4 °C. For 12 additional patients (group B) the analysis was performed immediately and repeated 10 and 30 days after collection of the samples, which were stored at room temperature in airtight containers.

The data were examined by Student's paired t-test and linear-regression analysis.

In neither group did storage conditions significantly influence the SCN measured two months (group A, t = 0.569, NS), 10 days (group B, t = 0.419, NS), or 30 days (group B, t = 0.01, NS) after sampling. Results of prompt and postponed analysis correlated well (Figure 1). In group B, culture of the samples confirmed in all cases the presence of polymicrobial flora normally present in oropharynx.

We conclude that SCN is stable in saliva stored in airtight containers for as long as one month at room temperature or two months at 4 °C—an additional advantage for the large-scale application of the test in epidemiological studies.

References

Fructosamine Assay Modified for the Estimation of Glycated Hemoglobin, B. L. Somani, R. Sinha, and M. M. Gupta (Departments of 1 Biochemistry and 2 Medicine, Armed Forces Med. College, Pune-411 040, India)

Glycated proteins reduce alkaline nitroblue tetrazolium chloride (NBT) reagent, and this property has been used for their estimation in fructose assays (1). Could the same property be used for estimation of globin-bound fructosamine, as an index of hemoglobin glycation?

We measured glycated hemoglobin by affinity chromatography (2) and hemoglobin by the cyanmethemoglobin method (3). To determine globin-bound and serum fructosamine, we modified the NBT reagent, using carbonate buffer (0.2 mol/L, pH 10.35) containing 500 μmol of NBT and 2 mmol of p-chloromercuribenzoic acid per liter.

Hemolysate, free of cell debris, was prepared from saline-washed erythrocytes. Hemolysate (0.5 to 1.0 mL) containing 10 mg of hemoglobin was added dropwise to 10 mL of cold acetone containing 30 μL of hydrochloric acid (2 mol/L), and mixed for 10 min. We then centrifuged (1000 x g, 10 min), decanted the acetone, and dissolved the globin precipitate in 1 mL of 9 g/L sodium chloride within 1 min.

We mixed 0.2 mL of test serum or standardized pooled serum with 1 mL of 9 g/L sodium chloride, incubated these and the globin solutions at 37 °C for 5 to 10 min, then added 1 mL of prewarmed NBT reagent to each sample at 1-min intervals. The globin solution initially precipitates, but redissolves when all of the reagent has been added. We measured the absorbance in a Beckman spectrophotometer (Model 25 UV/Vis) at 530 nm 10 (A1) and 20 min (A2) later, and calculated the concentration of globin-bound and serum fructosamine from ΔA(A2 minus A1), expressing the results as nmol/10 mg of globin and mmol/L of serum, respectively.

It is essential to centrifuge the globin precipitate at slow speed (1000 x g, 10 min) and dissolve it immediately (within 1 min) in 9 g/L sodium chloride, to prevent denaturation of globin from heat or air exposure. The p-chloromercuribenzoic acid in the NBT reagent is necessary for measuring the absorbance change ascribable to globin-bound fructosamine in the presence of -SH groups; otherwise these groups reduce NBT to a deep purple.

Results of this test for 30 normoglycemics and 41 diabetics were as follows:

<table>
<thead>
<tr>
<th>% Gly-Hb by affinity chromatog.</th>
<th>Globin-bound fructosamine, mmol/10 mg</th>
<th>Serum fructosamine, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoglycemics</td>
<td>4.38 (0.56)</td>
<td>124.81 (22.68)</td>
</tr>
<tr>
<td>Diabetics</td>
<td>10.65 (4.10)</td>
<td>298.21 (96.71)</td>
</tr>
</tbody>
</table>

Glycated hemoglobin, serum fructosamine, and globin-bound fructosamine were all significantly higher in diabetics than in normoglycemics (P < 0.001).

Intra-assay CVs for globin-bound fructosamine assay at mean values of 103.7 and 244.3 mmol/10 mg were 6.27% and 2.67%, respectively. For these 71 subjects, globin-bound fructosamine values (y) correlated well with glycemic hemoglobin (y = 24.23x + 26.94; Sx = 0.96) and serum fructosamine (y = 171.7x - 104.5; Sx = 0.92).

Unlike other methods (4, 5) this method avoids the hydrolysis of globin and eliminates interference from residual heme without requiring a separate control; the same solution acts as control and test (absorbance measured at 10 and 20 min). Moreover both long- and medium-term control of blood glucose can be monitored simultaneously, as globin-bound and serum fructosamine. The method also offers the possibility of automated analysis.

References

Comparison of Seven Sensitive Thyrotropin (TSH) Assays to Predict a Suppressed Thyrotrophin (TRH) Test, J. J. Body, F. Seraj, and V. Keymolen (Service de Médecine et Laboratoire d'Investigation Clinique H. Tagnon, Unité d'Endocrinologie, Institut J. Bordet Centre des Tumours de l'Université Libre de Bruxelles, 1 Rue Héger-Bordet, 1000 Bruxelles, Belgium)

Newer, more-sensitive TSH assays represent a considerable advance in the evaluation of thyroid disorders (1). Much literature exists on technical performances of numer-
ous assays and on their value for distinguishing hyperthyroid from euthyroid conditions (1–5). It has also been suggested that basal TSH measurement could replace the TRH stimulation test (1, 4), a step that would be most important for the prediction of a blunted TSH response. However, very few comparative studies between commercially available assays for that have been performed. We have compared in seven assays the value of an undetectable basal TSH in predicting a suppressed TRH test.

Our study population consisted of 275 patients who suffered from multi-nodular goiter (55%), single thyroid nodule (26%), thyroid cancer (11%), or miscellaneous problems (8%). The mean age was 48 (SD 13) years. They all underwent a TRH stimulation test (200 μg i.v.; blood sampling at 20 and 40 min) and the TSH response was “suppressed” [i.e., TSH increased by <1 milli-int. unit/L (5)] in 90 patients. Basal TSH levels were measured with seven assays: kit 1, TSH Amerwell IRMA (Amersham, U.K.); kit 2, Simultrac Free T4/TSH (Becton-Dickinson, Rutherford, NJ); kit 3, RiagnoST hTSH (Behring, Marburg, F.R.G.); kit 4, TSH IRMA (IRE-Medgenix, Fleurus, Belgium); kit 5, Delfia hTSH (LKB, Turku, Finland); kit 6, Pharmacia TSH RIA (Pharmacica, Uppsala, Sweden); kit 7, Spectria TSH IRMA (Farmos, Oulu, Finland). Moreover, free T4 and free T3 were measured in 47 of the 90 patients having a suppressed TRH test (Amerlex FT3 and FT4, Amersham).

The tabulation below summarizes the assay detection limits (defined as the mean + 2 SD of 32–40 replicate measurements of the zero standards), and the sensitivity, specificity, and accuracy (6) of an undetectable basal TSH to predict suppressed TSH response to TRH injection.

Kits 2 and 6 clearly perform less well. Differences between the five other kits were negligible. Except for kit 6, 90.6% to 93.5% of the patients were correctly classified (last column of the table). Adding FT3 measurement did not improve the prediction value of an undetectable TSH, whereas measurement of FT4 increased the sensitivity by a maximum of 4.3%, except for the Simultrac assay, where the large improvement (see table) was due to the very poor sensitivity of the TSH assay itself.

In conclusion, if measured with a sensitive assay, the existence of an undetectable basal TSH is a good predictor of a suppressed TRH test. Under these conditions, the value of supplementary determination of FT4 and FT3 seems limited.

References