INVESTIGATION FOR PAEDIATRIC CUSHING'S SYNDROME USING 24-HOUR URINARY FREE CORTISOL DETERMINATION

Shapiro L[1], Elahi S[1], Riddoch F[2], Perry LA[3], Martin L[1], Akker SA[1], Monson JP[1], Drake WM[1], Grossman AB[4], Savage MO[1], Storr HL[1]

[1]Centre for Endocrinology, William Harvey Research Institute, Queen Mary University of London, Barts and the London School of Medicine and Dentistry, London, EC1M 6BQ, UK. [2]Department of Clinical Biochemistry, Barts Health NHS Trust, Whitechapel, London, E1 1BB, UK. [3]Pathology Department, Croydon Health Services, 530 London Road, Croydon, CR7 7YE, UK. [4]Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford OX3 7LJ, UK.

Correspondence:
Dr Helen L. Storr,
Reader and Honorary Consultant in Paediatric Endocrinology,
Centre for Endocrinology, William Harvey Research Institute,
Barts and the London School of Medicine,
Queen Mary University London,
First Floor, John Vane Science Centre,
Charterhouse Square,
London EC1M 6BQ.
Email: h.l.storr@qmul.ac.uk
Fax: +44 (0)20 7882 6197

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Abstract

Objective Paediatric Cushing's syndrome (CS) remains a challenge to diagnose and exclude. We assessed the accuracy of 24-hr urinary free cortisol (UFC) determination in children referred for suspected CS.

Design Retrospective study of paediatric patients referred to our centre with suspected CS between 1982 and 2014.

Patients 66 subjects (mean age 12.9 yr; range 4.4-16.9): 47 cases of CS (29M), which included: Cushing's disease (CD) (39 patients, 25M), primary pigmented nodular adrenocortical disease (PPNAD) (8 patients, 4M) and 19 'controls' (6M) in whom the diagnosis of CS was excluded.

Measurements Subjects had between one and five 24-hr UFC collections analysed by radioimmunoassay, chemiluminescent immunoassay or liquid chromatography-mass spectrometry. Data were normalised, corrected for body surface area (m^2), and assessed using the Receiver Operating Characteristics (ROC) analysis and an independent 2-tailed t-test.

Results Diagnostic accuracy of 24-hr UFC for CS was excellent (AUC 0.98, 95% CI 0.946-1.00, sensitivity 89%, specificity 100%).

Conclusions 24-hr UFC is a reliable and practical investigation with high diagnostic accuracy for paediatric CS. However, further investigations may be required if the UFC is normal but there is a high diagnostic suspicion of CS.

Abbreviations: ACTH, adrenocorticotropic hormone; AUC, area under the ROC curve; PPNAD, primary pigmented nodular adrenocortical disease ; BSA, body surface area; CI, confidence interval; CD, Cushing's disease; CRH, corticotrophin-releasing hormone; CS, Cushing's Syndrome; HDDST, high-dose dexamethasone suppression test; IA, immunoassay; BSIPSS, bilateral simultaneous inferior petrosal sinus sampling; LCMS, liquid chromatography mass spectrometry; LDDST, low-dose dexamethasone suppression test; NR, normal range; p, value of probability; PCOS, polycystic ovarian syndrome; r, regression coefficient; RA, radioimmunoassay; ROC, receiver operating characteristic; SDS, standard deviation score; SEM, standard error of mean; UFC, urinary free cortisol.

Introduction

Endogenous Cushing's syndrome (CS) is extremely rare in childhood (1) and is characterised by a deceleration in height velocity in association with significant weight gain. Despite these hallmark features, CS may be difficult to recognise because of its very low incidence in the paediatric population and its insidious onset in many individuals (1, 2). The increasing incidence of paediatric obesity is well recognised (3), and underlying pathological causes need to be excluded. Diagnosing CS in children is particularly challenging as its presenting features are variable and can be subtle, especially in young patients (4). As a result, there is often a significant delay in diagnosing CS in the paediatric age range (5), which can result in significant morbidity, mortality and a reduction in quality of life (6-9). Therefore, it is important to have a readily available and robust investigation to accurately identify cases of CS amongst children with unexplained weight gain.

Published diagnostic guidelines for CS (10-13) are heavily based upon adult studies, as data in children with this disorder are scarce. However, there are important differences between paediatric and adult CS. Pubertal delay or arrest is frequently seen in paediatric CS patients caused by suppression of the hypothalamo–pituitary–gonadal axis by hypercortisolaemia.

Frequently reported symptoms and signs in childhood include a change in facial appearance, lethargy and emotional lability, whereas in adults, headaches, hypertension, striae and acne are common additional findings. There are also recognised aetiological differences between adult and paediatric CS groups; for example, pituitary macroadenomas are much less common in paediatric patients (5) and ectopic ACTH-secreting tumours are exceptionally rare (14, 15).

Existing investigations for the diagnosis and differential diagnosis of CS include 24-hour urinary free cortisol (24-hr UFC) determination, 09.00 plasma ACTH, sleeping 00.00 cortisol, late-night salivary cortisol, low- and high-dose dexamethasone suppression tests (LDDST and HDDST), corticotrophin-releasing hormone (CRH) test, pituitary MRI, abdominal imaging and bilateral simultaneous inferior petrosal sinus sampling for ACTH (BSIPSS) (16-19). The 24-hr UFC measurement has been widely used as an investigation for hypercortisolaemia and is reported to have a sensitivity of >90% in adult populations (20, 21). 24-hr UFC excretion is not affected by changes in cortisol binding globulin, which was reported to be a potential disadvantage in the measurement of serum cortisol (6). The Endocrine Society recommended 24-hr UFC as an initial investigation for CS (10); however, more recent studies have shown that its value in adults with suspected CS is limited because of high variability, particularly in cases of subclinical or cyclical CD (22, 23).

In paediatric practice, controversy regarding the value of UFC measurement still exists. Additionally, the 88% reported sensitivity of UFC determination for children with CS is slightly lower in the paediatric than adult age range (17). It has also been suggested that increased UFC excretion may not be a consistent finding in paediatric CS, and therefore serial measurements are necessary to rule out this diagnosis (17).

The objective of the present study was to assess the accuracy of 24-hr UFC excretion in paediatric patients referred for evaluation of suspected CS in our centre. We also evaluated

24-hr UFC levels in two different diagnostic groups, CD and PPNAD, and assessed the effect of gender and severity of CS on UFC concentrations.

Materials and Methods

Patients and data collection

A retrospective review of paediatric cases with suspected CS referred to Barts Health NHS Trust between 1982 and 2014 was undertaken. The series comprised 66 subjects: 39 cases of Cushing's disease (CD), 8 with CS due to primary pigmented nodular adrenocortical disease (PPNAD) and 19 subjects, in whom CS was subsequently excluded, who served as a control group. The patient characteristics are shown in **Table 1**. Clinical informed consent was obtained from the patients and/or their parents and, as part of an ongoing prospective audit into the investigation and outcome of patients with Cushing's syndrome, institutional review board permission was granted for the release of anonymised data for publication.

Symptoms and signs

The clinical features of the patients at diagnosis are shown in **Tables 2 and 3**. Mean duration of symptoms was 2 yrs (0.25-5.0 yrs) before diagnosis of CS and 1.7 yrs (0.4-5.0 yrs) before CS was excluded in the control group. Hypertension (HTN) in the paediatric subjects was defined as a diastolic or systolic blood pressure (BP) >95th centile for age and sex on more than two occasions (24).

Diagnosis of Cushing's syndrome (CS)

All subjects (total 66 patients; 35 males), mean age 12.9 yr; (range 4.4-16.9) had clinical features suggestive of hypercortisolaemia and diagnostic tests were performed according to published paediatric and adult endocrinology protocols (25-28) (**Table 4**). These included 09.00 plasma ACTH, sleeping 00.00 cortisol, LDDST, CRH testing, radiological investigations (pituitary MRI or adrenal imaging) and BSIPSS. CS was excluded in all

subjects in the control group (total 19 patients; 6 males), mean age 11.5 yr (range 4.4-15.7) by at least one undetectable (<50nmol/L) sleeping midnight serum cortisol with suppression of serum cortisol to <50nmol/L during a LDDST (**Table 4**) and by subsequent clinical follow-up.

Auxology and puberty staging

Height and weight were measured in all patients using standardised techniques (29). Puberty was assessed using Tanner's criteria (30, 31).

24-hr UFC collection

All patients had one to four completed 24-hr UFC collections performed in the hospital. Some of the 19 control subjects had UFC collections on non-consecutive days. The mean value was calculated for those having more than one collection and used in further analysis.

Assays

24-hr UFC was measured by one of three methods, reflecting the change in laboratory practice over the 30-year period. Until 1995, in-house radioimmunoassay (RA) was used (NV <240 nmol/24-hr). Between 1995 and February 2012, Siemens 2000 immunoassay (IA) was used (NR 40-340 nmol/24-hr). Since February 2012, a liquid chromatography-mass spectrometry (LC-MS) method has been used (NR <124nmol/24-hrs). To account for the differences in the reference ranges of the 3 assays, each data point has been normalised to generate unitless values, as described elsewhere (32). The patient's 24-hr UFC value was then expressed as a fraction of the upper limit of the assay's normal range. Therefore, values less <1.0 are within the normal range and values >1.0 are higher than the upper limit of the assay reference range. 24-hr UFC values were then corrected for body surface area (BSA, m^2).

Statistical analysis

Data were analysed by SPSS (version 22; IBM Corp. Armonk, NY). All values were assessed against the corresponding normal range (NR) and included in calculating the diagnostic accuracy of the 24-hr UFC as a test for CS. The diagnostic accuracy was depicted by the AUC values from receiver operating characteristic (ROC) analysis performed against the control group. The area under the ROC curve (AUC) quantifies the overall ability of the test to discriminate between those individuals with the disease and those without the disease. A perfect test i.e. one that has zero false positives and zero false negatives has an area of 1.00. The results of ROC analyses were classified according to the AUC values as fail (AUC=0.50-0.59), poor (0.60-0.69), moderate (0.70-0.79), good (0.80-0.89) and excellent (0.90-1.00). An independent 2-tailed t-test was performed to calculate the differences between the mean 24-hr UFC values.

Results

24-hr UFC measurements in the CS group

In the subjects with CS, 53% (n = 25) had one, 30% (n = 14) had two, 13% (n=6) had three and 4% (n=2) had four 24-hr UFC collections. In the control group, 11% (n = 2) had one, 47% (n = 9) had two, 31% (n = 6) had three, and 11% (n = 2) had five collections. 24-hr UFC/BSA values for all patient groups are shown in **Figure 1**. When corrected for BSA, the diagnostic accuracy for CS, as measured by area under the curve (AUC) was high (0.98, 95% CI 0.946-1.00), with sensitivity and specificity for CS being 89% and 100% respectively.

Five patients with CD had normal mean 24-hr UFC/BSA ratio. All but one had typical Cushingoid phenotypes. Patient 1 had CD with cyclical hypercortisolaemia. She was an 11.9 yr old female with height -2.0 SDS, BMI 2.5 SDS, excessive virilisation, striae and headaches. She had a pituitary macroadenoma and her UFC/BSA ratios were 0.17, 0.06,

and 0.06 (mean 0.10). Patient 2 was a 15.6 yr old pubertal male (Tanner stages PH 4, genitalia 4, testes 12 ml) with striae, height -1.1 SDS and weight 3 SDS. His UFC/BSA ratios were 0.33 and 0.52 (mean 0.42). Patient 3, a male, aged 6.4 yr was excessively virilised (Tanner stages PH 2, Genitalia 2, testes 2ml), with height - 1.6 SDS and BMI 5.1 SDS and had UFC/BSA ratios of 1.00, 0.68 and 0.73 (mean 0.8). Patient 4, a female aged 13.2 yr had Cushingoid appearance, height - 2.8 SDS, BMI 2.3 SDS, hypertension, striae and headaches and UFC/BSA ratios of 0.86 and 0.92 (mean 0.89). Patient 5, a 13.2 yr male had normal height (0.1 SDS) , BMI (- 0.4 SDS), with normal puberty (Tanner stages Genitalia 3, PH 2, AH 2, testes 8, 10 ml and hypertension. with a single UFC/BSA ratio of 0.94.

24-hr UFC measurements in the control group

In the control group, all subjects had mean 24-hr UFC concentrations within the normal range when corrected for BSA, demonstrating specificity of 100%.

24-hr UFC measurements in CD versus PPNAD

A comparison of 24-hr UFC measurements in patients with CD and PPNAD was undertaken. When adjusted for BSA, no significant difference was found; CD mean 2.32, PPNAD mean 2.53, p value 0.7, 95% CI -1.28 to 0.88.

Discussion

In the context of the increasing incidence of paediatric obesity and associated increase in the number of children referred with possible CS, it is useful to have a simple, reliable, non-invasive and inexpensive investigation, which provides information relevant to hypercortisolaemia. The advantage of 24-hr UFC determination is that the collections can be arranged at home or locally without the need for referral to a tertiary unit.

In contrast to the adult CS literature, few studies focus on investigations in the paediatric age range. We report the utility of 24-hr UFC determination in paediatric patients referred with possible CS. Our results demonstrate a high sensitivity and specificity of 24-hr UFC for the detection of hypercortisolaemia. This confirms a previous report which showed sensitivity and specificity of 24-hr UFC/BSA values of 88% and 90%, respectively in paediatric CS, and collections over more than one day improving accuracy (17). This suggests that measuring 24-hr UFC is a useful non-invasive test which helps differentiating CS from simple obesity in paediatric practice. We found higher 24-hr UFC concentrations in the subjects with PPNAD compared to those with CD but the difference was not significant when adjusted for BSA. This has not previously been examined in adults or children with CS. Our results emphasise the need to take BSA into account when analysing 24-hr UFC concentrations.

The patient with cyclical CS had normal 24-hr UFC. These results are in agreement with published adult studies which suggest that 24-hr UFC might be a suboptimal test in mild disease, cyclical CS and in patients with renal impairment (23). UFC measurements may be negative or borderline when the hypercortisolaemia is mild or periodic. These borderline abnormalities in cortisol hypersecretion appear to be more common in the adult population and UFC measurements are less useful in this clinical situation. If 24-hr UFC is repeatedly normal but clinical suspicion remains, there is a small chance that CS the result is false positive and other investigations should be undertaken. Measurement of midnight salivary cortisol may be a more appropriate alternative screening test for these difficult cases (33).

The predictive power of the 24-hr UFC measurements may be further improved by collecting urine over 3 consecutive days (17) since increased UFC excretion may not be a consistent finding in children (17). Therefore, serial measurements are necessary to confidently exclude paediatric CS. Adult studies have also demonstrated that 24-hr UFC values can be variable and ideally a minimum of three measurements need to be performed if the first result is normal and the clinical suspicion of CS is high (34). An Endocrine Society Clinical Practice

Guideline recommends at least two UFC measurements as an initial investigation for CS (10). Patients also need to avoid excessive fluid intake and the complete urine collection volume should be sent for analysis (23). Urine collections can be particularly challenging in young children. Consistent with this, we show that 41% of all children (CS and controls) produced only one 24-hr UFC collection.

Our study has obvious limitations; in particular the follow-up period for the control cases was relatively short. Whilst we cannot be certain that there are no 'missed' cases of CS in the control group, a combination of undetectable midnight cortisol level with suppression of cortisol on LDDST and subsequent clinical follow up allowed us to confidently exclude CS. Our results support the contribution of 24-hr UFC to the diagnosis in children with overt features of CS. The increasing incidence of obesity in the paediatric population is likely to lead to more investigations of patients with less extreme clinical features in general paediatric clinics. In conclusion, our results suggest that measurement of 24-hr UFC has a high diagnostic accuracy and may contribute to the diagnosis or exclusion of CS in the paediatric age range.

Acknowledgement

All authors have no conflicts of interest.

Declarations of interest

No financial interest to declare.

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Table 1. Diagnostic characteristics

Patient group	Cushing's Disease	Primary Pigmented Nodular Adrenocortical Disease	Controls
No. of patients (gender)	39 (25M)	8 (4M)	19 patients (6M)
Mean age yrs (range)	11.7 (5.7-16.9)	12.9 (10.5-16.9)	11.5 (4.4-15.7)

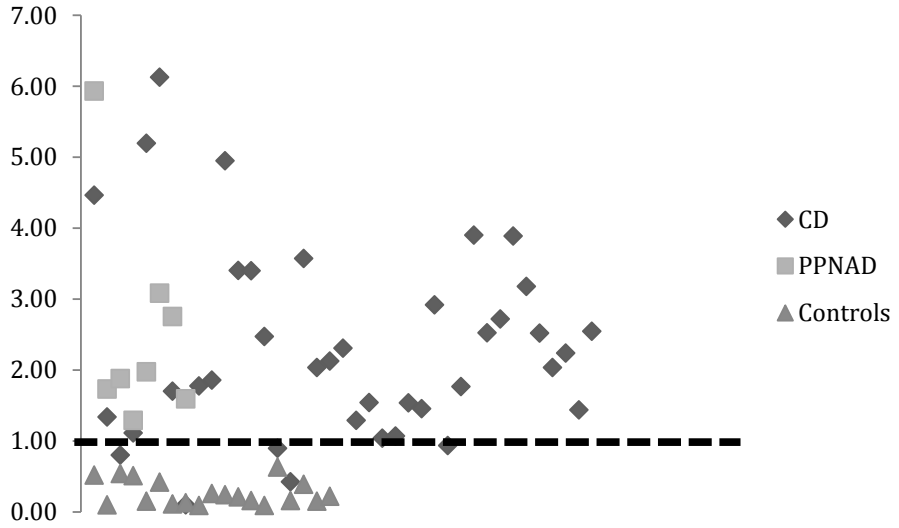
M, males.

Table 2. Height SDS and BMI SDS in the different diagnostic groups

	Mean height SDS (range) [p value and 95% CI vs controls]	Mean BMI SDS (range) [p value and 95% CI vs controls]
CD (n=39)	-1.7 (-5 to 1.2) [**p <0.01**; 95% CI 1.18 to 2.7]	3.0 (-0.4 to 9.2) [p = 0.5; 95% CI 1.08 to 0.49]
PPNAD (n=8)	-0.62 (-2.5 to 0.44) [p = 0.1; 95% CI -2 to 2.96]	2.05 (-0.6 to 4.6) [p = 0.3; 95% CI -2.2 to 0.15]
Controls (n=19)	0.2 (-3.4 to 2.3)	3.2 (2.8 to 4.8)

CD, Cushing's disease; PPNAD, primary pigmented nodular adrenocortical disease; CI, confidence intervals. P values represent comparison of the group (CD, PPNAD) and controls

Figure 1. 24-hr UFC/BSA in all patient groups



CD, Cushing's disease; PPNAD, primary pigmented nodular adrenocortical disease

Table 3. Clinical features of patients in the different diagnostic groups

Clinical symptom or sign	Controls (no.)	PPNAD (no. patients*)	CD (no. patients*)	Total CS (no. patients*)
Hypertension	13% (n=16)	43% (n=7)	51% (n=39)	50% (n=46)
Striae	37% (n=19)	50% (n=6)	54% (n=37)	53% (n=43)
Headaches	37% (n=19)	43% (n=7)	58% (n=38)	56% (n=45)
Hirsutism	26% (n=19)	50% (n=8)	59% (n=39)	57% (n=47)

*The number of patients for whom this information is available.

Table 4. Diagnostic features of Cushing's Syndrome.

	CD	PPNAD
No. patients	39 (25M)	8 (4M)
Mean age (range)	11.7 yr (5.7-16.9)	12.9 yr (10.5-16.9)
0900h plasma ACTH	(10-50 ng/L)	(<10 ng/L)
Sleeping 0.00 h cortisol	Mean 530.3 nmol/L (146-1377 nmol/L)	Mean 507.9 nmol/L (400-622 nmol/L)
Serum cortisol at 48 h in LDDST LDDST (0.5 mg 6 hourly for 48 h)	Mean 325.2 nmol/L (22-1063 nmol/L)	Mean 519.6 nmol/L (400-622 nmol/L)
% increase (mean and range) in serum cortisol from baseline during CRH test (1µg/kg i.v) (27).	207%; (34-823%)	1.3%; (0-6%)
Bilateral simultaneous inferior petrosal sinus sampling (BSIPSS)	Central ACTH secretion confirmed in 28 patients (72%) Mean central to peripheral ACTH ratio after CRH 6.8 (1-34.6)	Not indicated

CD, Cushing's disease; PPNAD, primary pigmented nodular adrenal disease; M, males; LDDST, Low-dose dexamethasone suppression test.