A-dione level was 4.7 times higher, while median 17OHP was about two times higher in pubertal compared to prepubertal children. Correspondingly, the ratio 17OHP/A-dione was significantly lower in pubertal than in prepubertal children ($p < 0.05$).

Gender

There were no significant differences of A-dione levels between prepubertal boys and girls. A-dione levels were

twice as high in girls as in boys at 12 and 13 years, i.e., 0.29 (range <0.02–0.93) vs. 0.13 (range <0.02–0.46) nmol/L, respectively at 12 years ($p < 0.05$) and 0.41 (range <0.02–1.57) vs. 0.24 (range <0.02–0.91) nmol/L, respectively at 13 years ($p < 0.05$). At 14, 15 and 16 years of age, there were no significant differences between the sexes. At 17 years of age, A-dione levels were significantly lower in girls than in boys, i.e., 0.56 (<0.02–1.71) vs. 0.83 (0.08–5.00) nmol/L, respectively ($p < 0.05$). When patients were categorised by Tanner stage there were no significant differences of A-dione between boys and girls.

17OHP levels were significantly higher in girls than in boys at the age of 6 years, i.e., 1.48 (<0.02–7.90) vs. 0.39 (<0.02–5.60) nmol/L, but were about two times lower in girls than in boys during the pubertal period (significant at 11, 15, 16 and 17 years; $p < 0.05$). When categorised by pubertal stage, the difference of 17OHP levels between boys and girls was still present.

Salivary androgen levels suggesting optimal versus undertreatment

In Table 3 the salivary data are divided in three subgroups, i.e., 1) A-dione below URL and 17OHP above URL suggesting

Table 3 Morning salivary A-dione and 17OHP levels (nmol/L) and the ratio 17OHP/A-dione in patients with classic 21-hydroxylase deficiency, divided in 3 subgroups: 1) A-dione below URL and 17OHP above URL suggesting optimal treatment; 2) A-dione and 17OHP below URL suggesting overtreatment; 3) A-dione and 17OHP above URL suggesting undertreatment.

	n	A-dione	17OHP
4–11 years			
Total	1024	0.10 (<0.02–0.55)	1.07 (<0.02–6.83)
1. Optimal	429	0.11 (<0.02–0.24)	1.43 (0.46–4.41)
2. Overtreated	342	0.02 (<0.02–0.09)	0.12 (<0.02–0.45)
3. Undertreated	252	0.40 (0.24–1.02)	4.70 (0.49–14.50)
12–19 years			
Total	868	0.44 (<0.02–1.97)	2.02 (<0.02–11.51)
Male	485	0.41 (<0.02–2.20)	2.32 (<0.02–15.04) ^a
Female	383	0.47 (<0.02–1.71)	1.78 (<0.02–8.50)
1. Optimal	464	0.45 (<0.02–1.10)	2.21 (0.32–7.70)
Male	238	0.37 (0.02–0.96) ^a	2.36 (0.63–8.89) ^a
Female	226	0.50 (<0.02–1.10)	1.99 (0.32–6.98)
2. Overtreated	211	0.10 (<0.02–0.45)	0.15 (<0.02–0.60)
Male	121	0.10 (<0.02–0.41)	0.24 (<0.02–0.60) ^a
Female	90	0.10 (<0.02–0.52)	0.07 (<0.02–0.38)
3. Undertreated	193	1.53 (0.96–3.73)	7.64 (1.48–25.00)
Male	126	1.60 (0.96–4.60)	9.52 (1.55–27.11) ^a
Female	67	1.47 (1.11–2.32)	5.69 (1.48–17.00)

Results are expressed as medians and ranges. A-dione, androstenedione; 17OHP, 17 α -OH progesterone; URL, upper reference limit.

^aSignificant difference between male and female; $p < 0.05$.

optimal treatment; 2) A-dione and 17OHP below URL suggesting overtreatment; 3) A-dione and 17OHP above URL suggesting undertreatment.

In the group representing optimal treatment median A-dione levels were 0.4–0.5 times the URL, i.e., 0.11 (<0.02–0.24) nmol/L for children of 4–11 years of age, 0.37 (0.02–0.96) nmol/L for boys and 0.50 (<0.02–1.10) nmol/L for girls of 12–19 years of age. Median 17OHP was 1.43 (0.46–4.41) nmol/L for the age group 4–11 years, which corresponds with three times the URL. Median 17OHP was 2.36 (0.63–8.89) nmol/L for boys and 1.99 (0.32–6.98) nmol/L for girls of 12–19 years of age, corresponding with four and six times URL, respectively.

A-dione and 17OHP levels suggesting undertreatment were two and 10 times URL, respectively for the age group 4–11 years. For the age group 12–19 years median A-dione levels were also up to two times URL, while 17OHP levels were 16–18 times URL for girls and boys. When categorised by age, about 16% of the androgen concentrations met the criteria for undertreatment during 4–8 years of age, which substantially increased to 31% for girls at 16 years and 46% for boys at 17 years of age.

Discussion

We retrospectively evaluated morning salivary A-dione and 17OHP levels in nearly 2000 samples of a large paediatric cohort of classic CAH patients (21-hydroxylase deficiency). The patients were treated with glucocorticoids and monitored in our outpatient clinic from childhood to adulthood. We described changes of salivary A-dione and 17OHP during childhood and puberty. In addition, we evaluated the levels in light of optimal treatment and undertreatment by combination of A-dione and 17OHP. To the best of our knowledge no published data on salivary A-dione and 17OHP levels during long-term treatment of CAH patients are available. The present analysis is based on the same, accurate and validated steroid hormone assays throughout the observation period of about 30 years. This method employs preliminary solvent extraction and chromatography prior to the immunoassay, preventing interference from cross reacting steroids [10, 17, 18].

Moderate median glucocorticoid dosages of 11–15 mg/m²/day were provided, which is in line with current international guidelines [5, 6]. Our data show that slightly higher dosages of hydrocortisone were needed in boys than in girls and in SV than in SW patients to suppress adrenal androgens. Probably, in these patients higher hydrocortisone dosages are necessary to normalise the chronically

activated pituitary-adrenal axis. Bonfigli et al. [21] advised not to exceed the hydrocortisone dosage above 17 mg/m²/day during puberty as supraphysiological dosages of glucocorticoids may have adverse effects on pubertal growth and final height. The balance between overtreatment and undertreatment is still a clinical challenge.

During prepuberty A-dione and 17OHP levels maintained constant with no sex-related differences. In healthy children adrenarche starts around 6 years of age, initiated by an increase in CYP17 activity within the adrenal cortex. Especially the 17,20-lyase component of the CYP17 enzyme is enhanced due to increase in cytochrome B5 activity that serves as a co-factor for 17,20-lyase. Consequently, adrenal androgen production [DHEA(S), and A-dione] increases with a decrease in 17OHP/A-dione ratio during childhood. Our data showed no change of the 17OHP/A-dione ratio, suggesting no increase in CYP17 activity and therefore absence of clear adrenarche in CAH patients, corresponding to other studies [22, 23]. Chronic hydrocortisone treatment may suppress the zona reticularis activation with consequently absence of adrenarche.

Significant increases of salivary A-dione and 17OHP levels were observed during puberty indicating gonad activity and steroid production within the testes or ovary. The pattern of A-dione and 17OHP changes in CAH patients should be compared with a reference population of healthy children, categorised by sex and age or Tanner stage. Our reference values were not detailed in this respect. In literature only few reports are available on serum and even less on saliva reference values [9, 24–26]. Additionally, there is a lack of international standardisation of A-dione and 17OHP assays, cross-reactivity in immunoassays causing overestimation, and use of different techniques (i.e., immunoassay vs. LC-MS/MS), complicating comparison of studies.

Kushnir et al. [25] studied morning serum A-dione levels in more than 2500 healthy children. Concentrations of A-dione increased during puberty reaching a maximum at the age of 20–30 years and gradually declined afterwards. In smaller studies (n=132–337) comparable changes of A-dione and 17OHP in serum or saliva were found during puberty versus childhood [9, 24, 26]. Kushnir et al. [25] observed that serum A-dione levels reached adult values at Tanner stage 3 for girls and at Tanner stage 4–5 for boys. Our results in CAH patients correspond with findings of Kushnir et al. [25] that A-dione levels increase sooner in girls reflecting earlier gonad maturation [27]. This suggests that reference values of A-dione and 17OHP based on Tanner stage rather than age have to be used for monitoring of glucocorticoid therapy in CAH patients.

In literature, serum A-dione levels are about 2–3 times higher in healthy females than in males during the age period of 12–19 years [9, 25, 26]. In our CAH patients no sex differences in A-dione levels were visible when categorised by pubertal stage. This may be explained by the contribution of A-dione levels representing undertreatment in the overall group. In the same line, we found gender-different levels of 17OHP during puberty with higher 17OHP in boys than in girls, whereas no gender-related differences of 17OHP were described in healthy pubertal subjects [9, 24–26]. When only the subgroup with levels suggesting optimal treatment was considered, A-dione levels were higher in girls than in boys during puberty corresponding to the literature [9, 25, 26]. The sex difference of 17OHP levels, though, was still present.

According to clinical guidelines A-dione levels should be normalised by treatment, but 17OHP levels within the normal range indicate overtreatment [5, 6]. We found normalised A-dione levels associated with 17OHP levels three times URL during prepuberty and 4–6 times URL during puberty. A-dione levels suggesting undertreatment were up to two times URL in combination with 17OHP levels ≥ 10 times URL during prepuberty and puberty. The percentage of A-dione levels representing undertreatment was 16% during the age interval of 4–8 years and increased to 31% for girls at 16 years and 46% for boys at 17 years of age, suggesting less adrenal suppression during puberty [13, 27]. Several studies indicated that the cortisol clearance rate increases during puberty due to either inhibition of the activity of 11 β hydroxysteroid dehydrogenase type 1 or 5 α reductase activity [13, 27, 28]. In CAH patients these changes may result in decrease of cortisol concentrations, subsequently inadequate suppression of the adrenal gland and increased production of 17OHP and A-dione. Lack of compliance may further contribute to increases in adrenal androgen levels. During puberty, adherence to medical therapy is more difficult due to psychosocial factors. Hirsutism and acne may be important drives for girls to better adhere to medical therapy than boys. Moreover, the presence of testicular adrenal rest tumours in boys may contribute to enhanced production of 17OHP [29]. Further studies are necessary to investigate this hypothesis.

It is important to note that in clinical laboratory liquid chromatography-tandem mass spectrometric methods (LC-MS/MS) are replacing immunoassays for steroids. Various LC-MS/MS methods on serum A-dione and 17OHP are described [9, 25, 26, 30], but only very few reports on salivary A-dione or 17OHP are available [31, 32]. High sensitivity is needed as concentrations in saliva are low. Future research is needed to extrapolate our results to LC-MS/MS methods.

In summary, the ratio 17OHP/A-dione remained constant during prepuberty suggesting the absence of adrenarche. During puberty, A-dione and 17OHP levels increased at earlier age in girls than in boys, suggesting that reference values of A-dione and 17OHP based on Tanner stage rather than age have to be used for monitoring of glucocorticoid therapy in paediatric CAH patients. Normalised A-dione levels in combination with 17OHP three times URL during prepuberty and normalised A-dione levels in combination with 17OHP 4–6 times URL during puberty could be obtained with moderate glucocorticoid dosages of 11–15 mg/m²/day. A-dione levels suggesting undertreatment were up to two times URL, while 17OHP levels were ≥ 10 times URL during prepuberty and puberty. During puberty the highest percentage of androgen levels consistent with undertreatment was found in boys of 17 years of age.

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