Letters to the Editor

Measurement of Late-Night Salivary Cortisol and Cortisone by LC-MS/MS to Assess Preanalytical Sample Contamination with Topical Hydrocortisone

To the Editor:

Measurement of increased late-night salivary cortisol (LNSC) is now a mainstay in the diagnosis of endogenous Cushing syndrome (1). Patients typically sample their saliva at home and are carefully instructed to avoid using potential contaminants, such as topical creams or ointments containing hydrocortisone (authentic cortisol). Using these creams or ointments can cause preanalytical contamination of the saliva sample that cannot be distinguished from endogenous cortisol as measured by either immunoassay or liquid chromatography–tandem mass spectrometry (LC-MS/MS). With endogenous cortisol production, the cortisone concentration in the saliva is usually higher than the cortisol concentration, and typically the cortisol-to-cortisone ratio is <1, owing to the transfer of cortisone from the plasma and the conversion of cortisol to cortisone by 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) in the salivary gland (2, 3). Contamination of the saliva with topical or oral hydrocortisone (cortisol) during sample collection would not be expected to increase salivary cortisone because there is no 11β-HSD2 activity in collected saliva (3). Furthermore, topical hydrocortisone is usually not absorbed into the plasma compartment in substantial quantities. Therefore, topical hydrocortisone constitutes true contamination of the saliva and is not converted to cortisone. We hypothesized that a very high cortisol-to-cortisone ratio would be strong evidence that an increased salivary cortisol value was due to contamination with topical or oral hydrocortisone. Such a finding is prima facie evidence of contamination and is justification for the clinician to reinterview the patient.

We evaluated samples referred to the Endocrine Research Laboratory at Aurora St. Luke’s Medical Center for standard LNSC analysis. These samples yielded very high values by ELISA, raising the suspicion of contamination (4) (Table 1). ELISA was performed as previously described (5) with both undiluted and diluted (1 volume of sample in 19 volumes of diluent) LNSC samples (reference interval for LNSC by ELISA, <4.2 nmol/L). We used LC-MS/MS analysis (2) and our previously published algorithm (4) to evaluate whether the patient might have contaminated samples with topical hydrocortisone during collection. The LC-MS/MS reference interval is <2.8 nmol/L for LNSC and <28 nmol/L for late-night salivary cortisone. The reference interval for the salivary cortisol-to-cortisone ratio is 0.2–1.1 at awakening (when the cortisol concentration is typically at its circadian peak) and 0.1–1.2 for late-night samples (the circadian nadir).

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References


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1 Nonstandard abbreviations: LNSC, late-night salivary cortisol; LC-MS/MS, liquid chromatography–tandem mass spectrometry; 11β-HSD2, 11β-hydroxysteroid dehydrogenase type 2.

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The LNSC LC-MS/MS results demonstrated very high cortisol concentrations, confirming the ELISA results (Table 1); however, salivary cortisone concentrations were not increased. Thus, the cortisol-to-cortisone ratios for patients 1–6 were much higher than the reference interval. Patients 7 and 8 had appropriately increased salivary cortisone concentrations and cortisol-to-cortisone ratios within the reference interval, suggesting endogenous hypercortisolism in these 2 patients. The physicians who referred the samples for clinical analyses were subsequently contacted. After intensive interviews of the patients, the physicians confirmed topical hydrocortisone use by 5 of the 6 patients with high cortisol-to-cortisone ratios. Patient 4 was evasive in her response to the physician, and subsequent ELISA data for LNSC obtained after this interview were all <4.2 nmol/L. The physician concluded that the patient had surreptitiously been using exogenous corticosteroids but was unable to confirm this suspicion in interviews with the patient. Patients 7 and 8 were subsequently reported by the referring physician to have endogenous hypercortisolism.

We conclude, as we hypothesized with our previously proposed algorithm (4), that contamination of saliva samples with topical hydrocortisone should be considered when LNSC results obtained by immunoassay are markedly increased, particularly when they are out of proportion with respect to other biochemical test results or clinical findings. In our experience as a reference laboratory, 1 of approximately 200 patients who submit samples has a dramatically increased salivary cortisol value by ELISA, warranting analysis for contamination with topical hydrocortisone. In that scenario, contamination with topical hydrocortisone would be suggested by a normal salivary cortisone concentration as measured by LC-MS/MS and a high cortisol-to-cortisone ratio.

**Table 1. LNSC analysis by ELISA and subsequent detection of contamination by analysis of salivary cortisol and cortisone and calculation of the cortisol-to-cortisone ratio.**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Cortisol (ELISA), nmol/L</th>
<th>Cortisol (LC-MS/MS), nmol/L</th>
<th>Cortisone (LC-MS/MS), nmol/L</th>
<th>Cortisol-to-cortisone ratio</th>
<th>Contamination source</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>&gt;1600</td>
<td>14324</td>
<td>16</td>
<td>895</td>
<td>Hydrocortisone ointment</td>
</tr>
<tr>
<td>2</td>
<td>130</td>
<td>245</td>
<td>5</td>
<td>49</td>
<td>Hydrocortisone cream</td>
</tr>
<tr>
<td>3</td>
<td>1256</td>
<td>1110</td>
<td>10</td>
<td>111</td>
<td>Hydrocortisone cream</td>
</tr>
<tr>
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<td>&gt;1600</td>
<td>208107</td>
<td>10</td>
<td>20810</td>
<td>Hydrocortisone suspected</td>
</tr>
<tr>
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<td>800</td>
<td>8</td>
<td>100</td>
<td>Hydrocortisone cream</td>
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<td>6</td>
<td>316</td>
<td>226</td>
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<td>38</td>
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</tr>
<tr>
<td>8</td>
<td>29</td>
<td>27</td>
<td>66</td>
<td>0.4</td>
<td>None</td>
</tr>
</tbody>
</table>

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**References**


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**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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**Inaccurate 25-Hydroxyvitamin D Results from a Common Immunoassay**

**To the Editor:**

The great increase in the volume of vitamin D testing over the past several years has created a demand for