Is Salivary Cortisol a Better Index than Free Cortisol in Serum or Urine for Diagnosis of Cushing Syndrome?

To the Editor:

During the past few years, interest has been growing about the utility of saliva as a biological fluid for quantifying steroids, especially cortisol, in the study of adrenal function (1). The main potential advantages of saliva include its being available through a stress-free and noninvasive sampling procedure; the medium also reflects the biologically active, unbound (free) cortisol concentration in serum (2). Measurements of plasma cortisol in basal conditions are of limited value in the diagnosis and differentiation of Cushing syndrome. Likewise, the measurement of free cortisol in 24-h urine is accepted as a better marker than serum cortisol (3).

To assess the diagnostic value of this analyte, we have compared the salivary, serum, and free urinary cortisol concentrations determined in a group of patients with untreated Cushing syndrome.

We studied 38 patients (10 men and 28 women), ages 24-66 years (mean age 48 years). Fourteen of them (3 men, 11 women) had Cushing syndrome, the diagnosis having been established by the appropriate dynamic tests as well as morphological studies and confirmed by surgery and histological examination. Of these 14 patients with Cushing syndrome, 11 had Cushing disease, 2 had adrenal adenoma, and 1 had an ectopic corticotropin secretory tumor. The other 24 patients studied had other pathologies: obesity (8 patients), hypertension (4 patients), incidental adrenal mass (4 patients), hirsutism (6 patients), Bartter syndrome (1 patient), and syndrome of inappropriate antidiuretic hormone secretion (1 patient).

Venous blood was drawn from the forearm antecubital vein after a 30-min resting period. Serum samples were stored at -20°C until analysis. After rinsing the mouth thoroughly with water, saliva samples were collected directly into disposable tubes by unstimulated flow; after centrifugation, they were stored at -70°C until analysis. Both saliva and serum samples were collected at 0800 for basal determinations. Urine samples (24 h) were also collected and aliquots were stored at -70°C. Reference values are described in a previous study (3).

Serum, saliva, and urine cortisol concentrations were measured by RIA with 125I-labeled cortisol as the labeled ligand and an antibody immobilized into the lower inner wall of the tube (Gamma Coat; Clinical Assays, Baxter, Cambridge, MA); details of the assay have been published previously (4). Intra- and interassay imprecisions (CVs) were respectively: serum, 5.4% and 6%; saliva, 6.5% and 6.8%; and urine, 5.5% and 7.4%. Statistical analysis was performed by the Mann-Whitney test. The significance level was set at P < 0.05. All the results are expressed as mean ± SD.

Basal serum, urine, and salivary cortisol concentrations are listed in Table 1, as are the results of suppression tests with low and high doses of dexamethasone. The diagnostic sensitivity calculated from basal values of the overall group (n = 38) was 73.3% for serum, 93.3% for saliva, and 93.3% for urine. Diagnostic specificity was 91.7%, 79.2%, and 79.2%, respectively.

We obtained a significant correlation between serum and salivary (r = 0.81, P < 0.001), serum and urine (r = 0.64, P < 0.001), and urine and salivary (r = 0.64, P < 0.001) cortisol concentrations for results obtained in basal conditions and after dexamethasone administration (n = 101).

Advantages of saliva over serum as a biological fluid to measure cortisol are: (a) stress-free and noninvasive collection, (b) simple collection that allows multiple sampling for monitoring rhythmic changes of adrenal activity as well as circadian rhythm, as we previously stated (4, 5) and (c) independence from transcortin fluctuation.

Advantages of salivary determination over urinary cortisol include easier sampling and avoiding a 24-h collection; moreover, salivary cortisol can detect rapid changes in stimulation or suppression test results, whereas urine cannot (4, 5).

Our results show that the diagnostic sensitivity of salivary cortisol is similar to that of free urinary cortisol and exceeds that of serum cortisol. The diagnostic specificity of cortisol concentration is greater in serum samples than in either saliva or urine.

In the present study the results obtained for basal serum cortisol and after dexamethasone administration in the group of patients with Cushing syndrome were well correlated with salivary values. The correlation between serum and salivary cortisol values was similar to what we obtained in previous studies (5).

We conclude that basal salivary cortisol is a good index to study adrenal function and should be one of the first markers to be determined in screening patients with suspected Cushing syndrome.

Table 1. Basal Serum, Urine, and Salivary Cortisol Concentrations (nmol/L) in the Groups Studied and after Dexamethasone (DXM) Suppression Tests in Patients with Cushing Syndrome

<table>
<thead>
<tr>
<th>Serum</th>
<th>Urine</th>
<th>Saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>n</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Cushing syndrome</td>
<td>774 ± 193</td>
<td>14</td>
</tr>
<tr>
<td>DXM 2 mg</td>
<td>589 ± 209</td>
<td>14</td>
</tr>
<tr>
<td>DXM 8 mg</td>
<td>233 ± 258</td>
<td>14</td>
</tr>
<tr>
<td>Other pathologies</td>
<td>417 ± 168*</td>
<td>24</td>
</tr>
<tr>
<td>Reference interval</td>
<td>165-689</td>
<td>207-745</td>
</tr>
</tbody>
</table>

* Significantly different from results for Cushing syndrome: P < 0.01.

References

Influence of Hematocrit on Quantitative Analysis of “Blood Spots” on Filter Paper

To the Editor:

Peripheral blood “spotted” onto special filter paper offers an economical method for collecting samples in mass screening programs. Uniform punches removed from the dried blood spots are extracted for analysis. The precise volume of blood accommodated by these punches can be preestimated for individual batches of paper by using blood labeled with [125I]thyroxine (1). Factors that contribute to imprecision include the paper batch (2), the volume of blood added, and the location of the punch in the blood spot (3). Arens (3) also noted that a decreasing area of paper was covered by the same volume of blood as the hematocrit increased. This “spreadability factor” was shown to influence the estimation of thyrotropin (TSH) from paper punches, TSH being exclusively associated with the serum.

Our study of hematocrit effects on the distribution of whole blood in paper was prompted by the desire not only to measure hemoglobin but also to develop blood-spot assays for growth factors such as folic acid, which are concentrated in the erythrocytes. A fresh normal blood sample (25 mL) was collected in acid–citrate–dextrose. Plasma was separated and then recombined with cells in different proportions to give four simulated “blood samples” having a range of hematocrit values (Coulter Electronics, Hialeah, FL). The mean cell volume of each sample was also noted as an index of sufficient mixing of cells. [125I]Thyroxine (Amersham, Bucks, UK) was added to each sample. Samples were mixed and spotted onto S&S Grade 903 filter paper (Schleicher and Schuell, Keene, NH) in 75- and 150-µL aliquots (n = 20 each). A portion of each sample was retained as a control. The paper was air-dried overnight, and the gamma radiation in the 1/4-in. (6.35-mm)-diameter punches taken from the center of each spot was counted. The radioactivity in measured volumes of blood (100 µL) from each mixed control sample was also counted and used to calculate the volume of blood in the punches. For hematocrit values of 0.503, 0.456, 0.36, and 0.237 these calculated punch volumes were 10.8, 10.5, 8.8, and 8.2 µL, respectively (CV <5.5%) for 75-µL applications and 11.9, 10.3, 10.5, and 8.8 µL (CV <7%) for 100-µL ones. These results demonstrate how both sample hematocrit and volume added may influence the absorbancy of blood on paper.

Similar normal blood samples (n = 3) were dual-labeled, each in separate experiments. Plasma was separated and the erythrocytes were labeled with sodium chromate ([51Cr; Amersham] by a Reference Method (4). Sodium chromate binds predominantly to hemoglobin (5). Iodinated human serum albumin (Amersham) was mixed with the plasma. The cells and plasma for each sample were then recombined to give “blood samples” with a range of hematocrit values. These samples were spotted (100 µL) onto plain S&S paper as described. In this case, the punches (1/4-in. diameter), again from the center of the spots, and a control for each were counted for both [51Cr and 125I]radioactivity. The volumes of blood in the punches were calculated for each sample from its own control, based on the counts of both isotopes (Figure 1).

The results (Figure 1) confirm that the calculated volume of blood in a 1/4-in. punch will vary according to the sample hematocrit. If both isotopes were distributed evenly throughout the blood spot, then the total blood volumes as calculated by either isotope would be similar and independent of the hematocrit. In fact, however, the serum absorbancy of the paper ([125I] counts) became less as the sample hematocrit was decreased, whereas the erythrocyte absorbancy ([51Cr counts) remained relatively constant under the same conditions (Figure 1).

Of course, these experiments cannot compare with blood collection from donors in the field, where clotting and other biological changes may influence the spreading of blood components. However, they do allow us to attempt to examine the effect of hematocrit in isolation. Given that the results (Figure 1) show variability among the three samples under similar study conditions, further studies will be necessary to determine whether hematocrit influence is predictable, or whether factors such as plasma viscosity are also significant.

Dried blood spots are used primarily for screening tests on selected populations, and a considerable degree of imprecision is allowed for. The identification of the individual factors contributing to this imprecision is essential. At present, however, hematocrit could have a significant influence on precision in screening populations at risk of hematocrit deficiencies.

Although estimations of analytes...