Usefulness and Limitation of Measurement of Insulin-Like Growth Factor Binding Protein-3 (IGFBP-3) for Diagnosis of Growth Hormone Deficiency

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Abstract. To analyze the utility of insulin-like growth factor binding protein-3 (IGFBP-3) radioimmunoassay for diagnosis of growth hormone deficiency (GHD) we measured IGFBP-3 in sera from normal children, short children and patients with GHD. The sensitivity (true positive ratio) of IGFBP-3 for complete GHD (cGHD) was 93%, while the specificity (true negative ratio) for normal short children (NS) was 88%. In contrast, the sensitivity of IGFBP-3 for partial GHD (pGHD) was only 43%. The poor discrimination between patients with pGHD and NS may be the result of their relatively similar GH level, as compared to cGHD, or due to the limitations of GH stimulation tests. The specificity of IGFBP-3 for NS was excellent in children of all ages: less than 10 years old (87%) and older than 10 (88%). However, sensitivity for GHD was good for children less than 10 years old (84%) but poor for children older than 10 (64%). IGFBP-3 may be less sensitive for diagnosing GHD in older children because IGFBP-3 levels may also increase during puberty due to mechanisms independent of the GH-IGF-I axis.

Key words: IGFBP-3, GH, Growth hormone deficiency (GHD), Normal short children (NS).

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THE INSULIN-like growth factors I and II (IGFs), important growth factors both *in vivo* and *in vitro* [1, 2], are known to have at least six binding proteins (IGFBP-1~6) [3, 4]. In human serum,

four IGFBPs (IGFBP-1~4) are reported to exist [5, 6]. Among them, IGFBP-3 is a major binding protein in serum and is considered to be GH-IGF-I axis dependent [6, 10].

When IGFBP-3 was analyzed by western ligand blot, Cohen and Rosenfeld *et al.* reported that there was some overlap between control subjects and patients with growth hormone deficiency (GHD) [6]. In our recent study, we found that by

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western ligand blot analysis, there is no overlap between prepubertal control subjects and patients with complete GHD (cGHD), whereas there is some overlap between these groups during puberty [11].

Recently, using IGFBP-3 RIA, Blum and Ranke reported more than 95% sensitivity and specificity for diagnosis of GHD [12]. However, Martha *et al.* and Phillip *et al.* reported that there was a definite overlap between short children without any hormonal problems and patients with GHD [13,14].

In this study, we measured serum IGFBP-3 levels by RIA in sera from normal children, from normal short children (NS) and from patients with GHD to clarify the utility of IGFBP-3 level as a diagnostic marker for GHD.

Methods and Subjects

Method

1) Anti-human IGFBP-3 antisera and purified human IGFBP-3 for iodination were obtained from Dr. W.F. Blum. IGFBP-3 was iodinated and purified by one of us (M.S.), as modified from the method of Blum. A serum standard calibrated to purified IGFBP-3 was used to prepare a standard curve. Five microliters of serum standard, control or unknown were diluted (1: 456) with assay buffer before analysis using a dilutor (Compulabor, Wheaton Instruments). Serum levels demonstrated parallelism with purified human as well as recombinant human IGFBP-3. The antisera did not cross react with IGF-I, IGF-II, IGFBP-1 or IGFBP-2.

The sensitivity of the assay was 0.3 ng/ml and half-maximal displacement occurred at 5 ng/ml. After correction for dilution this corresponds to 0.14 and 2.3 mg/L respectively. The intraassay variation ranged from 8–10% at 1 mg/L to 0.9%–4% at 3–4 mg/L. The interassay variation was 16% at 1 mg/L, 11% at 2 mg/L, 6.2% at 4.5 mg/L and 8.6% at 7.5 mg/L. Measurements were performed in duplicate and in batches of 80 samples by 3 different technicians. To insure the greatest accuracy, samples with initial levels less than 1 mg/L were repeated at a dilution of 1: 121.

2) GH was measured by various RIA and IRMA

assays. The definition of GHD and NS (defined later) was determined after all the GH levels were converted into our IRMA (Eiken kit) according to the relationships between various GH assays determined by Japanese Growth Foundation and Tokyo Metropolitan Kiyose Children's Hospital. For example, there was a significant relationship between GH levels by Eiken RIA and those by Eiken IRMA ($\text{GH IRMA} = 0.877 \times \text{Eiken RIA} - 1.39$, $r = 0.989$, $n = 183$).

Subjects

1) Normal control adults ($n = 32$, age 18–40) and children ($n = 272$, age 1 month–17 years) participated in this study at Tokyo Metropolitan Kiyose Children's Hospital. The heights of the control persons are within the mean ± 2 SD. Informed consent was obtained at the hospital.

2) Seventy-three patients with GHD (43 complete GHD and 30 partial GHD) and 93 patients with NS (height < -2 SD) were also involved in this study. Among them, about 50% of the patients have been followed up at Tokyo Metropolitan Kiyose Children's Hospital. Among 73 patients with GHD 54 were idiopathic GHD and 19 were secondary GHD due to other diseases such as brain tumor, radiation, etc. The associated hormonal deficiencies were treated appropriately with thyroxine, hydrocortisone and antidiuretic hormone. Secondary hypogonadism was not treated in this study. The definitions of complete GHD (cGHD), partial GHD (pGHD) and NS were the following: cGHD; all the GH peaks of GH stimulation tests (usually arginine and insulin tolerance tests) are less than 5 ng/ml, pGHD; the highest GH peaks of GH stimulation tests (usually arginine and insulin tolerance tests) are from 5 to 10 ng/ml, NS; at least one GH peak in GH stimulation tests is more than 10 ng/ml. These definitions of GHD and NS are basically the same as in Blum's and Ranke's paper [12]. GH stimulation tests were done at least twice. IGFBP-3 levels of GHD were measured either before GH therapy was started or after GH therapy was discontinued. The sera for IGFBP-3 were taken and frozen at -80 or -120°C for at most 1 year until assayed.

Statistics

Statistical analysis were carried out by Mann-

Whitney test and Chi-square test. All data are shown as the mean $\pm$ SD.

Results

1) Figure 1 shows IGFBP-3 levels of normal controls. Table 1 shows the mean $\pm$ SD, normal range, cutoff level for GHD at each age and sex. The normal range was determined after thirteen outlier values (beyond ± 2.2 SD) were omitted from a set of 285 normal children IGFBP-3 values. IGFBP-3 levels less than 2 standard deviations below the mean, at each age were considered to be the cutoff for GHD. There was no significant difference between male and female children. Unlike Blum and Ranke [12], we did not observe

Fig. 1. IGFBP-3 (mg/L) of normal control children.

Table 1. Normal IGFBP-3 (mean $\pm$ SD, range) and cutoff IGFBP-3 levels (mg/L) for diagnosis of GHD

AGE	M $\pm$ SD	Range	Cutoff for GHD
1W-2Y;	1.80 $\pm$ 0.39 (n=58)	0.98-2.91	1.0
2Y-4Y;	2.22 $\pm$ 0.45 (n=32)	1.45-3.35	1.2
4Y-6Y;	2.66 $\pm$ 0.43 (n=41)	1.68-3.85	1.6
6Y-8Y;	M: 2.67 $\pm$ 0.53 (n=17)	1.60-3.54	1.6
(M&F)	F: 3.00 $\pm$ 0.50 (n=29)	2.12-4.03	2.0
8Y-10Y;	M: 2.98 $\pm$ 0.49 (n=20)	2.28-4.05	2.0
(M&F)	F: 3.10 $\pm$ 0.46 (n=19)	2.54-3.80	2.2
10Y-12Y;	3.42 $\pm$ 0.60 (n=23)	2.45-4.58	2.2
12Y-14Y;	3.88 $\pm$ 0.59 (n=23)	2.93-4.62	2.7
14Y-18Y;	3.53 $\pm$ 0.49 (n=11)	2.90-4.55	2.6
18Y-40Y;	3.25 $\pm$ 0.49 (n=33)	2.59-4.26	2.3

M, male; F, female.

significantly different IGFBP-3 values in male and female children during puberty.

Among the peri- and post-pubertal normal control children (male; 9-17 years old, female; 8-17 years old), their pubertal stages (Tanner I-V) were recorded in seventy-one children. Normal IGFBP-3 levels for each pubertal stage were determined as follows: Tanner I (n=27); 2.97 $\pm$ 0.49 mg/L (range 1.84-4.05), Tanner II (n=15); 3.52 $\pm$ 0.60 mg/L (2.58-4.5), Tanner III (n=13); 3.88 $\pm$ 0.68mg/L (2.25-4.62), Tanner IV (n=10); 3.79 $\pm$ 0.52 mg/L (3.19-4.55), Tanner V (n=6); 3.84 $\pm$ 0.47 mg/L (3.18-4.55). There were no significant differences between male and female children at each stage.

2) Figure 2 shows IGFBP-3 levels of patients with cGHD and pGHD. The lines are the cutoff levels for each age as determined above. IGFBP-3 levels of patients with cGHD were almost completely below the cutoff level for each age and sex, whereas a significant number of patients with pGHD had IGFBP-3 levels that were above the cutoff level as stated precisely below.

3) Figure 3 shows IGFBP-3 levels of patients with NS and the cutoff levels for each age and sex. The IGFBP-3 levels of patients with NS were almost always more than the cutoff levels. When we use the control values at each pubertal level for peri- and pubertal patients with NS (male; >9 years old, female; >8 years old), 4 of 8 patients below the cutoff line in Fig. 3 had IGFBP-3 levels above the puberty-related cutoff level (which was similarly determined to the cutoff level at each age and sex, data not shown).

4) Sensitivity (true positive ratio) and specificity (true negative ratio) were calculated by the results of IGFBP-3 in patients with NS and GHD (Figs. 2 and 3). The sensitivity and specificity were determined based upon whether the IGFBP-3 levels were below or above the age-related cutoff level. The results were expressed separately depending on whether the patients are below or above the age of ten (Table 2). Overall, the specificity and sensitivity for the diagnosis of GHD are 88 and 73%, respectively. When we evaluate the specificity and sensitivity, separately before and after the age of ten, the results are as follows (Table 2): specificity : 87% (<10 years) and 88% (> 10 years);

Fig. 2. IGFBP-3 (mg/L) of patients with GHD. The lines are the cutoff level at each age and sex. The dotted line means the cutoff levels for female children only. The closed circle (●) and double circle (⊙) mean cGHD and pGHD, respectively.

Fig. 3. IGFBP-3 (mg/L) of patients with NS. The lines are the cutoff level at each age and sex. The dotted line means the cutoff levels for female children only.

Table 2. Sensitivity and specificity of IGFBP-3

	10 Y>	10 Y<	total
GHD sensitivity (%)	84%	64%	73%
(cGHD+pGHD)	(n=31)	(n=42)	(n=73)
cGHD sensitivity (%)	100%	88%	93%
(n=19)	(n=24)	(n=43)	
pGHD sensitivity (%)	58%	33%	43%
(n=12)	(n=18)	(n=30)	
NS specificity (%)	87%	88%	88%
(n=69)	(n=24)	(n=93)	

NS, normal short children. Percentage of patients with NS and GHD (cGHD and pGHD) whose IGFBP-3 levels were above and below the cutoff level at each age and sex. The results are shown separately depending on whether the patients are below or above the age of ten.

sensitivity: 84% (< 10 years), 64% (> 10 years). When cGHD and pGHD are analyzed separately, the sensitivity for GHD is as follows: cGHD: 100% (<10 years) and 88% (>10 years); pGHD: 58% (<10 years) and 33% (> 10 years).

5) Among the patients with GHD older than 15 years old, 22 patients were evaluated for secondary hypogonadism. Of these patients, only 1 of 12 with untreated secondary hypogonadism (7

cGHD and 5 pGHD) had an IGFBP-3 level above the cutoff level. In contrast, out of 10 pubertal patients without secondary hypogonadism (3 cGHD and 7 pGHD), 9 patients showed IGFBP-3 levels above the cutoff level at each age. There is a significant difference between the ratios ($P<0.005$) for GHD patients with hypogonadism and those without hypogonadism. The IGFBP-3 levels of these GHD patients together with normal levels for each Tanner stage are shown in Fig. 4.

Fig. 4. IGFBP-3 levels (mg/L) of older GHD patients with (●) and without (○) secondary hypogonadism. * represents cGHD. The Tanner-matched normal values (mean $\pm$ SD) are also shown.

Discussion

The specificity for diagnosis of GHD was about 90%, which is similar to the results obtained by Blum *et al.* [12]. The sensitivity for diagnosis of cGHD was more than 90%. Therefore the usefulness of IGFBP-3 measurement for screening GHD was proved.

As shown in Table 2, IGFBP-3 measurement for the diagnosis of pGHD is not so useful as cGHD. The sensitivity for pGHD was much lower than that reported by Blum *et al.*, although they did not classify GHD into two types as we did into cGHD and pGHD. One reason for the difference in the usefulness of IGFBP-3 in cGHD and pGHD is easy to understand: the severity of deficiency is greater in cGHD than in pGHD, by definition. Another possibility is the limitation of GH stimulation tests such as poor reproducibility [15, 16] and false positive ratios. We reported that the false positive ratio of arginine and insulin tests in short children was 22–23% [17].

The difference in the sensitivity between younger (<10 years) and older (>10 years) GHD patients may reflect the higher ratio of pGHD to cGHD in the older group. However, two other possibilities may explain the lower sensitivity for GHD after the age of ten: (1) some patients with a

constitutional delay in growth and adolescence (CDGA) who had transient GHD [18, 19] were included, although we think this type of transient GHD is not a spectrum of GHD in a true sense but a physiological event. (2) IGFBP-3 levels increase during puberty, independent of the GH-IGF-I axis.

Speculation (1) is likely to be true because we did not perform so-called testosterone or estrogen priming with GH stimulation tests in necessary cases. In our personal experience (not published), these CDGA patients with transient GHD are usually partial GHD by our definition. Thus, speculation (1) partially explains the fact that the sensitivity of IGFBP-3 testing for pGHD after the age of 10 is lower than that for pGHD before that age. However, we cannot explain the limitation of IGFBP-3 measurement after the age of ten solely by speculation (1), since two patients who were thought to have pGHD and cGHD before GH therapy was started at prepubertal age and who showed IGFBP-3 levels above the cutoff levels at pubertal age, were ascertained to have pGHD and cGHD when GH therapy was discontinued after puberty had developed almost fully.

Speculation (2) is supported by the following fact about GHD patients with or without secondary hypogonadism. Among the twenty-two pa-

tients with GHD evaluated for secondary hypogonadism, the ratio of patients with GHD whose IGFBP-3 levels were above the age-matched cutoff level for GHD was significantly lower in the patients with GHD associated with secondary hypogonadism than in those without secondary hypogonadism. A similar difference in IGFBP-3 levels between GHD with hypogonadism and that without hypogonadism using the Tanner-matched normal values, as shown in Fig. 4, also supports the speculation (2). This speculation is also consistent with our previous report where we showed an overlap in IGFBP-3 levels on western ligand blot between pubertal control and untreated pubertal or sex-hormone treated (but not treated with GH)

pubertal patients with GHD [11]. Furthermore, this speculation is analogous to partial dependence of the serum IGF-1 concentration upon gonadal steroids (independent of GH) as reported by Attie and Grumbach [20].

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