in diabetic patients. In the present study, we have found that a measurement of serum fructosamine would give similar information as an Hb A1c estimation. Although some physicians may prefer the longer integration of blood glucose concentrations offered by measurement of Hb A1c, we find that measuring fructosamine has some advantage, the assay being technically simple and easily automated. Indeed, the kit assessed here gave a precise and reliable quantification of fructosamine concentration in serum.

The fructosamine kits were kindly supplied by Roche Diagnostics Ltd. (NZ). We thank Dr. B. J. Linehan, Hamilton Medical Labs., for the specimens and for information regarding the glucose tolerance tests.

References

Rate Nephelometry and Radial Immunodiffusion Compared for Measuring Serum Prealbumin

Barbara M. Goldsmith1,2 and Sara Munson1

We compared a rate-nephelometric method and a radial immunodiffusion (RID) assay for measurement of prealbumin (transthyretin) in 55 samples of serum from healthy children. The mean prealbumin concentration as measured by the Beckman Auto ICS nephelometer was 188 mg/L (range 128–350); the mean by RID was 221 mg/L (range 125–419). This difference was statistically significant by Student's t-test (p < 0.05), but the correlation coefficient (r) was 0.95. To determine a reference interval for prealbumin in children by the Auto ICS method, we assayed samples from 93 healthy children between the ages of one day and 18 years (55 boys, 38 girls). The mean was 191 mg/L, the reference interval (mean ± 2 SD) 109–273 mg/L. There was no significant difference in prealbumin concentrations between girls and boys (Student's t-test, p > 0.05). Evidently the Beckman Auto ICS method measures prealbumin in serum rapidly and accurately.

Additional Keyphrases: pediatric chemistry · reference interval · nutritional status · transthyretin

In light of estimates that up to half of hospitalized patients may be malnourished (1, 2), assessment of protein-energy status is becoming an increasingly important component of medical practice. Treatment by enteral and parenteral routes have improved the nutritional status of patients, but there continues to be a need more accurately to monitor patients receiving therapy. Nutritional assessment of patients has included anthropometric, biochemical, and clinical measurements and dietary control (1). Biochemical assessments have consisted of measuring albumin and transferrin in serum; however, the relatively long biological half-lives of these analytes (20 and nine days, respectively) limit their usefulness (3–5). In the last few years, prealbumin has received increasing attention in the literature as a potentially better nutritional marker with a shorter biological half-life (3, 6–7).

Prealbumin, also known as transthyretin or thyroxin-binding prealbumin, is a 54 000-Da glycoprotein synthesized in the liver; its half-life is two days. It is a carrier of retinol-binding protein and transports about a third of the thyroxin in blood (7). Radial immunodiffusion (RID) is the method most often

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reported for measuring prealbumin. Recently, commercial reagents have become available for measuring prealbumin by rate nephelometry. We have compared results obtained by RID and by nephelometry for serum samples from healthy children, and we used the rate-nephelometric method to determine a reference interval for prealbumin in children.

Materials and Methods

Samples

Three milliliters of blood, drawn by syringe, were transferred to a tube containing no preservatives or anticoagulants. The blood was allowed to clot, and the samples were centrifuged. The serum was separated and stored at -20 °C until analysis.

To establish a reference interval, we collected blood samples from 93 children (55 boys, 38 girls), ages one day to 18 years, who had been fasting for at least 6 h. Only those children being admitted for minor surgical procedures such as ear-tube insertions, tonsillectomies, adenoidectomies, and orthopedic surgeries were asked to participate; these children were considered healthy from a nutritional standpoint. Samples from 55 of these children (31 boys, 24 girls) were also used in the method-comparison study.

Equipment and Reagents

Rate nephelometer and reagents. We used an Auto Immunochromatography System (ICS) and ICS Diluent and Calibrator from Beckman Instruments, Brea, CA 92621.

Radial immunodiffusion plates, standards, and controls. Human prealbumin "M-Partigen" RID plates, "Standard Serum B," and "Partigen Control Plasma" were purchased from Behring Diagnostics, La Jolla, CA 92037. The antisera used with the RID plates was a mixture of rabbit, goat, and sheep antisera. Standards contained 370 mg of prealbumin per liter, and were diluted three- and sixfold in isotonic saline to prepare the standard curve.

Human prealbumin (>97% pure, from Behring Diagnostics). We dissolved this in isotonic saline before using it in analytical-recovery studies (see below).

Bovine serum albumin in saline. We dissolved 40 g of bovine serum albumin (Cohn Fraction V; Sigma Chemical Co, St. Louis, MO 63178) per liter of isotonic saline.

Methods

RID. Add 5 μL of calibrator, control, and samples to each pre-cut cylindrical agar well containing antibody to human prealbumin. Incubate for 48 h at room temperature to allow formation of precipitin rings from the complexing of prealbumin in the sample with the anti-prealbumin antibody in the agar. Then measure the diameter of the precipitin rings for each unknown and compare it with those produced by standards of known concentrations.

Rate nephelometry. Place 150 μL of calibrator, control, and serum in the sample cups and position them on the turntable for automatic 36-fold dilution with ICS Diluent (phosphate-buffered saline). After an antibody card defining the program and calibrator parameters is inserted into the card reader, 42 μL of the 36-fold diluted samples is transferred to a reaction cup containing 600 μL of ICS Buffer solution (phosphate-buffered saline containing a polymer enhancer). The light scatter of the ICS Buffer solution is read and 42 μL of antibody to prealbumin solution is added. The formation of prealbumin-anti-prealbumin complexes causes an increase in light scatter. The scatter is converted to a peak rate signal, which is proportional to concentration. The instrument checks for antigen excess by analyzing a 72-fold dilution of each patient's sample, and samples with over-range results are diluted further.

Results and Discussion

Comparison of Methods

Table 1 summarizes the concentrations obtained with the ICS (y) and by RID (x). The overall mean concentrations by the two methods differed significantly (Student's t-test, p <0.05), but the results correlated highly: y = 0.68x + 36.08 mg/L, r = 0.96, Sxy = 12.38, n = 55.

We assayed the ICS Calibrator and RID control by both methods. The ICS Calibrator gave mean concentrations of 242 and 274 mg/L by ICS and RID, respectively (expected concentration, 246 mg/L); the RID control was 216 and 246 mg/L (expected concentration, 240 mg/L). Interassay precision studies with the Auto ICS and the 246 mg/L Calibrator yielded a CV of 2.6% (n = 14, mean = 242 mg/L, SD = 6.2).

To see which method of measurement gives the more nearly correct result, we added to serum human prealbumin dissolved in isotonic saline and assayed. Figure 1 shows the differences in slope and y-intercepts between the Auto ICS and RID. The RID slope was larger, and the y-intercept of 24 mg/L by the ICS is expected.

Table 1. Prealbumin Concentrations (mg/L) in Serum as Determined by RID and Beckman Auto ICS

<table>
<thead>
<tr>
<th></th>
<th>RID</th>
<th>SD</th>
<th>Range</th>
<th>ICS</th>
<th>SD</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>Boys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>31</td>
<td>228</td>
<td>53</td>
<td>141–344</td>
<td>190</td>
<td>35</td>
<td>133–272</td>
</tr>
<tr>
<td>Girls</td>
<td>211</td>
<td>63</td>
<td>125–419</td>
<td>185</td>
<td>49</td>
<td>128–350</td>
</tr>
<tr>
<td>Total</td>
<td>221</td>
<td>57</td>
<td>125–419</td>
<td>188</td>
<td>42</td>
<td>128–350</td>
</tr>
</tbody>
</table>

Fig. 1. Analytical recovery of human prealbumin added to serum.

Pre-addition concentrations of prealbumin were 200 mg/L by RID and 174 mg/L by the ICS. For RID, y = 1.32x + 200 mg/L; for ICS, y = 1.98x + 175.6 mg/L.

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Table 2. Analytical Recovery of Prealbumin by RID and Auto ICS

<table>
<thead>
<tr>
<th>Prealbumin, mg/L</th>
<th>Recovered</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RID</td>
<td>ICS</td>
</tr>
<tr>
<td>25</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>50</td>
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<td>150</td>
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<td>160</td>
</tr>
<tr>
<td>200</td>
<td>260</td>
<td>217</td>
</tr>
</tbody>
</table>

Figure 2 shows prealbumin concentrations in serum of 93 children. The Kolmogorov–Smirnov test for normality suggests that this is a normally distributed population (p <0.05) with a mean of 191 (SD 41) mg/L. Therefore, we used the mean ±2 SD to calculate the reference interval for this population: 109–273 mg/L.

There was no significant sex-related difference (p >0.05) in values for prealbumin as tested by the F-test for variance ratios and Student's t-test for means.

We used the ICS to determine a reference interval for prealbumin because most reported values have been based on RID and because there are few reports of reference intervals for prealbumin in children (8). One study (9), based on results for 50 adults (30 men, 20 women) by the ICS method, described a normal reference interval of 170–420 (mean 290) mg/L, with men having higher concentrations (mean 325 mg/L) than women (mean 248 mg/L). Our data suggest that prealbumin concentrations approach adult values after age 12 (Figure 2).

We conclude that use of the ICS yields results comparable with those by the RID method. However, the RID method is a manual procedure that takes 48 h to complete, whereas the Auto ICS method can yield results within several hours. Routine measurement of prealbumin is unlikely unless results can be quickly obtained.

References