Thyroid-Function Assessment by Use of Enzyme Immunoassays

Maria Teresa Proença and Luis Simões de Moura

We used an enzyme immunoassay to determine the normal reference intervals for thyroxin, triiodothyronine, and thyrotropin in groups of children, ranging from newborns to 15-year-olds. Results compared well with those by radioimmunoassay. There were no substantial differences between plasma and serum samples for thyroxin and triiodothyronine, but thyrotropin concentrations differed significantly (p < 0.05, n = 20 each).

Additional Keyphrases: pediatric chemistry · reference interval · age-related effects · plasma vs serum as sample · immunoenzymometric assay

In face of the limitations imposed by the strict regulatory controls on the use of isotopes, radioimmunoassay (RIA) tests are being replaced by enzyme immunoassays, especially the enzyme-linked immunosorbent assay (ELISA).

In this heterogeneous enzyme immunoassay, enzyme-labeled antigen is mixed with the test sample containing antigen, which competes for a limited amount of antibody coated onto the inside of the test vial. This is the competitive test used for assay of triiodothyronine (T3) and thyroxin (T4). Because thyrotropin (TSH) has several haptenic groups, assays for the hormone require an alternative method, based on the double-antibody "sandwich" technique: TSH in the sample is first bound by solid-phase-bound antibodies, then the addition of an enzyme-labeled specific antibody leads to the formation of "sandwich" TSH-complexes, in proportion to the TSH content of the sample. In both kinds of assays the bound fraction is then separated from the free material and the activity of the solid-phase-bound marker enzyme is determined photometrically after the addition of a substrate and a chromogen (1).

The purpose of our study was to establish normal concentrations for T3, T4, and TSH in serum by using an enzymic immunoassay, to compare them with the values for plasma, and to see whether there are significant differences between results by ELISA and by RIA.

Materials and Methods

Serum was sampled from 108 healthy children, divided into six groups matched for sex. We measured T3, T4, and TSH. Group 1 samples were cord bloods from 20 neonates. Group 2 comprised 24 neonates, ages one to five days old (mean, two days); group 3, 11 infants from one to 12 months old (mean, four months); group 4, 16 children from one to five years old (mean, four years); group 5, 22 children from five to 10 years old (mean, eight years); and group 6, 15 children from 10 to 15 years old (mean, 13 years).

In 20 of the 108 children we compared plasma vs serum with respect to mean concentrations of T3, T4, and TSH by ELISA; we also compared their serum T3, T4, and TSH concentrations as determined by ELISA and by RIA.

Serum and plasma treated with EDTA were stored at -20°C; each sample was frozen no more than once.

The kit used was an immunodiagnostic kit from Boehringer Mannheim, in which peroxidase is the enzyme and diaminonitrobenzoic acid (ABTS*) is the chromogen. Test samples were analyzed in duplicate and the results obtained from a calibration curve constructed from six standards. Control serum provided in the kit was also used for quality control. Absorbance at 420 nm was measured in a Beckman Model 42 spectrophotometer equipped with a suction cuvette and printer.

Student's t-test was used to assess differences between mean values for T3, T4, and TSH in the groups' plasma and serum, and by ELISA/RIA. Correlation coefficients were obtained as part of a regression analysis.

Results and Discussion

Figure 1 depicts the differences between mean serum concentrations of T3, T4, and TSH in the different age groups.

Table 1 shows serum concentrations (mean ± 2SD) for T3, T4, and TSH from birth to age 15 years.

There was a significant correlation between concentrations in serum as determined by ELISA and RIA: T3 (r = 0.55; p < 0.05), T4 (r = 0.78; p < 0.01), and TSH (r = 0.98; p < 0.01), respectively (n = 20 each). Results by the two techniques were not significantly different for the mean values of serum T3, T4, and TSH.

Comparing serum and plasma concentrations by ELISA, we also found good correlations for T3 (r = 0.77; p < 0.01), T4 (r = 0.88; p < 0.01), and TSH (r = 0.83; p < 0.01) (n = 20 each). There was no significant difference for mean values of T3 and T4 in the two kinds of samples, but TSH values differed significantly (p < 0.05).

Endocrinological applications are the focus of much of the current ELISA research (2, 3). Given the mean age of the

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1. Nonstandard abbreviations: ELISA, enzyme-linked immunosorbent assay; T3, thyroxin; T4, triiodothyronine; TSH, thyrotropin.

2. Received January 14, 1985; accepted February 25, 1984.
Table 1. ELISA-Measured Concentrations of T3, T4, and TSH (Mean ± 2 SD) in Serum, from Birth to Age 15 Years

<table>
<thead>
<tr>
<th>Group</th>
<th>T4, µg/L</th>
<th>T3, ng/L</th>
<th>TSH, mili-int. units/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>93 ± 29</td>
<td>600 ± 530</td>
<td>8.7 ± 7.7</td>
</tr>
<tr>
<td>Group 2</td>
<td>165 ± 64</td>
<td>1560 ± 1020</td>
<td>7.6 ± 9</td>
</tr>
<tr>
<td>Group 3</td>
<td>102 ± 47</td>
<td>1770 ± 1000</td>
<td>2.3 ± 1.7</td>
</tr>
<tr>
<td>Group 4</td>
<td>110 ± 48</td>
<td>1560 ± 640</td>
<td>2.9 ± 2.2</td>
</tr>
<tr>
<td>Group 5</td>
<td>103 ± 40</td>
<td>1500 ± 580</td>
<td>2.7 ± 2.6</td>
</tr>
<tr>
<td>Group 6</td>
<td>90 ± 27</td>
<td>1410 ± 620</td>
<td>2.5 ± 2.1</td>
</tr>
</tbody>
</table>

See text for age range of subjects in each group.

infants in group 2 (two days) we were not able to demonstrate the postnatal surge of TSH, but transient thyrotoxicosis is clearly suggested by the significant increase of T4 (Figure 1).

After the second postnatal day, serum concentrations of T3 remained stable in all groups. TSH mean value did not change significantly in the periods from birth to five days and from one month to 15 years. T4 is stable between one month and 10 years, but decreases significantly ($p < 0.05$) at puberty.

For determinations of T4 and T3 one can use serum or plasma treated with EDTA, but the same is not true for TSH.

The good correlation between ELISA and RIA techniques supports the use of enzymes as promising new labels, making them particularly suitable in small laboratories with limited technical and financial resources.

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References