NOTE

Spot Determinations of Urinary Cortisol for the Screening of Cushing’s Syndrome

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Abstract. The usefulness of spot determination of urinary cortisol in the screening of Cushing’s syndrome was evaluated by measuring the cortisol concentration in randomly sampled urine in 68 normal subjects and in 9 patients with Cushing’s syndrome. The urinary cortisol concentration in the morning was significantly higher in patients with Cushing’s syndrome but some overlap existed between normal subjects and patients with Cushing’s syndrome. In contrast, there was a clear discrimination between two groups when urinary cortisol was measured in the late evening: urinary cortisol was lower than 75 µg per gram creatinine (µg/gCr) in normal subjects but higher than 150 µg/gCr in patients with Cushing’s syndrome. When 1 mg dexamethasone was administered at 2300 h in the evening, spot urinary cortisol the next morning was less than 80 µg/gCr in normal subjects while it was above 100 µg/gCr in patients with Cushing’s syndrome. Dexamethasone-induced suppression of urinary cortisol in normal subjects lasted until late in the afternoon, which allows sampling of urine at any time in the morning and possibly in the afternoon. These results suggest the usefulness of spot determination of urinary cortisol in the screening of Cushing’s syndrome.

Keywords: Cushing’s syndrome, Cortisol, Dexamethasone.

CUSHING’S SYNDROME is characterized by hypersecretion of cortisol and abnormality in the feedback regulation of cortisol secretion [1]. Thus, a diagnosis of Cushing’s syndrome is made by demonstrating the hypersecretion of cortisol and the inability of exogenously added dexamethasone to suppress endogenous secretion of cortisol [2].

In normal subjects, the secretion of cortisol is episodic and exhibits a diurnal rhythm [3, 4]. In addition, plasma cortisol concentration fluctuates remarkably in a short time and it is sometimes above the normal range under stressful conditions, for example, repeated venipuncture and exercise. Because of such fluctuations in the plasma cortisol concentration, it is rather difficult to assess hypersecretion of cortisol in only a single determination of plasma cortisol [5]. For these reasons, the measurement of urinary cortisol is widely used for the assessment of hypercortisolism and the diagnosis of Cushing’s syndrome [2]. Nevertheless, this method has disadvantages in that 24-h urine collection is required and that the inaccurate collection of urine samples lead to underestimation of adrenocortical function. This method is particularly inconvenient for the screening of Cushing’s syndrome in the outpatient clinic.

In this regard, Contreras et al. [6] reported that the measurement of cortisol in 1-h urine samples provided a simple and practical method for assessing hypersecretion of cortisol. They measured cortisol in 1-h urine samples obtained in the morning (0700 h–0800 h) and in the evening (2200 h–2300 h). They showed that cortisol in 1-h
urine in the evening is considerably increased in
patients with Cushing’s syndrome.
From a practical point of view, it would be more
convenient if we could assess adrenocortical func-
tion by measuring urinary cortisol in randomly
collected urine samples. In the present study, we
examined the usefulness of the measurement of
cortisol in randomly collected urine for the screen-
ing of Cushing’s syndrome. We expressed urinary
cortisol as micrograms per gram creatinine (μg/
gCr). The results indicate that spot determination
of urinary cortisol, which can be easily done in the
outpatient clinic, is an extremely useful marker for
the screening of Cushing’s syndrome.

Subjects and Methods
The present study was performed in 68 normal
subjects (35 men and 33 women, aged 24–63), 18
subjects with simple obesity whose body weight was
more than 120% of their own ideal body weight (9
men and 9 women, aged 15–72), and 9 patients
with untreated Cushing’s syndrome (2 men and 7
women, aged 23–58) of whom 8 had Cushing’s
disease and one had ectopic ACTH secretion from
small cell carcinoma of the lung. The diagnosis of
Cushing’s syndrome was made by demonstrating
an excess of urinary cortisol and the absence of
suppression by dexamethasone. The diagnosis was
confirmed by surgery in 8 patients with Cushing’s
disease. The diagnosis of ectopic ACTH syndrome
was confirmed by immunohistochemistry of the
metastatic lymph node obtained at biopsy. Normal
and obese subjects were not hospitalized and were
studied during their normal activities.
To collect urine samples in the morning, first of
all, urine was voided and discarded immediately
after they woke up. Urine samples were then
collected spontaneously between 0800 h and 1100
h. Late evening samples were obtained between
2100 h and 2400 h. When an overnight dex-
amethasone suppression test was done, spot
urine and plasma were obtained in the morning at
0900 h. 1 mg of dexamethasone was then adminis-
tered orally at 2300 h in the late evening. The next
morning, a urine sample was collected at 0900 h. A
sample for plasma cortisol was also collected
simultaneously. To determine the time course of
suppression by dexamethasone, urine samples
were also collected at 1400 h and 1800 h.

Urinary cortisol was measured by radioimmu-
noassay with a radioimmunoassay kit from Baxter
after extraction with methylene chloride. The
inter- and intraassay variations were 8.2 and 7.3%,
respectively. Urinary cortisol is expressed as
micrograms per gram creatinine (μg/gCr). Statis-
tical analysis was done by means of Student t-tests.

Results
In normal subjects, urinary cortisol ranged
40–417 μg/gCr (151 ± 10.2, mean ± SE, n=68) in
the morning (Fig. 1). In obese subjects, urinary
cortisol in the morning ranged 40–371 μg/gCr
(156 ± 19.8, mean ± SE, n=18), which is not differ-
ent from that in normal subjects. In patients with
Cushing’s syndrome, urinary cortisol in the morn-
ing ranged from 349 to 3235 μg/gCr (1240 ± 313,
mean ± SE, n=9), which was significantly higher
than that in normal subjects (p<0.001). However,
there was some overlap between the two groups
(Fig. 1). When urinary cortisol in the late evening
was compared in normal subjects and in patients
with Cushing’s syndrome, no overlap was ob-
served. Urinary cortisol in normal subjects was
below 75 μg/gCr while it was above 150 μg/gCr in
all patients with Cushing’s syndrome (Fig. 1).
An overnight dexamethasone suppression test
was then performed with spot urinary cortisol and
plasma cortisol as indicators. Spot urinary cortisol
and plasma cortisol were determined in the morn-
ing and then 1 mg dexamethasone was adminis-
tered at 2300 h. Spot urine sample and plasma
were obtained at 0900 h the next morning. Before
the administration of dexamethasone, morning
plasma cortisol ranged from 4.4 to 26.8 μg/dl in
normal subjects and from 13.5 to 56.7 μg/dl in
patients with Cushing’s syndrome. After the admi-
nistration of dexamethasone, morning plasma cortisol was higher than 5 μg/dl in all patients with Cushing’s
syndrome, but below 5 μg/dl in normal subjects. It
should be mentioned that, in seven normal sub-
jects, plasma cortisol ranged between 2 and 5 μg/dl
after the administration of dexamethasone (Fig.
2). Before the administration of dexamethasone,
there was some overlap in urinary cortisol in
normal subjects and patients with Cushing’s syn-
drome, as mentioned above. After the administra-
tion of dexamethasone, urinary cortisol in the
morning was suppressed to below 80 μg/gCr in
CORTISOL IN SPOT URINE

Fig. 1. Comparison of spot urinary cortisol in normal subjects and in patients with Cushing's syndrome. Spot urine was collected in the morning (left) and in the late evening (right) and cortisol was measured as described in “Methods”. Bars represent the mean±SE.

Fig. 2. Plasma cortisol and spot urinary cortisol after the administration of dexamethasone. Dexamethasone (1 mg) was administered at 2300 h in the evening. Then spot urinary cortisol (right) and plasma cortisol (left) were measured the next morning.

Fig. 3. Time course of suppression of urinary cortisol after the administration of dexamethasone. Urinary cortisol in spot urine was measured at 0900 h in the morning and 1 mg dexamethasone was administered at 2300 h. The next day, spot urine was collected at 0900 h, 1400 h and 1800 h and cortisol was measured. (●), normal subject; (○), patient with Cushing's syndrome.

Discussion

In the present study, we evaluated the usefulness of spot urinary cortisol in the screening of Cushing's syndrome. In particular, we measured cortisol in spontaneously voided urine for the convenience. Our results clearly indicate that adrenocortical hyperfunction can be assessed by measuring cortisol in spot urine. Specifically, our results agree in three respects with those in two previous reports [6, 7], which assessed the validity
of the determination of cortisol in 1-h or 2-h urine samples. First, spot urinary cortisol exhibits a diurnal rhythm. Second, cortisol in spot urine obtained in the late evening clearly discriminates Cushing’s syndrome from normal subjects. In patients with Cushing’s syndrome, urinary cortisol in the evening is more than twice as high as that in normal subjects. Hence, spot determination of urinary cortisol in the evening provides a simple way to screen Cushing’s syndrome. Thirdly, an overnight dexamethasone suppression test can be performed by using spot urinary cortisol as an indicator. Our present study further indicates that spot urinary cortisol has an advantage over plasma cortisol as an indicator for dexamethasone suppression test. As shown in Fig. 2, plasma cortisol after the administration of dexamethasone ranged between 2 and 5 µg/dl in 7 of 68 normal subjects. Blethen et al. [8] reported that plasma cortisol was less than 2 µg/dl after the administration of dexamethasone in normal subjects. They proposed a cut-off value of 2 µg/dl. According to their criteria, values between 2 and 5 µg/dl indicated the possibility of Cushing’s syndrome, and the plasma cortisol concentrations in our seven subjects therefore demonstrate a false-positive. In contrast, when spot urinary cortisol was measured after the administration of dexamethasone, there was a clear distinction between the two groups: it was below 80 µg/gCr in normal subjects but above 100 µg/gCr in patients with Cushing’s syndrome. Given the fact that urinary cortisol reflects an active portion of plasma cortisol [9], it is conceivable that urinary cortisol is a better marker for the dexamethasone suppression test.

In addition, the results in Fig. 3 indicate that, after the administration of dexamethasone, urinary cortisol remains suppressed until late in the afternoon. This is of significance from a practical point of view because it makes sampling of urine possible at any time in the morning and even in the afternoon. Such a procedure is indeed suitable for the screening of Cushing’s syndrome in the outpatient clinic.

In summary, spot determination of urinary cortisol is a simple and practical method for the assessment of adrenocortical hyperfunction. It is easily done in the outpatient clinic and avoids the stress of venipuncture. The determination of urinary cortisol in the evening is a simple and convenient way of screening for Cushing’s syndrome. Finally, overnight dexamethasone suppression test can be done by utilizing spot urinary cortisol as an indicator.

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References