

A Novel Tool in the Diagnosis and Follow-Up of (Cyclic) Cushing's Syndrome: Measurement of Long-Term Cortisol in Scalp Hair

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Background: Measurement of cortisol in 24-h urine collections and midnight saliva are standard screening tests for Cushing's syndrome (CS). These tests reflect cortisol levels during a maximum of 24 h and do not provide historical information. Therefore, they can yield normal results in case of cyclic CS, which is a rare disorder that is characterized by alternating episodes of endogenous cortisol excess and normal cortisol secretion. The measurement of cortisol in scalp hair is a novel tool that might be helpful to establish the diagnosis of (cyclic) CS. Our aim was to study whether hair cortisol timelines correspond with clinical course in patients with CS and whether we could create retrospective timelines of cortisol exposure that correspond with symptomatic periods in patients suspected of cyclic CS.

Methods: Scalp hair was collected in 14 patients with confirmed CS and six patients suspected of cyclic CS. Cortisol was extracted from the hair samples with methanol, and an ELISA was used to measure cortisol levels in hair extracts. A group of 96 nonobese individuals were used as a control group.

Results: Hair cortisol levels were significantly elevated in CS patients ($P < 0.0001$). Sensitivity and specificity of hair cortisol measurements for CS were 86 and 98%, respectively. Hair cortisol timelines of patients with CS and cyclic CS corresponded with clinical course.

Conclusion: Hair samples can provide a historical timeline that corresponds with clinical course in patients with (cyclic) CS. This new diagnostic tool can contribute significantly to early recognition of patients suffering from cyclic CS. (*J Clin Endocrinol Metab* 97: E1836–E1843, 2012)

Cortisol, the most important glucocorticoid in man, is secreted by the adrenal glands and has many effects throughout the human body. It is, for example, involved in lipid and glucose metabolism, immune response, and the development and functioning of numerous organs. Cortisol excess, as present in patients with Cushing's syndrome (CS), has many negative effects on the body. Symptoms caused by cortisol excess are weight gain, with ac-

cumulation of fat especially in the neck, face, and abdomen; insulin resistance; muscle weakness, usually of the proximal muscles; bone loss; and infections (1, 2). Also, psychological symptoms such as loss of emotional control, irritability, and depression are common (1). The presence and severity of these symptoms depend on the level and duration of cortisol excess. Major causes of endogenous hypercortisolism are excessive ACTH production by a pituitary adenoma, excessive

cortisol secretion from an adrenocortical tumor or hyperplasia, and ectopic ACTH production by a nonpituitary tumor (1). Standard screening tests for CS are the measurement of cortisol in 24-h urinary collections, the measurement of cortisol in midnight saliva and the 1-mg overnight dexamethasone suppression test (2). Urinary and salivary cortisol levels should be measured at least twice, because hypercortisolism in CS can be variable (2).

Cyclic CS is thought to be a rare disorder that is characterized by alternating episodes of endogenous cortisol excess and normal cortisol secretion. The cycles can occur regularly or irregularly with periods of normal cortisol secretion ranging from days to years (3). This makes cyclic CS a diagnosis that is difficult to make and easy to miss. The standard screening tests such as the measurement of cortisol in 24-h urine collections or in saliva represent cortisol levels during a maximum of 24 h and do not provide information on cortisol exposure in the previous weeks or months. Therefore, these methods are not optimal to diagnose cyclic CS or require multiple sample collections to confirm the diagnosis. The measurement of cortisol in scalp hair is a novel tool that might be helpful to establish the diagnosis of cyclic CS. Several studies have validated hair cortisol measurements and have shown that hair can be used to create a retrospective timeline of cortisol exposure, with every 1 cm of hair corresponding to a period of approximately 1 month (4–7). Thomson *et al.* (7) already showed promising results of hair cortisol measurements in five patients with endogenous CS. In that study, hair cortisol levels corresponded with clinical course of the disease. The measurement of cortisol in scalp hair is currently the only method that provides information on cortisol exposure in the previous months to years (depending on the length of the hair) and could therefore be very valuable in patients suspected of cyclic CS.

The aim of the present study was to investigate whether hair cortisol timelines correspond with clinical course in patients with CS and whether we could create retrospective timelines of cortisol exposure that corresponded with symptomatic periods in patients suspected of cyclic CS. Therefore, we measured hair cortisol levels in patients with confirmed CS and patients suspected of cyclic CS and compared our results with those of a large group of controls.

Patients and Methods

Patients

Patients admitted to the clinic or outpatient clinic of the Erasmus Medical Center, the Sophia Children's Hospital, the Leeuwarden Medical Center, or the St. Radboud Medical Center with confirmed CS or suspected of cyclic CS were included. Patients

could not participate if there was insufficient hair growth at the posterior vertex of the scalp. This study was approved by the local ethics committee, and all patients gave written informed consent. In case the patients were younger than 18 yr, informed consent was given by both the patients and their parents or guardians.

Healthy controls

To compare hair cortisol levels in CS patients with hair cortisol levels in healthy individuals and to calculate a reference range of normal hair cortisol levels, we selected healthy individuals with BMI between 18.5 and 24.9 kg/m² and without abdominal obesity (n = 96) (6). From the measurements in these 96 controls, we calculated the reference range of normal hair cortisol levels defined as mean \pm 1.96 SD and found a range of 9.9–75.9 pg/mg hair. We calculated sensitivity and specificity of the hair cortisol measurements for CS based on this reference range. In addition, we calculated specificity in overweight and obese individuals from our large group of healthy individuals and in the group of healthy individuals with abdominal obesity. The characteristics of the total group of healthy individuals are extensively described elsewhere (6).

Hair collection

A lock of approximately 100–150 hairs was cut from the posterior vertex of the scalp, as close to the scalp as possible. The hair samples were taped to pieces of paper and the proximal side of the hairs was marked. The hair samples were stored at room temperature in an envelope until analysis.

Hair preparation

From all controls and patients with CS we measured cortisol levels in hair segments of 1–3 cm, roughly corresponding with a period of 1–3 months. In addition, in a number of patients with long hair and in all patients suspected of cyclic CS, we divided the total length of the hair in segments of 1 cm, to create a retrospective timeline of monthly hair cortisol levels. Additional preparation was performed as described by Sauv e *et al.* (8). In brief, a minimum of 10 mg of hair was weighed and put into a glass vial. The hair samples were cut into small pieces of 1–2 mm in length, and methanol was used to extract cortisol from the hair samples. After incubation, the methanol was transferred to another vial and evaporated under a stream of nitrogen. Subsequently, the samples were redissolved in PBS and vortexed for 1 min. Before analysis, the samples were vortexed again for 30 sec.

Hair analysis

A commercially available ELISA Kit for salivary cortisol (DRG GmbH, Marburg, Germany) was used to measure cortisol levels. Cross-reactivity of other steroids with the kit's antibodies was reported as follows: corticosterone (29%), cortisone (3%), 11-deoxycortisol (<1%), 17-OH progesterone (<0.5%), and other hormones (<0.1%). Intraassay variation was below 5% and the interassay variation below 8%, as reported by the supplier. The recovery of the assay was tested and described previously (6).

Urine cortisol and serum cortisol

The measurement of cortisol in serum, saliva, and urine was performed as part of the diagnostic process for the establishment

TABLE 1. Overview of the patients included in this study

	Gender	Age (yr)	Cause	Urinary cortisol at diagnosis (nmol/24 h)
CS				
Patient 1	F	45	Pituitary microadenoma	332
Patient 2	F	35	Pituitary microadenoma	482
Patient 3	M	23	Pituitary microadenoma	973
Patient 4	M	36	Pituitary microadenoma	1,156
Patient 5	F	45	Pituitary microadenoma	436
Patient 6	F	65	Pituitary macroadenoma	2,061
Patient 7	F	48	Pituitary macroadenoma	10,588
Patient 8	M	57	ACTH-producing metastasized prostate carcinoma	45,430
Patient 9	M	57	ACTH-producing gastric carcinoid	51,590
Patient 10	M	63	ACTH-producing small-cell lung carcinoma	697
Patient 11	F	48	ACTH-producing neuroendocrine tumor pancreas	31,732
Patient 12	M	63	ACTH-producing neuroendocrine tumor pancreas	21,831
Patient 13	F	50	ACTH-producing metastasized mammary carcinoma	5,213
Patient 14	M	76	Adrenal carcinoma	1,955
Cyclic CS				
Patient 15	F	56	Unknown	6,000
Patient 16	F	23	Unknown	
Patient 17	F	33	Thymus carcinoid	16,009
Patient 18	F	26	Thymus carcinoid	47,446
Patient 19	M	10	Pituitary microadenoma	563
Patient 20	M	35	Ectopic, source of ACTH production unknown	5,132

F, Female; M, male.

of CS. Serum cortisol was determined using a competitive immunoassay (Immulite 2000; Siemens Healthcare Diagnostics B.V., Breda, The Netherlands). Serum cortisol levels of less than 718 nmol/liter were considered as normal. Urinary cortisol was measured using an in-house method, using UV detection after HPLC. Urinary cortisol levels were measured in 24-h urine collections and levels of less than 119 nmol/24 h were considered as normal.

Results

Cortisol levels were determined in scalp hair of 14 patients with CS and six patients suspected of cyclic CS. Clinical data of all patients have been summarized in Table 1.

Long-term hair cortisol measurements

Mean hair cortisol levels were significantly elevated in patients with CS (cyclic CS patients excluded) compared with healthy individuals [399.7 pg/mg hair (95% confidence interval = 171.8–930.0) vs. 27.3 pg/mg hair (95% confidence interval = 24.6–30.4, *P* < 0.0001] (Fig. 1). There was no significant effect of age, gender, and hair treatment (dyeing and bleaching of hairs) on hair cortisol levels in the control group or in the CS patients, although hair cortisol levels were slightly lower in healthy women who treated their hairs (*P* = 0.06). Adjusting for these factors did not influence the results. Based on the upper limit of the reference range of nonoverweight healthy controls (75.9 pg/mg hair),

the sensitivity and specificity of hair cortisol measurements for CS were 86 and 98%, respectively. The cutoff value of 75.9 pg/mg hair resulted in a specificity of hair cortisol measurements for CS of 98 and 93% in overweight and obese individuals, respectively, and 93% in individuals with abdominal obesity (Fig. 1).

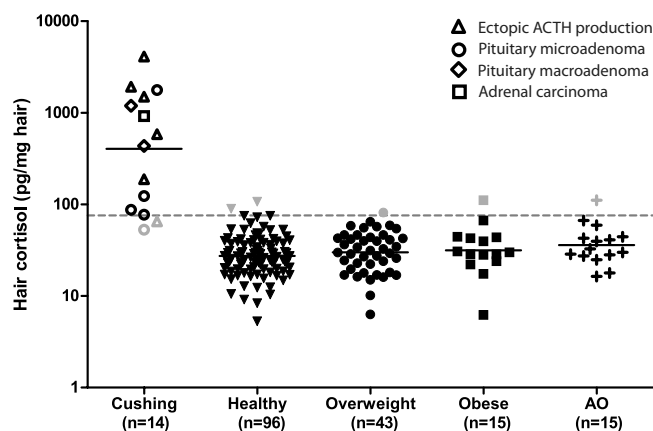


FIG. 1. Hair cortisol levels in patients with CS and healthy overweight and obese controls and individuals with abdominal obesity. The dotted line represents the upper limit of normal hair cortisol levels (75.9 pg/mg hair). The gray symbols represent the individuals with cortisol levels below the upper limit of normal (in case of confirmed CS) or above the upper limit of normal (no CS). Based on this upper limit, the sensitivity for hair cortisol measurements in CS is 86%. This cutoff value of 75.9 pg/mg hair for the diagnosis of CS resulted in a specificity of 98% in healthy individuals, 98% in overweight individuals, 93% in obese individuals, and 93% in individuals with abdominal obesity (AO). The nature of the CS is indicated by the different symbols used for the patients (see also Table 1).

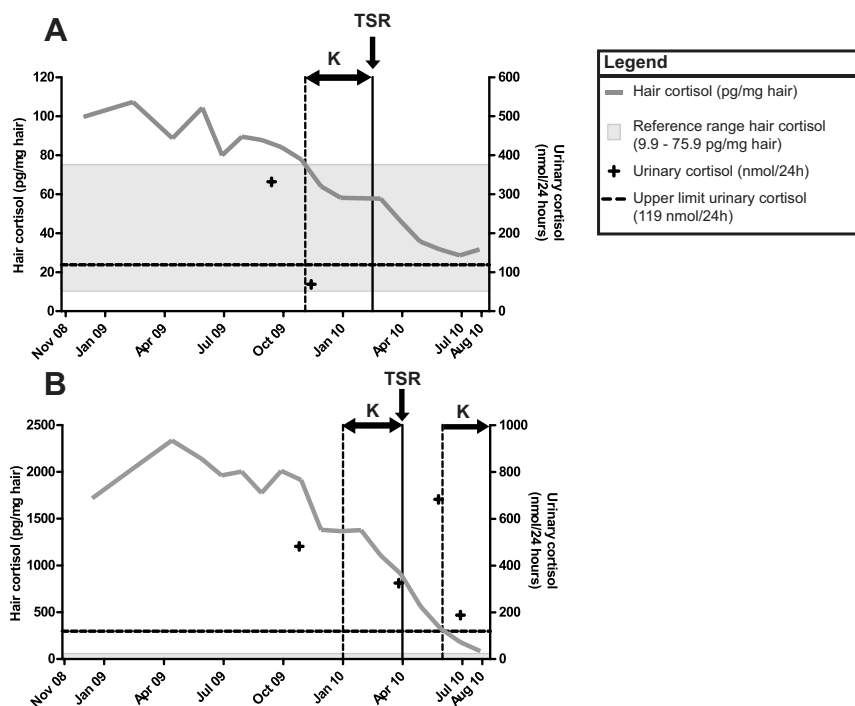


FIG. 2. Hair cortisol timelines of patients with CS before, during, and after treatment. A, Hair and urinary cortisol levels of patient 1; B, hair and urinary cortisol levels of patient 2. Cortisol was measured in adjacent 1-cm fragments derived from a single lock of hair. K, Ketoconazole treatment.

Hair cortisol timelines in CS patients

We selected three CS patients with hair of sufficient length to evaluate the effect of treatment of their CS on hair cortisol levels. The hair samples of these patients were divided into 1-cm segments to measure cortisol exposure per month.

The hair cortisol timeline and 24-h urinary cortisol levels of patient 1, a 45-yr-old woman, are presented in Fig. 2A. This patient was diagnosed with Cushing's disease in October 2009 and underwent transsphenoidal resection (TSR) of a microadenoma in February 2010. Three months before surgery, she started with ketoconazole therapy. Urinary cortisol levels were elevated before the start of ketoconazole (332 nmol/24 h; upper limit of normal, 119 nmol/24 h) and were normalized after ketoconazole was started (69 nmol/24 h). Her hair cortisol timeline showed elevated cortisol levels already a year before the start of ketoconazole and a decline in cortisol levels after the start of ketoconazole. After TSR, cortisol levels decreased even more (Fig. 2A).

Figure 2B shows the hair and urinary cortisol levels of patient 2. This 35-yr-old woman was also diagnosed with Cushing's disease in October 2009 (urinary cortisol level, 482 nmol/24 h; upper limit of normal, 119 nmol/24 h). In January 2010, she started with ketoconazole. She underwent TSR at the end of March 2010. Despite surgery, she continued to have elevated 24-h urinary cortisol levels (683 nmol/24 h), and ketoconazole was restarted in June

2010. With this treatment, urinary cortisol levels decreased (188 nmol/24 h) but remained above the upper limit of normal (119 nmol/24 h). Hair cortisol levels were already elevated more than a year before the diagnosis of CS was established and decreased after ketoconazole treatment and surgery (85.4 pg/mg hair) but remained above the upper limit of normal, which is the same pattern as the multiple urinary collections show.

Patient 3 (Supplemental Fig. A, published on The Endocrine Society's Journals Online website at <http://jcem.endojournals.org>), a 23-yr-old man, was diagnosed with medullary thyroid carcinoma during childhood, as part of multiple endocrine neoplasia type 2A. In 2009, he developed CS, caused by a pituitary microadenoma. After a 3-month period of ketoconazole treatment, the patient underwent TSR of the microadenoma in November 2010. Around the start of ketoconazole, his hair cortisol level was 123.4 pg/mg hair and his urinary cortisol levels ranged from 70–137 nmol/24 h (upper limit of normal, 119 nmol/24 h). Four months after TSR, his hair cortisol level was decreased to 50.1 pg/mg hair (measured during hydrocortisone replacement therapy) (see Supplemental Fig. A).

Hair cortisol timelines in patients suspected of cyclic CS

In the six patients with (suspected) cyclic CS, we created timelines of cortisol exposure. Again, hair samples were divided into 1-cm segments to measure monthly cortisol exposure.

Patient 15 is a 56-yr-old woman who was recently diagnosed with cyclic CS. She noticed a moon face in August 2011 and was admitted to the hospital with hypertensive crisis in November 2011. In March and April 2011 and January 2012, she suffered from muscle pains. During the hypertensive crisis, her urinary cortisol level was extremely elevated (6000 nmol/24 h). In the months January to March 2012, urinary cortisol measurements were repeated during several occasions, and they were all found to be normal (54, 80, and 44 nmol/24 h; upper limit of normal, 119 nmol/24 h). In March 2012, a hair sample was collected, which was of sufficient length to measure hair cortisol levels from April 2011 onward. Her hair cortisol levels were elevated during the period in which she had a moon face and during the hypertensive crisis. In the

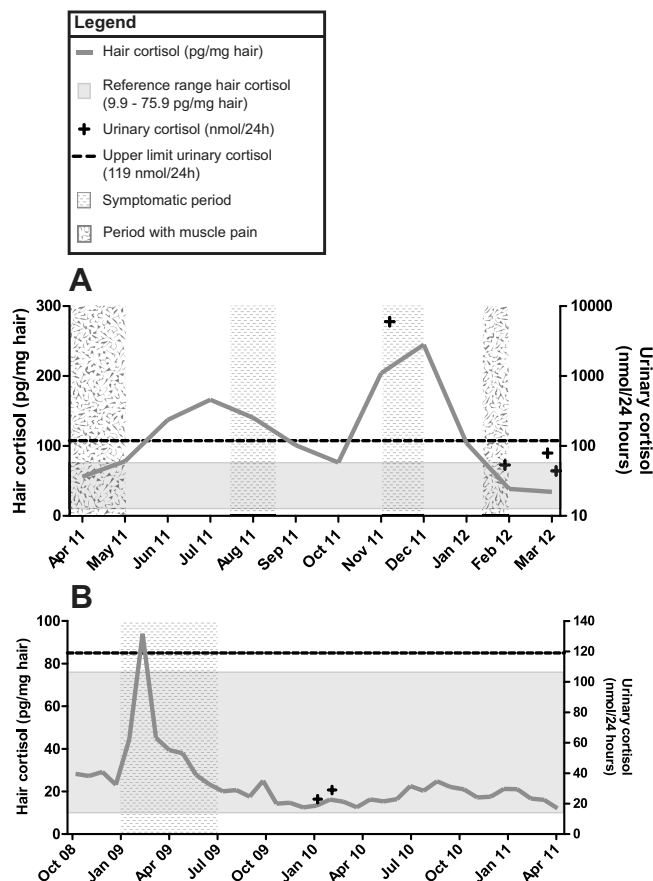


FIG. 3. Hair cortisol timelines of patients suspected of cyclic CS. A, Hair cortisol levels of patient 15; B, hair cortisol levels of patient 16. Cortisol was measured in adjacent 1-cm fragments derived from a single lock of hair.

periods with hair cortisol levels within the normal range, she suffered from muscle pain, which might be caused by the relative cortisol deficiency in that period (Fig. 3A).

Patient 16, a 23-yr-old woman, was frequently seen at the outpatient clinic from January 2010 onward. In the period of January to July 2009, she gained approximately 30 kg of weight, primarily located at the abdomen and face, despite being on a diet. On examination, she had an evident Cushingoid appearance. Urinary cortisol excretion was within the normal range on two occasions (January 2010, 23 nmol/24 h; February 2010, 29 nmol/24 h; upper limit of normal, 119 nmol/24 h), and CS could not be confirmed. It was decided that urinary cortisol levels would be measured again upon return of the symptoms, but until now, no additional episodes of weight gain or other Cushingoid symptoms have occurred. In April 2011, a hair sample was obtained that was of sufficient length to retrospectively measure cortisol levels from October 2008 onward. In this hair sample, we found a peak (94.1 pg/mg hair) in the hair segment corresponding with the symptomatic period in 2009. Hair cortisol levels during asymptomatic periods ranged from 12.1–29.7 pg/mg hair (Fig. 3B).

Patient 17 (Supplemental Fig. B), a 33-yr-old woman, was diagnosed with CS due to an ACTH-producing thymus carcinoid in September 2010. She had abdominal obesity, moon face, buffalo hump, hypertension, and hypokalemia, all starting in August 2010. Laboratory examination showed an elevated urinary cortisol level (16,009 nmol/24 h; upper limit of normal, 119 nmol/24 h) and absence of cortisol suppression after 1 mg dexamethasone. Her medical history suggested a cyclic pattern of hypercortisolism with previous symptoms of cortisol excess, such as moon face, proximal muscle weakness, acne, and hirsutism from July to September 2009 and a period of minor hirsutism and acne in January 2010. Between these episodes, she had no complaints. Unfortunately, no urinary cortisol measurements were performed in the symptomatic and asymptomatic periods to confirm cyclic CS. However, the retrospective hair cortisol timeline of this patient, which reflected the period September 2009 to October 2010, showed a cyclic pattern with 4-fold higher cortisol levels during symptomatic periods (around 80 pg/mg hair) compared with the asymptomatic periods (around 20 pg/mg hair, Supplemental Fig. B). Hair cortisol levels in the symptomatic periods were above the upper limit of normal (75.9 pg/mg hair).

Patient 18 (Supplemental Fig. C), a 26-yr-old woman, was diagnosed with cyclic CS in October 2009. Since 2008, she suffered from repeated episodes of acne, hirsutism, low potassium levels, edema, and weight gain. Her general practitioner found elevated serum cortisol levels during two of these episodes and referred the patient to an endocrinologist, who measured normal 24-h urinary cortisol excretion in December 2008 (58 nmol/24 h; upper limit of normal, 119 nmol/24 h), February 2009 (25 nmol/24 h), and June 2009 (47 and 36 nmol/24 h). In addition, there was normal suppression of cortisol after 1 mg dexamethasone. In October 2009, she developed symptoms of cortisol excess again, and her 24-h urinary cortisol level was extremely elevated (47,446 nmol/24 h). Sinus petrosus sampling revealed a pituitary cause of hypercortisolism, and magnetic resonance imaging showed a small lesion in the pituitary. Despite transsphenoidal surgery in December 2009, the patient remained hypercortisolemic (urinary cortisol level, 1103 nmol/24 h) and was treated with ketoconazole and later with the combination of ketoconazole and cabergoline. During this period, urinary cortisol levels ranged from 73–181 nmol/24 h. Additional examination revealed a small ACTH-producing thymic carcinoid as the cause of hypercortisolism. Two hair samples were collected, one in October 2009 during a short period of hypercortisolism and one in August 2010 after a period of ketoconazole, cabergoline, and hydrocortisone treatment. The first hair sample, reflecting the

period August to October 2009, shows a timeline in which the cyclic pattern is very clear with elevated hair cortisol levels during the active episode (150.8 pg/mg hair) and normal cortisol levels in the asymptomatic period (24.4 and 36.7 pg/mg hair). The second hair sample, reflecting the period February to August 2010, shows continuously elevated hair cortisol levels from March 2010 until the moment of sample collection (ranging from 94.5–135.8 pg/mg hair), with no response to the addition of cabergoline to the treatment. This corresponds with the elevated urinary cortisol levels during combined treatment with ketoconazole and cabergoline (181 and 140 nmol/24 h; upper limit of normal; 119 nmol/24 h) (Supplemental Fig. C).

Patient 19 (Supplemental Fig. D), a 10-yr-old boy, was first seen in 2009 because of growth retardation and weight gain. Serum cortisol was elevated, but this was attributed to his fear of blood and needles. After a repeated measure in December 2010, his serum cortisol level was normal (502 nmol/liter; upper limit of normal, 718 nmol/liter). However, the patient had progressive symptoms and in June and July 2011, multiple elevated serum (861, 734, and 931 nmol/liter) and urinary cortisol levels (563 nmol/24 h; upper limit of normal, 119 nmol/24 h) were found in combination with absent suppression of serum cortisol after 1 mg dexamethasone. Photographs of the patient revealed that he had developed a Cushingoid appearance in the period between 2009 and July 2011. The retrospective timeline of hair cortisol in this patient reflected the period September 2010 to July 2011 and revealed a cyclic pattern with 3-fold higher levels in September 2010 (75.1 pg/mg hair) and May and July 2011 (65.5 pg/mg hair) compared with the period in between (25.8 pg/mg hair) (Supplemental Fig. D).

Patient 20 (Supplemental Fig. E), a 35-yr-old man, had been frequently seen at the outpatient clinic for gastrointestinal diseases because of chronic pancreatitis with endocrine and exocrine insufficiency, which was, at that time, attributed to marijuana abuse. During 2009–2011, the patient was admitted to the hospital several times because of severe abdominal pain and jaundice. During these admissions, examination revealed hypertension, hypokalemia, proximal muscle atrophy, easy bruisability, and a moon face. CS was suspected, but urinary cortisol levels were normal (12 nmol/24 h; upper limit of normal, 119 nmol/24 h). In June 2011, the patient was admitted again and the diagnosis of CS was established by multiple elevated 24-h urinary cortisol levels (3352, 4622, and 7422 nmol/24 h). After 1 wk of elevated urinary cortisol levels, cortisol levels returned to normal (28, 10, 23, 4, 2, and 15 nmol/24 h), confirming cyclic CS. The cause of hypercortisolism was unknown, and the patient underwent bi-ad-

renalectomy. Hair cortisol levels of this patient were increasing over time and above the normal range from February 2011 onward. In contrast to his hair cortisol levels, his urinary cortisol levels were normal during hospitalization in February 2011. In total, seven of 10 urinary cortisol levels were normal, whereas hair cortisol levels were continuously elevated (Supplemental Fig. E).

Discussion

In this study, we show that hair cortisol levels were significantly elevated in scalp hairs of patients with CS compared with healthy controls. In addition, timelines of cortisol exposure, constructed by measuring cortisol in consecutive hair segments, corresponded with clinical course of the disease, both in the noncyclic and in cyclic CS patients. In recent literature, the measurement of cortisol in scalp hair has been well validated. Most studies have used this method to study long-term cortisol levels in patients with chronic pain and stress or in healthy individuals (6, 9–11). Two previous reports also showed that hair cortisol timelines corresponded with clinical course in patients with CS (6, 7). Our study is the first study that also shows hair cortisol levels in patients suspected of cyclic CS, and our cases illustrate that retrospectively obtained cortisol measurements can be of great value in the diagnosis of cyclic CS.

The sensitivity and specificity of cortisol levels in hair for the diagnosis of CS on the basis of the upper limit of the reference range of our healthy individuals were 86 and 98%, respectively. These percentages are comparable with values described when using 24-h urinary cortisol or midnight salivary cortisol to screen for CS as described in a meta-analysis of Elamin *et al.* (12), who found that the sensitivity of cortisol measurements in multiple 24-h urine collections ranged from 38–100% (mean 84%), and specificity ranged from 44–100% (mean 92%), depending on the methods used and cutoff values set by the investigators. The sensitivity of midnight salivary cortisol ranged from 46–100% (mean 85%), with specificity ranging from 79–100% (mean 92%) (12). All of these values were based on the collection of a minimum of two samples. Furthermore, Friedman *et al.* (13) showed that when collecting up to six 24-h urinary samples, at least 88% of the patients with CS had at least one negative test. For midnight salivary cortisol, this was even 92% (13). This shows that it is required to collect multiple samples to establish or reject the diagnosis of CS. The advantage of measuring cortisol in hair is that only one sample needs to be collected to see whether cortisol levels were increased over longer periods of time. Furthermore, in cyclic CS or in patients with long hair, the

creation of a hair cortisol timeline might provide the opportunity to evaluate an individual's baseline cortisol level. It might be potentially more useful to compare an individual's high cortisol levels with his/her own baseline cortisol levels than comparing it with the reference range from a group or population.

The diagnosis of cyclic CS is difficult and can be easily missed when standard tests are used during normocortisolemic periods. As shown in our cases, it may take several months to years to establish the diagnosis of cyclic CS using standard tests. The patients described here were finally diagnosed with cyclic CS by collection of multiple serum and 24-h urine samples, which interferes with daily activities and is troublesome to obtain for prolonged periods of time. The collection of a single hair sample can provide cortisol levels at time points reaching back months or even years, depending on the length of the hair samples. We have shown that clear cyclic patterns of cortisol excess were found in hair of patients suspected of cyclic CS. Only in one patient, with very short periods of cortisol excess (patient 20), was no clear cyclic pattern detected. However, in this patient, hair cortisol measurements could have been of great value for earlier recognition of CS as well, because his hair cortisol levels were elevated from February 2011 onward, whereas his urinary cortisol levels were normal in seven of 10 samples, indicating rapidly cycling episodes of hypercortisolism. This discrepancy between hair and urinary cortisol measurements can be attributed to the different timeframes that hair cortisol and urinary cortisol measurements represent. In our study, hair samples were measured in minimally 1-cm segments, corresponding to a period of 1 month, whereas urinary cortisol levels represent maximally 24 h.

The use of hair samples as a historical record for cortisol exposure might be questioned if cortisol excess affects hair growth. Hair loss is a key feature of CS, but it is not known what the effect of cortisol excess is on hair growth. However, considering the clinical course in the patients described, hair cortisol levels seem to reflect treatment effect and the symptomatic periods very accurately when assuming a hair growth rate of 1 cm/month. This suggests that altered hair growth rate is not the case or at least does not play a major role in CS. In addition, in the study of Thomson *et al.* (7), a growth rate of 1 cm/month seemed to apply to patients with CS as well. This suggests that cortisol excess does not play a major role in changes in hair growth rate. Furthermore, some studies reported a decrease in cortisol levels in the more distal hair segments, indicating that hair could be used as a historical record for up to 6 months only (5). This is in contrast with the finding of Thomson *et al.* (7) and our own previous results in healthy individuals (6). We found no washout effect in

hairs with a length of up to 18 cm. In addition, in this study, our hair cortisol timelines showed variations corresponding with symptomatic periods in patients suspected of cyclic CS. Normal hair cortisol levels in asymptomatic periods were observed in hairs of more than 6 cm length, suggesting that there is no washout effect of cortisol from the distal hair segments. Finally, at present, the method to measure cortisol in scalp hair has been used in only a few laboratories worldwide, and the normal values vary between these institutions. To implement this method as a diagnostic tool in the clinical setting, further refinement and standardization of the technique would be valuable.

In conclusion, hair cortisol levels are increased in patients with CS, and the sensitivity and specificity of hair cortisol measurements for CS is high. Furthermore, hair can provide an historical timeline that corresponds with clinical course in patients with CS as well as in patients with cyclic CS. This new diagnostic tool can contribute significantly to earlier recognition of patients suffering from cyclic CS.

Acknowledgments

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