IN VITRO NUCLEAR MEDICINE

Salivary Thyroxine as an Estimate of Free Thyroxine: Concise Communication

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To test the hypothesis that the levels of salivary thyroxine (T_4) reflect those of circulating free T_4, we developed a radioimmunoassay (RIA) sensitive to low levels of T_4. Concurrent saliva and serum samples were obtained from 32 euthyroid volunteers, ages 19–64. Salivary and serum T_4 and cortisol levels were measured by RIA. Salivary albumin was measured by nephelometry. Salivary T_4 levels were higher than predicted, 4.2–35 ng/dl (normal range 0.6–2.0). No correlation was found between salivary T_4 and serum levels of free T_4 and total T_4 but there was a significant correlation between salivary T_4 and albumin ($r = 0.82$). Salivary cortisol levels agreed with reported results and showed no correlation with salivary albumin. We conclude that salivary levels of drugs and hormones may be strongly affected by protein binding, and caution must be exercised in using salivary levels as an estimate of circulating free levels.

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Free levels of hormones and drugs are generally thought to be the determinant for physiological action. Measurements of free hormones and drug levels present technical problems, particularly when the hormone or drug is strongly bound to serum proteins and the free levels are low compared with the bound level. Dialysis is the usual method for separating free from protein-bound fractions but it requires specialized skills and is time-consuming, so it is seldom used in the clinical laboratory. Intense clinical interest in free T_4 levels has prompted the marketing of several radioimmunoassays to measure free T_4 levels directly, without dialysis (*1*). Free T_4 levels determined with these assays correlate well with levels determined by dialysis on sera from normal subjects, but each assay seems to have its methodological biases when used for abnormal subjects. Saliva has been suggested as a "window" for free analyte levels with the underlying assumption that the salivary glands passively allow the free molecules into the saliva while retaining the fraction bound by serum proteins. This assumption has worked well for drugs such as salicylic acid, acetaminophen, theophylline, digoxin, and for steroid hormones such as cortisol, testosterone, aldosterone, and progesterone (*2,3*).

The possibility that salivary T_4 levels might reflect plasma free T_4 levels was intriguing and provided the impetus for this study.

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MATERIALS AND METHODS

A radioimmunoassay for the expected low levels of T_4 was developed, similar to assays we previously reported (*4,5*). A phosphate buffer was substituted for HEPES, and EDTA was added. The assay buffer was 0.01 *M* PO_4, pH 7.4, 0.01 *M* EDTA, 0.1% gelatin.* Standards were prepared by diluting a stock solution of T_4 in assay buffer.

Most available T_4 antisera are not adequate for the determination of T_4 at pg/ml levels, but two were located; one was a gift§, the other was a commercial product.† Both antisera were effective at the same dilution. They were diluted 1:40,000 in assay buffer. The I-125 T_4 was carrier-free with a specific activity of >5500 μCi/μg.‡ It was necessary to purify it by adsorption chromatography on sephadex (*6*).

The assay procedure was the following. One hundred μl of standard or sample, 100 μl of I-125 T_4 (approximately 10,000 cpm), and 100 μl anti-T_4 (1:40,000) were placed in 12- by 75-mm glass tubes. The assay was incubated overnight at 4°C. While on ice, one ml of cold 20% polyethylene glycol (diluted in H_2O) was added. The tubes were vortexed and centrifuged at 2000 *g* for 30 min at 4°C. The supernate was aspirated and the radioactivity measured in a gamma counter. T_4 levels were read from a plot of B/Bo × 100 against log of T_4 concentration. Samples and standards were run in duplicate. Tubes for nonspecific binding were run with the standards and each sample.

Serum cortisol was measured with a commercial kit. Standards were diluted in 10 m*M* phosphate-saline buffer, pH 7.4. Tubes were rotated overnight at room temperature, centrifuged, decanted, and radioactivity measured in a gamma counter. Cortisol levels were read from a plot of B/Bo × 100 against log of cortisol concentration. Samples and standards were run in duplicate.

Samples of saliva and serum were obtained from euthyroid volunteers, ages 19 to 64, with normal serum data for T_4, T_3, T_3U, and FTI (T_3U × total T_4). Saliva samples were collected by several methods. The most convenient used cotton dental rolls (*7*). Two of these rolls were placed in the subject's mouth to adsorb saliva and to give mechanical stimulation to increase flow. After several minutes, when the rolls were saturated, they were removed and placed into the barrel of a 10-cc syringe. The 2-3 ml of saliva was then expressed from the rolls with the plunger into a glass culture tube. The dental rolls were not effective with all volunteers, so other methods were also used involving chewing on wax or clean rubber bands and expectorating into the culture tube.

Salivary albumin levels were measured by nephelometry using an immunochemistry analyzer. Statistical analysis included linear regression and correlation coefficients.

RESULTS

The radioimmunoassay was used over the range of 2 to 100 pg T_4 per tube. B_0 was 35 to 40%. Sensitivity (B/B_0 = 90%) was 2 pg T_4/tube. Between-run coefficient of variation was 6.6% at B/B_0 = 50%. Within-run coefficient of variation was 7.6% at about 7 pg T_4/tube. Dilutions of saliva gave a response that was parallel to the standard curve. Dilutions of serum with assay buffer to the same T_4 range bound more tracer than antiserum alone and did not produce a dose-response curve.

Saliva levels of T_4 were higher than the expected normal range of serum-free T_4 of 0.6 to 2.0 ng/dl (*1*). The observed T_4 levels ranged from 4.2 ng/dl to 35 ng/dl and, from another series, up to 100 ng/dl. The higher level is about 50 times the upper limit of the normal range for serum-free T_4 as measured by dialysis. Generally the salivary T_4 levels were about 10 times the expected free T_4 level.

There was no correlation between serum T_4 and salivary T_4, r = 0.1, n = 30. Also there was no correlation between the FTI and salivary T_4, r = 0.05, n = 30. In a smaller group of seven, serum-free T_4 was determined by a direct radioimmunoassay of a dialysate obtained by ultrafiltration. There was no significant correlation between serum-free T_4 and salivary T_4, r = −0.3, n = 7.

One subject was sampled repeatedly throughout the day, and spikes of salivary T_4 levels (2-3 times basal) were observed at mealtimes.

Aliquots of saliva that were collected by the three methods (chewing on wax, chewing on rubber bands, and with cotton rolls) were incubated with fresh cotton rolls for 15 min. Measured T_4 levels in these aliquots were 13 to 17% lower than in the untreated saliva.

There was a positive correlation between salivary albumin levels and salivary T_4, r = 0.82, n = 27 (Fig. 1).

Salivary cortisol levels were within the expected range (*3*). There was a weak correlation between salivary cortisol and serum cortisol levels (r = 0.56) but there was no correlation between salivary cortisol and salivary albumin (r = −0.2).

DISCUSSION

The level of a therapeutic drug in saliva is generally considered to be the same as that of the free drug in

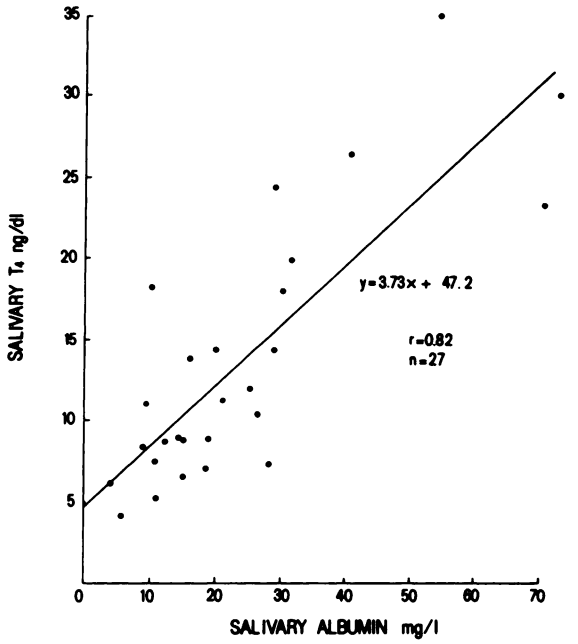

FIG. 1. Comparison of salivary T_4 levels with salivary albumin levels.

circulating plasma (*2*). Hormones such as cortisol and testosterone are present in saliva, and the salivary level reflects the free hormone level in plasma (*3*). Consequently, it was anticipated that salivary T_4 would correlate with the free T_4 level in plasma.

The observed salivary T_4 levels were about tenfold higher than anticipated. Also there was essentially no correlation between the salivary T_4 levels and serum total T_4, FTI, and serum-free T_4. Active transport, while unlikely, cannot be ruled out on the basis of the data of this report. The most likely explanation is the salivary albumin concentration. Salivary albumin levels correlate well ($r = 0.82$) with salivary T_4 levels.

Oral saliva contains a wide variety of materials such as food debris, bacteria, shed cells, leukocytes, secretory proteins, and proteins with smaller molecules from plasma. The composition of saliva varies with flow rate and differs between parotid and submandibular glands (*8*). Whole saliva, a mixture of parotid and submandibular gland secretions, was used for this study. Chewing wax or rubber bands gave a sample with a great deal of insoluble material that was somewhat difficult to handle. Centrifugation helped clarify the sample and did not affect the T_4 level measured. The use of the cotton dental rolls was most convenient and gave a relatively clear sample since the insoluble material was filtered by the cotton roll as the saliva was expressed from the syringe. However, the absorption of T_4 by the cotton rolls makes their use questionable.

The correlation of salivary T_4 levels with salivary albumin levels suggests that plasma proteins may cross the salivary glands and carry bound T_4 into the saliva. In saliva these proteins are at a very low concentration and do not compete with the T_4 antiserum in the assay of T_4. Cortisol is about 95% bound by serum proteins. This binding is low when compared with serum protein binding of T_4, and does not adversely effect salivary levels. The salivary cortisol levels we measured are in agreement with other reports and are not correlated with salivary albumin levels. The use of saliva levels as an index of free levels in circulating plasma may well be limited to molecules that are not strongly bound to plasma proteins.

We conclude that salivary levels of drugs or hormones may be strongly affected by salivary protein concentration, and caution must be exercised when using salivary levels as an estimate of circulating free levels.

FOOTNOTES

* Swine skin, 300 bloom, Sigma Chemical, St. Louis, MO 63178.
† Calbiochem, La Jolla, CA 92112.
‡ New England Nuclear, North Billerica, MA 01862.

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