Clinical utility of insulin-like growth factor binding protein-3 in the evaluation and treatment of short children with suspected growth hormone deficiency

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We have shown previously that serum insulin-like growth factor binding protein-3 (IGFBP-3) levels have good predictive value for complete, but not partial, growth hormone deficiency (GHD). In this study, we compare IGFBP-3 levels in short children previously divided into groups on the basis of their post-stimulation GH levels. Complete GHD (N = 59) included those children with peak post-stimulation GH < 5 µg/l. The partial GHD group (N = 49) had post-stimulation GH peaks of > 5 µg/l but < 10 µg/l. The normal children with short stature (N = 103) had post-stimulation GH peaks > 10 µg/l. Partial GHD and normal children with short stature also were divided into either low IGF-I or normal IGF-I subgroups. The clinical sensitivity of IGFBP-3 for complete GHD was 92%, whereas its sensitivity for partial GHD was 39%. For partial GHD, among those with low IGF-I (N = 19) 68% were also low for IGFBP-3, while 80% of those with normal IGF-I (N = 30) were also normal for IGFBP-3. The clinical specificity of IGFBP-3 for normal children with short stature was 69%. For these groups, among those with low IGF-I (N = 22) 73% also were low for IGFBP-3, while 80% of those with normal IGF-I (N = 81) also were normal for IGFBP-3. In addition, we tested whether IGFBP-3 can predict the response to GH treatment in prepubertal children by comparing pretreatment IGFBP-3 with the height gain achieved by 1 year of GH treatment. The incremental growth velocity during treatment correlated significantly with the pretreatment IGFBP-3 score (N = 46 r = −0.80, p < 0.005). The baseline IGFBP-3 score for all subjects correlated (N = 171, r = 0.51 p < 0.0001) with height. These data suggest that IGFBP-3 may reflect GH secretion status in most children being evaluated for GHD and that a low pretreatment IGFBP-3 score predicts improved growth during the first year of GH treatment.

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In clinical practice, the evaluation of GH secretion is complicated by fluctuations in basal and stimulated GH levels (1–11). In contrast, insulin-like growth factor-I (IGF-I) and IGFBP-3 are virtually invariable indices of GH secretion (9). Both IGF-I and IGFBP-3 are used now to facilitate the diagnosis of GH disorders, but little has been published concerning the clinical comparison between GH stimulation tests and IGFBP-3. In our previous reports, we have shown that IGFBP-3 has good predictive value for complete GH deficiency (CGHD) but not for partial GH deficiency (PGHD) (12–15). In this study, we tested whether IGFBP-3 is a useful index of GH secretion by comparing IGFBP-3 levels in short children, previously divided into CGHD, PGHD or normal children with short stature (NS) groups, on the basis of their post-stimulation GH levels. We also established a relationship between IGFBP-3 levels and height and growth rate achieved by 1 year of GH treatment.

Subjects and methods

Subjects

Fifty-nine CGHD, 49 PGHD and 103 NS Japanese children were studied. Among these, about 70% were
subjects of our earlier studies (12, 15). Growth hormone deficiency was defined by the peak GH level obtained by at least two stimulation tests (usually arginine and insulin); for CGHD group all GH peaks < 5 \( \mu \text{g/l} \); for PGHD group, highest GH peak > 5 but less than 10 \( \mu \text{g/l} \); for NS group (height less than \(-2 \text{SD}\)), highest GH peak > 10 \( \mu \text{g/l} \). These are the criteria that we used previously (12–15) and are similar to those of Blum et al. (16). The false positive ratios of arginine and insulin tolerance tests at Tokyo Metropolitan Kiyose Children’s Hospital (1) are 25% and 19%, respectively, in normal short children. Fifty-two of 59 CGHD children had IGF-I levels at or below the 5th percentile for each age (CGHD with low IGF-I). The other seven CGHD children had IGF-I levels above the 5th percentile for each age (CGHD with normal IGF-I). The PGHD children with IGF-I at or below the 5th percentile were similarly considered as PGHD with low IGF-I, while those above were PGHD with normal IGF-I. Similarly, the NS group was subdivided into NS with low and normal IGF-I. Among the 108 GHD patients, 69 were males and 39 were females. Among the NS children, 58 were males and 45 were females. Growth hormone deficiency was idiopathic in 81 patients and secondary to other diseases or radiation in 27 patients. Secondary hypogonadism was treated after the age of 17 years, and other hormonal deficiencies were treated appropriately with thyroxine, hydrocortisone or antidiuretic hormone.

Baseline IGF-I and IGFBP-3 levels in the GHD group were determined before GH therapy or at least 6 months after GH therapy had been discontinued. All samples (serum or plasma) were stored below \(-80^\circ\text{C}\) for less than 1 year before testing.

The GHD patients with a bone age (Greulich–Pyle method) of less than 15 years for girls and 17 years for boys were treated with GH (0.5 \( \text{U} \cdot \text{kg}^{-1} \cdot \text{week}^{-1}\), six or seven times a week). Some NS patients were treated similarly. The IGF-I levels of the prepubertal NS children who were treated (\(N = 19\)) had \(\text{SD}\) scores of \(-2.68\) up to 0.85 (mean \(\pm\) \(\text{SD}\) = \(-1.00 \pm 0.86\)). These children ranged in height from \(-3.86\) to \(-2.03\) \(\text{SD}\) score (mean \(\pm\) \(\text{SD}\) = \(-2.87 \pm 0.54\)).

Methods

The IGF-I level was measured in duplicate using a commercially available RIA kit (Medgenix and Chiba Corning, Tokyo, Japan), as reported previously (17). The samples were measured after acid–ethanol extraction. The lower and upper limits of this assay are 18 and 1750 \(\mu \text{g/l} \), respectively. The results of recovery tests were 99.4% (at the concentration of 30.9 \(\mu \text{g/l} \), 100.7% (136.9 \(\mu \text{g/l} \)) and 100.3% (210.7 \(\mu \text{g/l} \)). Intra-assay coefficients of variation were 3.4% (at the concentration of 35.4 \(\mu \text{g/l} \)), 3.7% (195.7 \(\mu \text{g/l} \)) and 4.0% (583.8 \(\mu \text{g/l} \)). Interassay coefficients of variation were 4.7% (at the concentration of 40.1 \(\mu \text{g/l} \)), 2.9% (196.8 \(\mu \text{g/l} \)) and 3.6% (604.6 \(\mu \text{g/l} \)). Normal IGF-I and low cut-off levels for each age (5th percentile) were determined in this paper based on 262 normal children who were the same as in our previous study (15). About 70% of these 262 subjects were used to determine normal IGFBP-3 levels. Previously we had determined the low cut-off levels of IGF-I at the ages of 8 and 9 years, separately in males and females (male 140 \(\mu \text{g/l} \), female 160 \(\mu \text{g/l} \)) (15). However, as there was no significant difference in IGF-I level between males and females, we set a single low cut-off level at the ages of 8 and 9 years for both males and females, based on the 5th percentile at those ages (150 \(\mu \text{g/l} \)). In other age groups, we used the 5th percentile as the low cut-off level; these were the same as in our previous study (15). The distribution of normal IGF-I levels in our study was not log-normal at all ages, as reported by Blum et al. (18). This difference is probably due to the small numbers of our normal subjects.

As reported previously (19), IGFBP-3 measurements were made by the method of Blum and Ranke (16) with a few minor modifications. We added aprotonin (5 \(\times 10^3\) U/l) and EDTA-2Na (10 mmol/l) to the dilution buffer. Intra-assay coefficients of variation were 6.4% (at the concentration of 1.46 mg/l), 9.8% (5.87 mg/l) and 4.3% (7.98 mg/l). Intrassay coefficients of variation were 13.2% (at the concentration of 1.40 mg/l) and 4.4% (3.91 mg/l). Using this assay, normal IGFBP-3 and low cut-off levels at each age (5th percentile) were determined from 312 normal subjects. The distribution of normal serum levels of IGFBP-3 was not log-normal in accordance with the report by Blum et al. (16).

Growth hormone was measured by an immunoradiometric assay (IRMA; Eiken, Tokyo, Japan) whose standard potency was calibrated to an RIA after studies in our laboratory (12). The definitions of GHD and NS were based on the IRMA GH levels.

A height study was done in prepubertal GHD and NS groups. Heights were determined before and after GH treatment. Patients with the onset of puberty during GH treatment and those with organic diseases were excluded from this prepubertal group. The pretreatment growth velocity (pre-GV, cm/year) was calculated if the patient was measured over a minimum of 1 year. Growth velocity after GH treatment (post-GV, cm/year) was based on measurements over an 11–14 month period. Incremental height gain 1 year after initiation of GH treatment (cm/year) was calculated by subtracting the growth velocity before treatment from the growth velocity after treatment (incremental height gain = post-GV – pre-GV). An incremental height gain of more than 2 cm/year was considered to be a good response in this study (20). Height data, except incremental height gain, are shown as \(\text{SD}\) scores and are based on previously reported height data in Japan (21).
**Table 1. Normal IGFBP-3 (mg/l), mean – 2 sd and 5th percentile (low cut-off level) for each age.**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Mean ± sd (N, range)</th>
<th>Mean – 2 sd</th>
<th>5th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>1.80 ± 0.39 (N = 58, 0.98–2.91)</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>2–3</td>
<td>2.22 ± 0.45 (N = 32, 1.45–3.35)</td>
<td>1.3</td>
<td>1.5</td>
</tr>
<tr>
<td>4–5</td>
<td>2.62 ± 0.49 (N = 47, 1.05–3.83)</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>6–7</td>
<td>2.88 ± 0.53 (N = 46, 1.60–4.03)</td>
<td>1.8</td>
<td>2.0</td>
</tr>
<tr>
<td>8–9</td>
<td>3.06 ± 0.46 (N = 39, 2.28–4.05)</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>10–11</td>
<td>3.42 ± 0.61 (N = 23, 2.45–4.58)</td>
<td>2.2</td>
<td>2.5</td>
</tr>
<tr>
<td>12–13</td>
<td>3.88 ± 0.59 (N = 23, 2.93–4.62)</td>
<td>2.7</td>
<td>3.0</td>
</tr>
<tr>
<td>14–17</td>
<td>3.53 ± 0.49 (N = 11, 2.90–4.55)</td>
<td>2.6</td>
<td>2.9</td>
</tr>
<tr>
<td>18–40</td>
<td>3.25 ± 0.49 (N = 33, 2.59–4.26)</td>
<td>2.3</td>
<td>2.6</td>
</tr>
</tbody>
</table>

**Statistics**

All data are shown as means ± sd. Statistical comparison were made by the Mann–Whitney U test and chi-squared analysis.

**Results**

To provide a comparison between serum IGFBP-3 and GH levels after stimulation, we measured IGFBP-3 in samples from short children previously grouped according to the GH stimulation test data. Individual IGFBP-3 levels were compared with those of our normal reference data (Table 1). As shown in Table 2, 92% of CGHD and 31% of NS children had a low IGFBP-3 level (less than the 5th percentile for each age in Table 1). Because of the limitations of GH stimulation tests (1, 9, 12–15), we subdivided GHD and NS groups into those with normal and low IGF-I as described earlier. Among CGHD children with low IGF-I (at or below the 5th percentile), only one patient had IGFBP-3 levels greater than the 5th percentile for each age, whereas four of the seven other CGHD children with normal IGF-I had IGFBP-3 levels greater than the low cut-off levels. Thirty-nine per cent of PGHD children had a low IGFBP-3 level. Sixty-one per cent of PGHD children had a normal IGF-I level. Among the PGHD children with low IGF-I, 68% had a low IGFBP-3 level. Among the PGDH children with normal IGF-I, 80% had a normal IGFBP-3 level. About 80% of NS children had a normal IGF-I level. Among these NS children with normal IGF-I, 80% had a normal IGFBP-3 level. Among the NS children with low IGF-I, 73% had a low IGFBP-3 level. Overall, the IGFBP-3 sd score in GHD and NS children correlated significantly with the IGF-I sd score (r = 0.65, p < 0.0005).

Individual data of the CGHD, PGHD and NS groups are shown in Fig. 1. These data are summarized in Table 2, along with the ratio of the patients whose IGFBP-3 was less than the mean – 2 sd (in parentheses). All secondary GHD (n = 27: 22 CGHD and 5 PGHD children) had IGFBP-3 levels below the low cut-off levels.

Longitudinal bone growth is partly dependent on GH secretion (22). To test whether IGFBP-3 correlates with growth, we compared IGFBP-3 with height. The IGFBP-3 sd score in prepubertal normal, NS and GHD children correlated with the height sd score (r = 0.51, p < 0.0001, N = 171). Similarly, the IGF-I sd score in the same prepubertal subjects correlated with the height sd score (r = 0.25, p < 0.005, N = 141), although this is a weaker correlation than for the IGFBP-3 sd score. Among these prepubertal subjects, the height sd score also was significantly lower in CGHD than in NS children.

**Table 2. Ratio of the patients who had IGFBP-3 less than 5th percentile.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage with low BP-3</th>
<th>Low BP-3 cases*</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGHD, N = 59</td>
<td>92</td>
<td>54/59 (53/59)</td>
<td></td>
</tr>
<tr>
<td>Low IGF-I, N = 52</td>
<td>98</td>
<td>51/52 (50/52)</td>
<td></td>
</tr>
<tr>
<td>Normal IGF-I, N = 7</td>
<td>43</td>
<td>3/7 (3/7)</td>
<td></td>
</tr>
<tr>
<td>PGHD, N = 49</td>
<td>39</td>
<td>19/49 (18/49)</td>
<td></td>
</tr>
<tr>
<td>Low IGF-I, N = 19</td>
<td>68</td>
<td>13/19 (12/19)</td>
<td></td>
</tr>
<tr>
<td>Normal IGF-I, N = 30</td>
<td>20</td>
<td>6/30 (6/30)</td>
<td></td>
</tr>
<tr>
<td>NS, N = 103</td>
<td>31</td>
<td>32/103 (21/103)</td>
<td></td>
</tr>
<tr>
<td>Low IGF-I, N = 22</td>
<td>73</td>
<td>16/22 (12/22)</td>
<td></td>
</tr>
<tr>
<td>Normal IGF-I, N = 81</td>
<td>20</td>
<td>16/81 (9/81)</td>
<td></td>
</tr>
</tbody>
</table>

* Ratio of cases in parentheses shows the ratio of patients who had IGFBP-3 less than – 2 sd; ** p < 0.001.

* CGHD: complete growth hormone deficiency; PGHD: partial growth hormone deficiency; NS: normal children with short stature.
(−4.19 ± 1.59 versus −2.92 ± 0.54, p < 0.0005). The PGHD children with low IGF-I also were shorter than the PGHD children with normal IGF-I (−4.15 ± 1.58 versus −2.84 ± 0.58, p < 0.005).

Children with the lowest endogenous GH levels respond most favorably to GH treatment (20). To evaluate IGFBP-3 levels as a predictor for GH response, we compared the pretreatment IGFBP-3 level with incremental height gain after 1 year of treatment (post-GV − pre-GV). Only prepubertal children were included. Among these children (N = 46), all 18 CGHD and PGHD children with low IGF-I grew well, with an incremental height gain of more than 2 cm/year. Fewer NS patients (10/19) and PGHD patients with normal IGF-I (6/10) responded well to treatment. Incremental height gain for the entire group correlated significantly with the pretreatment IGFBP-3 sd score (Fig. 2: r = −0.80, p < 0.005, N = 46). A similar correlation was obtained when incremental growth velocity was estimated using the Japanese growth velocity sd score (21) (data not shown). Patients with an IGFBP-3 sd score of less than −1 (26/28) responded well to treatment, while those with an IGFBP-3 sd score of more than −1 (6/18) did not (p < 0.001).

The IGF-I sd score also correlated with incremental height gain (r = 0.54, p < 0.001), but to a lesser degree than for the IGFBP-3 sd score.

Discussion
Formerly we used the mean − 2 sd and the 5th percentile as the low cut-off levels of IGFBP-3 and IGF-I, respectively. In this study the 5th percentile was used as the low cut-off level of IGFBP-3 because normal
IGFBP-3 levels did not distribute normally. As in Table 2, the sensitivity of IGFBP-3 for either CGHD or PGHD would not change much if we used the mean – 2 sd as the low cut-off level. However, the specificity of IGFBP-3 for NS children would be higher if we used the mean – 2 sd as the low cut-off level. Because some NS children have been reported to respond to GH treatment, a higher cut-off level, namely the 5th percentile, may be better in terms of screening for short children who may respond to GH treatment. Indeed, our data suggest that short children with either GHD or NS who had an IGFBP-3 sd score of less than –1 may respond to GH therapy.

As shown here and as reported previously by our group, IGF-I (17) and IGFBP-3 (12–14, 16) can be useful for screening patients for CGHD. In addition, this report shows that the PGHD and NS groups are heterogeneous with respect to IGF-I and IGFBP-3 concentration. Moreover, the variable incremental height gain during GH treatment in these groups confirms the diversity of the PGHD and NS groups. This finding may be due directly to the limitations of stimulation tests (1–9, 12–15), although GH stimulation tests have been used for diagnosis of GHD. These tests, while useful in absolute deficiency, are otherwise unreliable (9–11) and prone to false positive values (1–8).

The significant correlation between the pretreatment IGFBP-3 sd score and incremental height gain in our prepubertal subjects, including NS children, suggest that IGFBP-3 may reflect endogenous GH status more accurately than do stimulation tests if it is true that children with the lowest endogenous GH levels respond most favorably to GH treatment (20). That IGF-I, regulated by GH, correlates well with IGFBP-3 in our study further supports the dependency of IGFBP-3 on endogenous GH status. The correlation between the height sd score, which correlates with 24-h GH secretion (22), and the IGFBP-3 sd score in prepubertal children also provides indirect evidence that IGFBP-3 reflects current GH status. These findings suggest that IGFBP-3 reflects GH status in most children evaluated for GHD. Others have shown a direct correlation between 24-h GH secretion and IGFBP-3 sd score (18). In a clinical situation, however, we have to remember factors other than GH, such as liver function, renal function and malnutrition, which may have an effect on IGFBP-3 levels (23).

It has been reported that some short children with normal GH stimulation tests grow well with GH treatment, at least for a short time (24–28). Our data, which show a variable growth response to GH in NS and PGHD children, also suggest that stimulation tests may have limited value as response predictors in these children. Our study has shown that the IGFBP-3 sd score correlates with response and may facilitate the identification of children who could be expected to show good responses. Patients with an IGFBP-3 sd score lower than –1 responded most favorably to treatment, with an incremental height gain of at least 2 cm/year.

In summary, our data provide indirect evidence that IGFBP-3 reflects GH status in most short children evaluated for GHD. We have shown also that a low pretreatment IGFBP-3 level suggests a favorable short-term response to GH. Patients with an IGFBP-3 sd score of less than –1 may respond well.
References


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