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Circadian plasma cortisol measurements reflect severity of hypercortisolemia in children with different etiologies of endogenous Cushing syndrome

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Abstract

Background—The utility of circadian cortisol variation in estimating hypercortisolemia degree in different forms of endogenous Cushing Syndrome (CS) has not been evaluated in children yet.

Methods—A retrospective cohort study, including children who underwent surgery due to CS (n=115), were divided into those with a pituitary adenoma (Cushing disease) (n=88), primary adrenal CS (n=21), or ectopic adrenocorticotropin or corticotropin-releasing hormone (ACTH/CRH) secreting tumors (n=6).

Circadian plasma cortisol measurements were obtained at 11:30 PM and midnight and at 7:30 and 8:00 AM. The ratios between the morning and late-night concentrations were calculated.

Results—Plasma cortisol AM/PM ratios negatively correlated with 24 hour urinary free cortisol (UFC) collections among the full study population and in each of the individual etiologies. Plasma ACTH concentrations positively correlated with plasma cortisol AM/PM ratios among patients with ACTH independent CS. Finally, patients with primary pigmented adrenocortical disease (PPNAD) showed no correlation between UFC collections and plasma cortisol AM/PM ratio, in contrast with other etiologies for primary adrenal CS which showed strong negative correlation between them.

Conclusions—Our study shows the association between plasma cortisol AM/PM ratio with the degree of hypercortisolemia in children with CS.

Keywords

cortisol; Cushing; circadian

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Introduction

Endogenous Cushing syndrome (CS) is caused by excess secretion of adrenocorticotropin (ACTH) or cortisol leading to dysregulation of hypothalamic-pituitary-adrenal (HPA) axis. Thus, primary adrenal CS is due to autonomous secretion of cortisol from the adrenal gland; Cushing disease (CD) is caused by an ACTH-secreting pituitary adenoma, and ectopic CS is caused by ACTH and/or CRH secretion from a non-pituitary neoplasm [1].

The normal physiology of cortisol secretion follows a circadian pattern, peaking after awakening in the morning, and reaching a nadir around midnight [2]. However, among adult patients with CS, circadian cortisol variation is diminished [3]. Hence, the current guidelines for CS diagnosis in adult patients cite late night hypercortisolemia as measured by midnight salivary cortisol as a criterion for CS diagnosis [4], and similar tests are used for CS diagnosis in children [5]. Moreover, beyond its use for CS diagnosis, diminished circadian cortisol variation is both a cardiovascular [6] and metabolic risk factor [7], even among normocortisolemic subjects.

In the current analysis we investigated the circadian cortisol variation among children with different etiologies of CS, as expressed by the ratio between early morning and midnight (AM/PM) plasma cortisol. We analyzed the association between this variation and 24 hour urinary free cortisol collections among patients with different etiologies for CS.

Patients and methods

We conducted a retrospective cohort study, including consecutive patients aged ≤18 years, that were admitted to the National Institutes of Health for evaluation of suspected CS between 1995 and 2015. All patients were recruited through clinical protocols 97-CH-0076, 95-CH-0059, and 00-CH-0160 conducted by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) and their families gave written informed consent.

All patients were hospitalized and underwent screening tests for CS, including serial 24 hour urinary free cortisol (UFC) collections and circadian plasma cortisol measurements. Circadian plasma cortisol measurements were performed during hospitalization, using an indwelling venous catheter with designated extension to avoid sleep disturbance during the night measurements. Blood samples were drawn at 7:30 and 8:00 AM for the morning measurements and at 11:30 PM and midnight for the late-night samples (two measurements in the morning and two at late-night, respectively). Calculated mean values of the AM and PM samples, respectively, were used for the final analysis. The current analysis included patients ≤18 years with either elevated 24 hour UFC or elevated midnight plasma cortisol (>4.4 mcg/dL). Patients diagnosed with CS underwent further investigation in order to locate the source of hypercortisolemia. Patients then went on to surgical intervention, either adrenalectomy or transsphenoidal surgery, as appropriate.

All pathological reports were reviewed by the authors; CD was defined as a pituitary adenoma with positive ACTH immunohistochemistry, and adrenal lesions were defined as previously described [8].

Statistical analysis

Statistical calculations were performed with SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) and R statistics, version 3.2.2. Results are expressed as mean ± standard deviation (SD) unless otherwise indicated. For group comparisons, the independent Student's t-test or one-way analysis of variance (ANOVA) were used to analyze differences in numerical variables, the chi-square test was employed to analyze differences in categorical variables. Variables with a non-normal distribution were analyzed using non-parametric tests, as appropriate. The Pearson product was used for analysis of correlations between variables. The p value for significance was set at less than 0.05.

Results

Description of the cohort and overall data

The current analysis included 115 children, aged 11.7±3.7 years, 65 females (56.5%). The patients' mean time from first symptom to diagnosis was 2.1±1.4 years. Eighty eight patients (76.5%) had CD, 21 (18.3%) had primary adrenal CS (3 adrenal adenomas, 7 micronodular hyperplasia, 1 macronodular hyperplasia and 10 – primary pigmented nodular adrenocortical disease [PPNAD]), and 6 patients (5.2%) had ectopic ACTH/CRH secreting tumors.

Mean plasma cortisol concentrations for the whole cohort were 16.4±10.4 mcg/dL at midnight, and 18.2±9.9 mcg/dL when measured in the morning, with an AM/PM ratio of 1.3±0.8 and a mean 24 hour UFC of 7.9±11.6 times the upper limit of the reference range (xULN). The patients' mean BMI z-score was 1.9±0.9 and the mean height z-score was −0.7±1.3. Patients with ectopic CS had highest, and those with primary adrenal CS had lowest 24 hour UFC collections. Demographic and biochemical characteristics of the patients according to their histopathological diagnoses are detailed in Table 1.

UFC and AM/PM cortisol ratio correlation in the different etiologies of CS

Patients with a lower 24 hour UFC collections (<2 xULN, n=28) retained circadian cortisol rythmicity more than those having a higher 24 hour UFC collections (n=82, 1.8±1.2 vs. 1.1±0.6 xULN, p=0.01, respectively). This was also reflected in the negative correlation between 24 hour UFC collections and plasma cortisol AM/PM ratios (n=111, r=−0.3, p=0.001). Moreover, a subgroup analysis according to the CS etiology revealed a weak negative correlation among patients with CD (r=−0.3, p=0.002), a similar trend among those with primary adrenal CS (r=−0.4, p=0.06), and a strong negative correlation among subjects with CS due to ACTH/CRH secreting tumors (r=−0.9, p=0.007, Figure 1).

Morning ACTH concentrations positively correlated with plasma cortisol AM/PM ratio among patients with primary adrenal CS (r=0.9, p<0.001) but had no correlation (r=0) among those with either CD or ectopic ACTH/CRH secreting tumors (Figure 2).

Primary adrenal CS – comparison between different etiologies

Patients with PPNAD had comparable mean plasma cortisol AM/PM ratios (1.3±0.7 vs. 1.2±0.6, NS, respectively), mean 24 hour UFC collections (3.6±6.9 vs. 4.9±6.2 xULN, NS), mean midnight (12.1±6.4 vs. 16.9±8.5 mcg/dl, NS) and morning plasma cortisol

concentrations (14.2±6.2 vs. 17.6±7.9 mcg/dl, NS), and mean morning plasma ACTH concentrations (7.6±4.7 vs. 7.9±8.1, NS) with subjects having other etiologies for primary adrenal CS. In addition, a strong negative correlation (r=−0.8, p=0.01) was found among those with other etiologies for primary adrenal CS (figure 3).

Discussion

In the current analysis we investigated the association between plasma cortisol AM/PM ratio with 24 hour UFC values in children with different etiologies of CS. We found that patients with a higher degree of cortisolemia had more loss of circadian cortisol rythmicity (Figure 1), as expected. We found a strong positive correlation between ACTH concentrations and plasma cortisol AM/PM ratio among patients with primary adrenal CS, whereas no correlation was found among subjects with either CD or ectopic ACTH/CRH-secreting tumors (Figure 2). Finally, among patients with primary adrenal Cushing syndrome, subjects with a primary adrenal CS other than PPNAD showed strong negative correlation between these two measures (Figure 3).

Measurement of midnight plasma cortisol in adults was found to be highly sensitive for diagnosing CS [9]. Similarly, late night plasma cortisol measurement of less than 4.4 mcg/dL in children had 100% specificity for ruling out CS [5]. We used the plasma cortisol AM/PM ratio as an estimate for circadian variation, and showed that it negatively correlated with 24 hour UFC collections, a valid measure for hypercortisolemia, among all etiologies for CS (Figure 1).

Suppression of plasma ACTH concentrations due to elevated plasma cortisol concentrations is expected in a normally functioning HPA axis [10]. In patients with ACTH or CRH-secreting tumors, plasma ACTH concentrations mainly reflect the degree of the tumor's secretion, whereas among those with autonomous adrenal cortisol, the pituitary ACTH secretion is low due to negative feedback. Thus, it is not surprising to find lack of correlation between plasma ACTH concentrations and plasma AM/PM ratios among subjects with CD or ectopic CS, and a preserved correlation among those with primary adrenal CS (Figure 2), who obviously did not have a fully suppressed HPA axis.

PPNAD is caused by a germline mutation in the gene encoding the regulatory subunit of protein kinase A (*PRKAR1A*)[11], and usually presents as a part of Carney complex (CNC) [12]. We have shown a lack of correlation between 24 hour UFC collections and circadian cortisol rhythmicity in PPNAD (Figure 3), in contrast to high correlation between them in other forms of primary adrenal CS. This is in light of comparable plasma and urine cortisol concentrations between PPNAD and non-PPNAD primary adrenal CS. We suggest three possible explanations for the lack of circadian cortisol rhythmicity in PPNAD patients compared with other primary adrenal CS etiologies: First, the lack of unaffected adrenocortical cells in the PPNAD-affected adrenal cortex, compared with other etiologies for primary adrenal CS might have stronger suppression of the normal circadian rhythm. Second, there might be a higher endogenous activity of the PPNAD-affected adrenocortical cells compared with other etiologies. Third, adrenocortical cells in PPNAD might have altered expression of "clock genes"[13], that were demonstrated in unaffected adrenocortical

cells. However, the small number of patients in the PPNAD group limits our ability to draw definite conclusion.

The main strength of this study is the large sample size, and the full and uniform evaluation performed for all the patients. Moreover, all diagnoses for this study subjects were classified according to the histopathological evaluation.

In conclusion, we have shown that lower circadian cortisol rythmicity reflects higher degree of cortisolemia in general, and specifically in various forms of endogenous CS.

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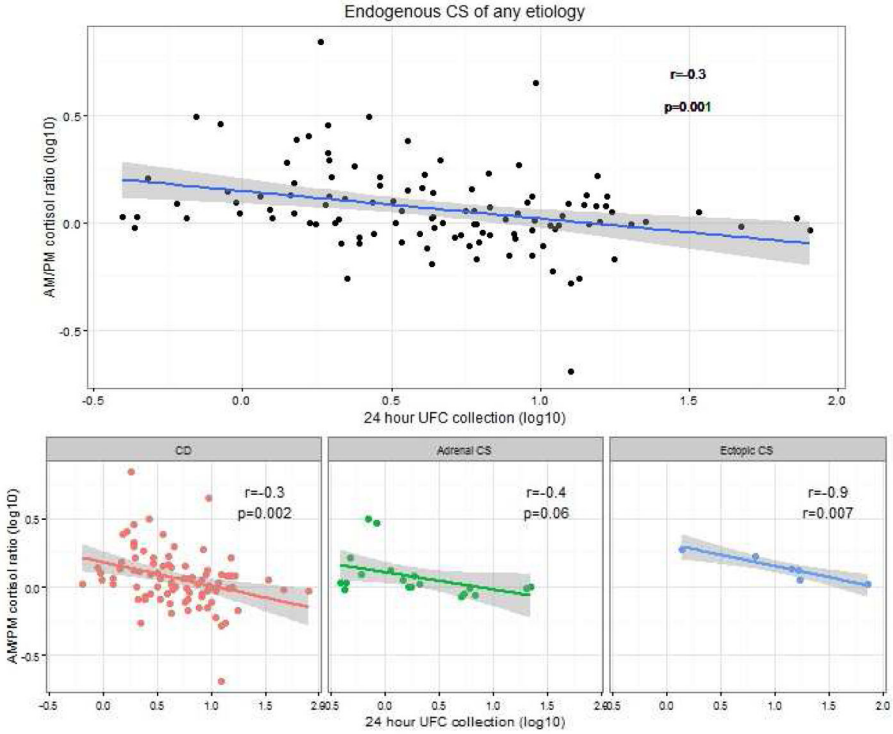

Figure 1.

Correlation analysis between 24 hour UFC collection and plasma cortisol AM/PM ratio according patient's CS etiology.

UFC, 24 hour urinary free cortisol collection; CS, Cushing syndrome

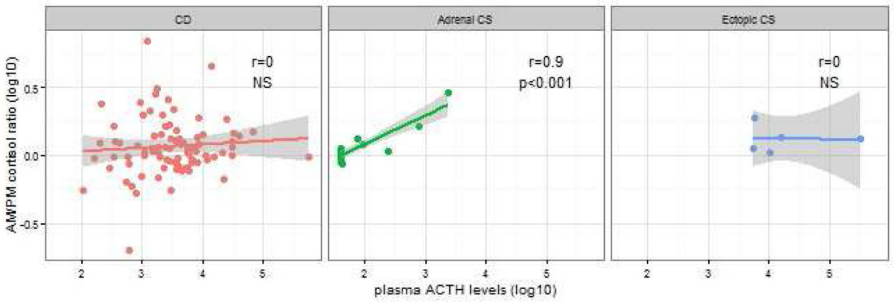

Figure 2.

Correlation analysis between morning plasma ACTH concentrations and plasma cortisol AM/PM ratio according to the etiology of CS.

CS, Cushing syndrome; ACTH, adrenocorticotropin; CS, Cushing syndrome

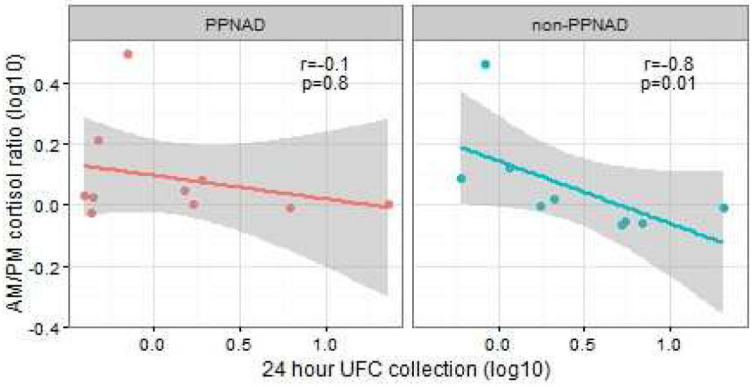

Figure 3.

Difference in circadian cortisol variation between patients with PPNAD vs. other primary adrenal CS: Mean plasma cortisol AM/PM ratio (A), and correlation between 24 hours UFC collection and plasma cortisol AM/PM ratio (B)

UFC, urinary free cortisol; CS, Cushing syndrome; PPNAD, primary pigmented nodular adrenocortical disease

Table 1

Patients' demographic and baseline biochemical characteristics according to patients' diagnoses

	CD	Primary adrenal CS	Ectopic CS	p value		
	n=88	n=21	n=6	CD vs. adrenal	CD vs. ectopic	Adrenal vs. ectopic
Female gender n(%)	48 (54.5)	14 (66.7)	3 (50.0)	NS	NS	NS
Age (years)	12.2±3.1	9.0±4.6	13.5±3.9	0.007	NS	0.04
Time from symptoms to diagnosis (years)	2.2±1.5	2.0±1.1	2.0±1.0	NS	NS	NS
BMI z-score	2.0±0.9	2.0±1.1	1.5±1.1	NS	NS	NS
Height z-score	−0.9±1.3	0±1.6	−0.6±1.0	0.02	NS	NS
Ethnicity n(%)				NS	NS	NS
Caucasian	65 (73.9)	18 (76.2)	3 (50.0)			
African-American	8 (9.1)	2 (9.5)	2 (33.3)			
Asian	5 (5.7)	0	0			
Hispanic/Latino	8 (9.1)	3 (14.3)	1 (16.7)			
Unknown	2 (2.2)	0	0			
Plasma cortisol						
AM concentrations (mcg/dL)	18.1±10.1	16.0±7.2	25.9±12.8	NS	NS	0.02
PM concentrations (mcg/dL)	16.5±10.8	14.6±7.8	20.7±13.2	NS	NS	NS
AM/PM ratio	1.3±0.9	1.3±0.6	1.4±0.3	NS	NS	NS
ACTH						
AM plasma concentrations (pg/mL)	40.9±37.9	7.8±6.5	91.5±89.2	<0.001	0.02 MW	<0.001 MW
PM plasma concentrations (pg/mL)	47.1±62.0	5.7±1.8	86.7±88.0	0.02	NS	<0.001 MW
AM/PM ratio	1.1±0.7	1.2±0.8	1.2±0.5	NS	NS	NS
24 hour UFC						
Mean level (xULN)	7.8±10.5	4.2±6.4	21.4±25.7	0.001 MW	0.045 MW	0.02 MW

Data are presented as mean±SD unless mentioned otherwise.

CD, Cushing disease; CS, Cushing syndrome; BMI, body mass index; ACTH, adrenocorticotropic hormone; UFC, urinary free cortisol; ULN, upper limit of the reference range

Comparisons were performed using ANOVA and chi square tests for continuous and categorical variables, respectively.