Mini Review

Clinical Utility of Total Insulin-like Growth Factor-I and Insulin-like Growth Factor Binding Protein-3 Measurements in the Evaluation of Short Children

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In this review paper, we discuss the clinical utility of total insulin-like growth factor-I (IGF-I) and that of IGF binding protein-3 (IGFBP-3). IGF-I and IGFBP-3 are well known to be dependent on GH and are thought to be useful in the evaluation of short children (1-10). We discuss methods of measurements of these parameters, and their clinical utility in the evaluation of short children. Part of this review paper has been published in our previous papers (6-11).

Methods of measurements of IGF-I and IGFBP-3

It is well known that IGFBPs can interfere with measurements of total IGF-I, since IGFBPs have a high affinity with IGF-I, similarly to anti-IGF-I antibody used in a radioimmunoassay (RIA) and immunoradiometric assay (IRMA) (12). Thus, it is necessary to separate IGFBPs from IGF-I completely by acid-extraction etcetera in order to measure total IGF-I accurately. Although almost all the methods so far used to separate IGFBPs from IGF-I have never been complete, they have been reported to be useful clinically to some extent (2, 8, 13, 14).

It is possible that if we could completely separate IGFBPs from IGF-I and thus could measure total IGF-I more accurately, all the data about total IGF-I which have been reported so far would be a little different. The method reported by Blum et al. (12), where total IGF-I was measured without the extraction step by using an excess amount of IGF-II, may be the best to measure total IGF-I levels in terms of the effect of IGFBPs. However, this method needs highly sensitive antibody with IGF-I (compared to the affinity with IGF-II) and the authors did not report much clinical data in the diagnosis of GH deficiency (GHD).

If an assay for free IGF-I is available widely, we may not have to think over accurate measurements of total IGF-I by removing IGFBPs. We have recently reported a newly developed IRMA for free IGF-I (15-17). There have been several reports on measuring free IGF-I (18-23) by using gel-chromatography, which can theoretically disturb the equilibrium. We have reported that the equilibrium can not be disturbed within the first incubation time of the reaction between the capture antibody and free IGF-I in plasma (16, 17). In our assay, the cap-
ture antibody was proved not to crossreact with IGF-I complexed with IGFBP (16, 17). The clinical utility of free IGF-I in the evaluation of short children was reported to be similar to that of total IGF-I. Similar IRMA for free IGF-I has been reported recently (24, 25).

There are several methods of IGFBP-3 measurements such as the affinity cross-linking method, western ligand blot, western immunoblot, and RIA. Among these assays, RIA or similar quantitative assays such as IRMA and enzyme-linked immunosorbent assay (ELISA) are clinically useful, because they can measure quantitatively IGFBP-3 of relatively many samples. So far all the commercially available quantitative methods we have tried (RIA with a minor modification to Blum's method, Diagnostic Systems Laboratories’ (DSL) RIA, DSL IRMA) are clinically useful in the evaluation of short children, as well as other assays reported (3, 7-10, 26, 27). The correlation in the results among either two of the three kits we tried are statistically significant; for example, the correlation between Blum's RIA and DSL IRMA kits is statistically significant, giving almost the same IGFBP-3 levels (r=0.97, P<0.001, n=41)(27). Dr. Blum's method and the RIA developed by DSL give almost the same results if the same standard is used (data not shown).

In our studies, the antibodies in DSL RIA, DSL IRMA (both capture and indicator antibody) can cross-react with not only intact IGFBP-3 but also proteolysed IGFBP-3 (27), which is typically present due to IGFBP-3 proteolytic activity in pregnant serum (28-30). Fig. 1 shows the characterization of the indicator antibody of DSL IRMA. Similar data were obtained by using the capture antibody of DSL IRMA (data not shown)(27). So far, other than in pregnant sera, IGFBP-3 proteolytic activity was reported to be present in seminal plasma (31), sera from patients with GH receptor dysfunction (32) and sera from various catabolic conditions (33-35).

In this review paper, we measured total IGF-I levels by RIA after acid-ethanol extraction (8, 9, 13). The standard of the IGF-I assay was recombinant IGF-I. IGFBP-3 was measured by Blum's RIA method with minor modifications (9, 36). The standards of IGFBP-3 assays were pooled serum calibrated to purified IGFBP-3 given by Dr. Mark Stene (Endocrine Sciences)(7-9, 36). The cutoff levels of IGF-I and IGFBP-3 for diagnosing GHD at each age (5th percentile) are shown in Table 1.
IGF-I and IGFBP-3 in Short Children

Table 1 The cutoff levels (5th percentile) of IGF-I and IGFBP-3 (ng/mL, mg/L, respectively)

<table>
<thead>
<tr>
<th>AGE</th>
<th>IGF-I</th>
<th>IGFBP-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1W-1Y</td>
<td>40</td>
<td>1.2</td>
</tr>
<tr>
<td>2-3Y</td>
<td>50</td>
<td>1.5</td>
</tr>
<tr>
<td>4-5Y</td>
<td>60</td>
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</tr>
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<td>6-7Y</td>
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</tr>
<tr>
<td>8-9Y</td>
<td>150</td>
<td>2.3</td>
</tr>
<tr>
<td>10-11Y</td>
<td>160</td>
<td>2.5</td>
</tr>
<tr>
<td>12-13Y</td>
<td>180</td>
<td>3.0</td>
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<tr>
<td>14-17Y</td>
<td>350</td>
<td>2.9</td>
</tr>
<tr>
<td>18-40Y</td>
<td>160</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Clinical utility of total IGF-I and IGFBP-3 in the evaluation of short children

In this section we would like to show: 1) total IGF-I and IGFBP-3 measurements are useful in the diagnosis of GHD and evaluation of GH secretion, 2) total IGF-I and IGFBP-3 measurements can replace growth hormone (GH) provocation tests as laboratory parameters in the diagnosis of GHD and evaluation of GH secretion, 3) comparison of total IGF-I and IGFBP-3 in the evaluation of GH secretion.

1) Total IGF-I and IGFBP-3 measurements are useful in the diagnosis of GHD and evaluation of GH secretion

In order to analyse the clinical utility of total IGF-I, we compared the results of total IGF-I measurements with those of GH provocation tests in short children (IGF-I means total IGF-I in the rest of this paper, unless defined differently). The short children subjects were divided into three groups: (1) complete GHD (CGHD): all the GH peaks of GH provocation tests were less than 5 ng/mL, (2) partial GHD (PGHD): the highest GH peak among more than two GH provocation tests was from 5 to 10 ng/mL (3) normal short children (NS): at least one GH peak was over 10 ng/mL. More than two kinds of GH provocation tests were done. The false positive ratio of arginine and insulin tests, which means that one of the GH peaks was more than 10 ng/mL, whereas the other GH peak was less than 10 ng/mL in short children, were 25 % and 19 %, respectively (37). Fifty-two out of 59 patients with CGHD (88 %) had IGF-I levels below the 5th percentile for each age, or in other words the sensitivity of IGF-I for CGHD was 88 %, whereas the sensitivity of IGF-I for PGHD (n=49) was only 39 %. As for NS, 81 out of 103 NS patients (79 %) had normal IGF-I levels (more than 5th percentile), namely the specificity of IGF-I for NS was 79 %. The relatively low sensitivity for PGHD can be explained by the following two facts; (a) by definition, PGHD is less severe than CGHD, (b) limitations of GH provocation tests as we will comment on briefly in the next paragraph are true in particular in the case of PGHD.

A similar study was done using IGFBP-3. Fifty-four out of 59 patients with CGHD (92 %) had IGFBP-3 levels below the 5th percentile for each age, or in other words the sensitivity of IGFBP-3 for CGHD was 92 %, whereas the sensitivity of IGFBP-3 for PGHD (n=49) was only 39 %. As for NS, 72 out of 103 NS patients (69 %) had normal IGFBP-3 levels (more than 5th percentile), namely the specificity of IGFBP-3 for NS was 69 %. The relatively low sensitivity of IGFBP-3 for PGHD can be similarly explained to the low sensitivity of IGF-I as mentioned.
Recently, concerns regarding the diagnostic accuracy of GH provocation tests have been raised (11). There are well-documented limitations of GH provocation tests (37-50) such as absence of reproducibility, no age- and sex-related normal values, and the fact that some short children with apparently normal results of GH provocation tests, which were judged based on the results of GH provocation tests, do have low IGF-I or IGFBP-3 and do grow with conventional GH treatment, etc. So we analysed IGF-I data of the following two groups of short children. One group is CGHD with low IGFBP-3 and the other is NS with normal IGFBP-3. Because of the limitations of GH provocation tests, we added IGFBP-3 levels to the results of GH provocation tests. The sensitivity of IGF-I for CGHD with low IGFBP-3 was 94% (n=54). The specificity of IGF-I for NS with normal IGFBP-3 was 91% (n=65). These data suggest that IGF-I is one of the best screening parameters in the evaluation of short children.

Similar studies were done by using two groups of short children to clarify the clinical utility of IGFBP-3. One group is CGHD with low IGF-I and the other is NS with normal IGF-I. The sensitivity of IGFBP-3 for CGHD with low IGF-I was 98% (n=52). The specificity of IGFBP-3 for NS with normal IGF-I was 80% (n=81). These data suggest that IGFBP-3 is also one of the best screening parameters in the evaluation of short children.

So far IGF-I and IGFBP-3 levels were analysed, basically, in comparison with the results of GH provocation tests, which we believe was not the best method to study IGF-I and IGFBP-3 utility in the evaluation of GH secretion. GH provocation tests have been used traditionally but they can not be a gold standard in the diagnosis of GHD (11). In addition, any cut-off criteria for GHD is arbitrary judging from the fact that GH secretion is continuous from zero to normal (or acromagalic). Indeed, we have reported that there are considerable overlaps in height, IGF-I levels, and IGFBP-3 levels between PGHD and NS, if those diagnoses are based on results of GH provocation tests (9). Thus, in order to check the clinical utility of IGF-I and IGFBP-3, we have analysed IGF-I and IGFBP-3 a little differently in the following paragraph.

The correlations of a response to a conventional GH treatment (0.5 U/kg/week, 6-7 time a week) with pretreatment IGF-I and IGFBP-3 levels (standard deviation scores; SDS), were studied. The subjects were 46 prepubertal short children (less than -2 SDS at the time of the initiation of GH treatment) who had been treated for at least 1 year. A significant correlation between pretreatment IGF-I SDS and incremental height gain (= height velocity after GH treatment − height velocity before GH treatment) was obtained (r=0.48, P=0.008). As shown in Fig. 2, a more significant correlation between pretreatment IGFBP-3 SDS and incremental height gain was also obtained (r=0.80, P=0.0001). If the assumption that the less the GH secretion in short children is, the more they grow with a conventional GH treatment is true, these significant correlations indicate that IGF-I and IGFBP-3, in particular IGFBP-3, can reflect GH secretion status, thus IGFBP-3 measurements are useful in the evaluation of GH secretion.

So far we have explained that IGF-I and IGFBP-3 measurements are useful in the evaluation of GH secretion. However, GH provo-
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Fig. 2 The relationship between incremental height gain (height velocity after GH treatment (cm/year) − height velocity before GH treatment (cm/year)) and pretreatment IGFBP-3 SDS. (This figure was reproduced from Ref (9) with authors' permission)

2) Can total IGF-I and IGFBP-3 measurements replace GH provocation tests as laboratory parameters in the diagnosis of GHD and evaluation of GH secretion?

Our simple answer to this question is yes. We can say “yes” very clearly in diagnosing severe type of GHD. For example, if he or she had (a) hard auxological data such as height velocity of less than 3.5 cm/year (arbitrary criteria) or height SDS of less than −3.5 SDS (arbitrary criteria) and/or (b) magnetic resonance imaging (MRI) abnormalities such as brain tumor in the hypothalamic-pituitary region, invisible pituitary stalk, or hypoplastic anterior pituitary lobe, etc., just measurements of IGFBP-3 (and perhaps IGF-I) could be enough to ensure the diagnosis. Indeed, all of the 14 patients with short stature in our hospital who showed invisible stalk on MRI together with a past history of abnormal delivery had low IGF-I and IGFBP-3 levels with no any exceptions (unpublished observations). Similar data in patients with Ecuador GH receptor dysfunction, namely the fact that all those patients had extremely low IGFBP-3 and IGF-I (for example, less than 0.1th percentile in a European study) have been reported (51, 52).

How about less severe type of GHD? We believe IGF-I and IGFBP-3, in particular IGFBP-3, may replace GH provocation tests even in less severe type of GHD, because (a) as we have shown in the previous paragraph, IGFBP-3 and IGF-I can replace GH provocation tests in severe type of GHD and (b) GH secretion status is theoretically continuous, from zero to normal. Indeed, the relationship between pretreatment IGFBP-3 SDS and a response to conventional GH treatment (Fig. 2 where the subjects of PGHD and NS as defined based on results of GH provocation tests were also included) suggests that IGFBP-3 is useful in the evaluation of GH secretion from zero to almost normal. Thus, IGFBP-3 is theoretically useful even in diagnosing less severe type of GHD.

Finally we can say IGFBP-3, probably together with IGF-I measurements, are very useful as laboratory parameters in the evaluation of GH secretion status so that we may not have to do GH provocation tests in the evaluation of short children. Furthermore, it must be recognized that there are patients with short stature, retarded height velocity, and low serum IGF-I and IGFBP-3 concentrations who can be considered to have disordered GH secretion,
even in the presence of "normal" results of GH provocation tests. Conversely, there are children who may "fail" GH provocation tests, whose growth patterns and serum IGF-I and IGFBP-3 concentrations argue against a diagnosis of GHD. In addition to the limitations of GH provocation tests as has been explained in this paper, theoretically IGFBP-3 (and IGF-I) measurements have advantages over GH provocation tests: (a) they are cost effective and not risky, (b) we can rely on one result of the IGF parameters because of minimal intradaily variation (45), (c) we do have normal values for each age and sex, (d) measurements of the IGF parameters are physiological tests, whereas most of the GH provocation tests are pharmacological. Of course, we have to remember every laboratory parameter (including IGF-I and IGFBP-3) has limitations in diagnosing diseases; IGF-I and IGFBP-3 may be low in patients with inanition and liver diseases, etc.

It is not surprising that these "functional tests" of GH secretion status such as IGF-I and IGFBP-3 can be very useful in the evaluation of GH secretion status and that those may replace other GH direct parameters including GH provocation tests. For example, you can make a diagnosis of insulin-dependent diabetes mellitus based on blood sugar levels ("functional tests of insulin secretion status"), not on direct measurements of insulin. Similarly, we can diagnose hyperinsulinism based on hypoglycemia with a low concentration of ketone body and non-esterified free acid even in the absence of an overtly high concentration of insulin. The other example is that you may make a diagnosis of diabetes insipidus based on urine osmolalities during water restriction tests ("functional tests of arginine vasopressin secretion status"), not necessarily on direct measurements of arginine vasopressin during hypertonic saline loading tests.

Lastly, we have to stress the importance of clinical data, especially auxological data. GHD is diagnosed based on both clinical and laboratory data as is true in other endocrine diseases. We have explained the utility of IGF parameters as laboratory data in the evaluation of GH secretion. However, clinical data, in particular, auxological data are much more important than laboratory data in the evaluation of GH secretion. No laboratory data can be justified in the absence of suggestive clinical data.

3) Comparison of total IGF-I and IGFBP-3 levels in the evaluation of GH secretion

IGF-I and IGFBP-3 classified subjects differently in 15% of patients with GHD and 21% of those with NS as will be explained in detail, although overall, the correlation of IGF-I and IGFBP-3 by using sera of 238 normal, normal short, and GHD children was significant (r=0.74, P<0.0005). Among 108 patients with GHD (9), 92 patients had concordant IGF-I and IGFBP-3 levels in terms of above and below the cutoff levels (28 patients are both above and 64 patients are both below), whereas 16 patients (15%) had discordant results (7 patients showed IGF-I levels below the cutoff levels with IGFBP-3 levels above the cutoff levels and 9 showed the opposite situation for IGF-I and IGFBP-3). The discordant ratio of CGHD (4/59) was lower than that of PGHD (12/49). Similarly, among 103 patients with NS (9), 81 patients (79%) had concordant results of IGF-I and IGFBP-3 (65 patients are both above and 16 are both below), whereas 22 patients (21%) had discordant results (6 patients showed IGF-I levels below
the cutoff levels with IGFBP-3 levels above the cutoff levels and 16 showed the opposite situation for IGF-I and IGFBP-3). These data suggest that measuring both IGF-I and IGFBP-3 may improve diagnostic accuracy for GHD. Another explanation of these discordant results of IGF-I and IGFBP-3 may be due to a limitation of GHD diagnosis based on GH stimulation tests, since about 20 % of GHD and NS had normal levels of both IGF parameters and low levels of them, respectively. The other possibility for these discordant results is a difference in intradaily variation and the effect of nutrition between these parameters.

The IGFBP-3 values may well be considered in the diagnosis of GHD rather than the IGF-I values in the younger age. Indeed, in CGHD patients of the younger age group (less than 10 years old) in our previous report (8), all the IGFBP-3 levels were below the age-related cutoff levels, whereas 79 % of IGF-I levels were below the age-related cutoff levels. This difference (100 % for IGFBP-3 vs 79 % for IGF-I) might be due to the relatively higher normal IGFBP-3 values at younger ages than IGF-I values or due to the limitation of IGF-I measurements in terms of removing IGFBPs specially at low levels of IGF-I. A similar advantage of IGFBP-3 over IGF-I especially at young ages was reported by Blum et al. and Yokoya et al. using different subjects (3, 26).

It remains to be established whether IGFBP-3 is less useful in the older age groups than in the younger. The difference in the ratio of the GHD patients who had IGFBP-3 levels above the age-related cutoff levels between the younger and older age groups (the ratio was lower in a young age group), again noted in our previous report (8), may be due to the influence of gonadal steroids, since there is a significant difference in the ratio of the patients who had IGFBP-3 levels above the age-related cutoff between GHD with secondary hypogonadism and that without secondary hypogonadism (1 out of 12, 9 out of 10, respectively). This may suggest that gonadal steroids increase IGFBP-3 levels directly without the effect of GH (8). However, since, except for patients with gene deletion of GH, etc., there are not any complete deficiencies of GH in a strict sense, the hypothesis of the direct effect of gonadal steroids on IGFBP-3 levels may be an oversimplified explanation. Furthermore, GHD patients with hypogonadism are generally more severely GH-deficient than those without hypogonadism. Indeed, we did not show any significant difference in the ratio of the GHD patients who had IGFBP-3 levels above the age-related cutoff levels between the younger and older age groups in our expanded analysis (9).

Based on all the various data of IGF-I and IGFBP-3 so far, we believe that IGFBP-3 is, generally, a more useful parameter in the evaluation of short children. The following three facts which have already been written in the previous paragraphs of this paper, may favor our hypothesis for IGFBP-3 over IGF-I, 1) at younger ages IGFBP-3 is probably more useful in the evaluation of short children than IGF-I, 2) there was a more significant correlation of IGFBP-3 with the incremental height gain than that of IGF-I in our study, 3) the specificity of IGF-I for NS with normal IGFBP-3 was higher than that of IGFBP-3 for NS with normal IGF-I in our study.

The possible reasons why IGFBP-3 is more useful in the evaluation of short children than IGF-I may be: 1) at young ages IGFBP-3 has relatively higher values than IGF-I, 2) IGFBP-
3 may reflect the sum of IGF-I and IGF-II (IGF-II is supposedly partially GH-dependent),
3) IGF-I concentrations may be more influenced by other factors such as nutrition than GH secretion status.

References

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45. Hasegawa Y, Hasegawa T, Kotoh S, Tsuchiya Y. Reproducibility of GH stimulation tests (arginine and insulin), IGF-I and


