Comparison of Two Methods for Measuring Salivary Cortisol

To the Editor:

The concentration of cortisol in the saliva reflects the activity of the hypothalamic-pituitary-adrenal axis (1), and an increased late-night salivary cortisol has >95% sensitivity and specificity for Cushing syndrome (1, 2).

We compared a commonly used modification of a RIA for serum cortisol with a new enzyme immunoassay (EIA) specifically designed to measure salivary cortisol in 352 saliva samples. The first samples (n = 195) were sent to our laboratory to screen for Cushing syndrome; all were collected at 2300 from 90 patients (age range, 17–77 years; 74 women and 16 men). The second set of samples (n = 104) was obtained at 2300 and 0700 from healthy, elderly patients (n = 52; age range, 67–82 years; 21 women and 31 men) who were part of a large, longitudinal study (3). The third set of samples (n = 53) was obtained at 2300 and 0700 from a group of apparently healthy individuals (n = 29; age range, 12–63 years; 16 women and 11 men). The study was approved by the appropriate Institutional Review Boards, and consent was obtained.

Saliva was sampled as described previously (2, 4) with a collecting device (Salivettes with no preservative; Sarstedt).

Salivary cortisol was measured by two methods. The serum cortisol RIA (Coat-a-Count TKCO; Diagnostic Products) was used as commonly modified for the measurement of salivary cortisol (2, 4). The salivary cortisol EIA (product no. 10-67100; Diagnostic Systems Laboratories) was used as instructed without modification. The sample volumes were 200 and 25 µL for the modified RIA and EIA, respectively, and the incubation times were 3 h for the RIA and 45 min for the EIA. The EIA calibrators ranged from 2.8 to 276 nmol/L and were provided as cortisol in a solution of bovine serum albumin.

The lower detection limit of the EIA was 0.4 nmol/L. The intraassay imprecision (CV) was 9.5% at 6.3 (SD, 0.6) nmol/L (n = 12), 4.4% at 10.6 (SD, 0.5) nmol/L (n = 9), and 5.6% at 53.7 (SD, 3.0) nmol/L (n = 12). Interassay (total) imprecision (CV) was 9.7% at 4.3 (SD, 0.4) nmol/L (n = 13), 11% at 7.4 (SD, 0.8) nmol/L (n = 13), and 7.8% at 59.6 (SD, 4.7) nmol/L (n = 13).

The regression lines for the data from the three groups of individuals were not different from each other, so the combined data are shown in Fig. 1. The slope of the regression for RIA salivary cortisol values <11 nmol/L was not significantly different from the slope for the complete data set (Fig. 1, inset). Because the EIA showed consistently higher salivary cortisol concentrations, we assayed the RIA calibrators in the EIA, and vice versa. The EIA gave values for the RIA calibrators that were the same as the RIA stated values, and vice versa. When we added the 27.6 nmol/L EIA calibrator to saliva, the RIA returned a value of 21.2 nmol/L. When we added the 13.8 nmol/L RIA calibrator to saliva, the EIA returned a value of 15.8 nmol/L.

To determine which method was correct, cortisol (Hydrocortisone; cat. no. H 4001; Sigma Chemical) was accurately weighed to create a stock solution of 2.76 mmol/L and then diluted. Cortisol (23 nmol/L) or vehicle was added to saliva and assayed 10 times. The difference between saliva with cortisol added and saliva with vehicle added was 22.4 (SD, 0.7) nmol/L in the RIA and 27.3 (SD, 0.6) nmol/L in the EIA. Therefore, the RIA was much closer to the expected result (23 nmol/L) than the EIA, and the EIA overestimated the concentration of cortisol in the saliva.

The most important finding is that the EIA returned consistently higher salivary cortisol values than the RIA. The RIA gave results much closer to the expected value of an independently created cortisol stock solution diluted in saliva. Because salivary cortisol measurement is increasing in popularity, it is important to be aware of the different results generated by these two commercially available methods and to interpret the published reference intervals appropriately.

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References


Caveats in Carbohydrate-deficient Transferrin Determination

To the Editor:

Carbohydrate-deficient transferrin (CDT) refers to a group of minor isoforms of transferrin that, according to most authors, includes asialo-, monosialo-, and disialo-Fe₂-
transferrin (1). CDT is a widely used marker of chronic alcohol abuse in Europe and, after its recent approval by the Food and Drug Administration, a candidate to become a popular diagnostic tool in the US (2). Arndt (1) recently concluded that “CDT is the most specific marker of chronic alcohol abuse to date”. Its specificity, however, is affected by the choice of analytical method, which must selectively determine only the CDT components, without interference from the other transferrin glycoforms (trisialo-, tetrasialo-, pentasialo-, and hexasialo-Fe₂-
transferrin), which are present in large excess.

Interference from trisialo-Fe₂-
transferrin has been reported for most commercial CDT tests, which are based on anion-exchange micro-
chromatographic separations followed by immunoassays. Unfortunately, much of the literature does not report any correlation between trisialo-transferrin and alcohol intake (3). Moreover, Dibbelt (4) recently demonstrated by HPLC that increased relative concentrations of di-
sialo- and asialo-transferrin associated with alcohol abuse are not correlated with increased trisialo-
transferrin concentrations and consequently stated that “trisialo-transferrin is obviously of no diagnostic value, I strongly recommend not including this iso transferrin in the CDT fraction measured for laboratory diagnosis of alcoholism”.

To avoid generation of false-positive results in the presence of isolated increases of trisialo-transferrin, data from immunoassays are currently confirmed by separative methods such as HPLC and isoelectric focusing (1). Recently, capillary electrophoresis methods have been developed and validated to provide selective determination of the individual CDT isoforms (3).

It appears quite unfortunate that a recent paper by Wuyts et al. (5) describes a capillary zone electrophoretic method for CDT determination that is clearly unable to separate disialo-transferrin, the major component of CDT, and trisialo-transferrin. By contrast, several capillary electrophoresis methods provide baseline separation and individual quantification of the two isoforms (6–10).

The claimed advantage of direct serum injection offered by the method reported by Wuyts et al. (5) is clearly of minor relevance if compared with the lack of selectivity, which may lead to false-positive results. On the other hand, most of the recently published capillary electrophoretic methods (7–9) require only minimal sample pretreatment, limited to iron saturation and dilution of the serum sample.

We suggest that assays for CDT be based on only specific methods and that analytical selectivity and diagnostic effectiveness should not be sacrificed in favor of operative simplicity, throughput, and automation.

References


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