ORIGINAL

Effects of GH assay standardization on evaluation of treatment outcomes for acromegaly in Japan

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Abstract. In Japan, the growth hormone (GH) assay has been standardized since April 2005 through use of a uniform recombinant human GH (rhGH) standard. Since then, GH values measured using the rhGH standard have been approximately 40% lower than previous values measured using kit standards based on the WHO standards for hGH of pituitary origin. However, the Japanese criteria for evaluating treatment outcomes for acromegaly have remained the same: a nadir GH during a 75 g OGTT $<1 \mu\text{g/L}$ is considered cured, $1 \leq \text{GH} < 2.5 \mu\text{g/L}$ is considered inadequately controlled, and $\geq 2.5 \mu\text{g/L}$ is considered poorly controlled, instead of these levels were lowered to 60%, i.e. from 1 to $0.6 \mu\text{g/L}$ for cured and from 2.5 to $1.5 \mu\text{g/L}$ for inadequately controlled (termed as “newly proposed criteria” in this study). We investigated the effects of standardization of the GH assay on the evaluation of post-surgical disease activity in 50 patients with acromegaly (M/F 19/31, 21-72 yr.). Post-surgical nadir GH levels during OGTT were positively correlated with the IGF-I SD score 3 months after TSS. Five of 6 patients whose post-surgical nadir GH levels ranged between 0.6 and $1 \mu\text{g/L}$ had normal serum IGF-I levels 3 months after TSS. Rates of improvement in glucose metabolism did not differ when patients were classified based on the present criteria vs. the newly proposed criteria. In conclusion, the current Japanese remission criteria for acromegaly still accurately reflect post-surgical disease activity in most patients, though long-term observation is still required.

Key words: Acromegaly, GH standardization, Diabetes mellitus

ACROMEGALY is caused by chronic growth hormone (GH) hypersecretion from a pituitary adenoma. Persistent GH excess leads to acral growth, facial changes and co-morbidities including glucose intolerance, hypertension, and sleep apnea syndrome. Abnormal regulation of GH secretion in acromegaly is reflected by impaired GH suppression during an oral glucose tolerance test (OGTT) and elevated circulating IGF-I levels. Transsphenoidal surgery (TSS) is the primary therapy for most patients with acromegaly. Cured acromegaly is defined as a complete remission of clinical signs and symptoms of GH excess, reversal of tumor mass effects with preservation of other anterior pituitary hormones, and the normalization of GH and IGF-I secretion. Monitoring of acromegaly disease activity relies upon measurement of both serum GH

and IGF-I. For GH, OGTT response is more important than random measurement of GH levels due to the pulsatile nature of GH secretion. In contrast, serum IGF-I is an excellent marker of 24-hour integrated GH secretion and a very sensitive indicator of GH excess. A post-surgical cure is defined by normalization of serum IGF-I and by recovery of normal GH suppression after an OGTT. An international consensus statement has proposed a post-glucose GH nadir of less than $1 \mu\text{g/L}$ as one component of the criterion for remission [1]. Concordance between GH assays is necessary to accurately evaluate post-surgical disease activity using international criteria. Until 2005, GH values obtained using different commercially available kits varied widely in Japan, as the standards used by each kit was not the rhGH standard but WHO standards for human GH of pituitary origin which had different potencies. Since April 2005, the GH assay has been standardized nationwide by using a uniform recombinant human GH (rhGH) standard. Since then, discrepancies in GH values measured using different commercial kits have

Submitted Mar. 23, 2011; Accepted Jun. 10, 2011 as K11E-099
Released online in J-STAGE as advance publication Jun. 30, 2011
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been resolved. However, GH values measured using the rhGH standard are on average 60% of the GH values measured using the previous standards for pituitary-derived GH, as pituitary hGH contains multiple forms of hGH and has lower potency than rhGH due to its impurity [2]. There was a linear regression between GH values (values obtained by calculation using the correction formula after measurements) measured by six hGH kits according to the previous standards and to the rhGH standard ($Y=0.6\pm0.017$) [2]. Since standardization, diagnostic cut-off levels for GH deficiency using peak stimulated GH have been lowered by 40% in Japan, namely, from 10 to 6 $\mu\text{g/L}$ for children and from 5 to 3 $\mu\text{g/L}$ for adults. However, for acromegaly, nadir GH criteria during the 75 g OGTT have remained at $<1 \mu\text{g/L}$ for cured, $<2.5 \mu\text{g/L}$ for inadequately controlled, and $\geq 2.5 \mu\text{g/L}$ for poorly controlled. In this study, we investigated the effects of standardization of the GH assay on current post-operative remission criteria for acromegaly.

Subjects and Methods

Fifty patients with active acromegaly (19 males and 31 females; mean age $49 \pm \text{SD } 13$ yr, range 21–72 yr), who were admitted to the Department of Medicine, Institute of Clinical Endocrinology, Tokyo Women's Medical University after the national standardization of GH measurement (April 2005) were studied. The diagnosis of acromegaly was confirmed by positive GH immunohistochemical staining of excised pituitary adenomas after TSS. We used medical records to retrospectively analyze glucose metabolism, endocrine data and post-surgical outcomes for each patient. Fifty patients with acromegaly who received TSS were divided into 3 groups based on nadir GH levels during a 75 g OGTT after TSS. First, the nadir GH cut-off levels were based on the present Japanese criteria for evaluating treatment outcomes: cured (group C: nadir GH $<1 \mu\text{g/L}$, $n=38$), inadequately controlled (group I: $1 \mu\text{g/L} \leq \text{nadir GH} < 2.5 \mu\text{g/L}$, $n=6$) and poorly controlled (group P: nadir GH $\geq 2.5 \mu\text{g/L}$, $n=6$). Then, the same patients were re-classified based on "the newly proposed criteria", which was set taking GH assay standardization into account, i.e. group C (st) (nadir GH $< 0.6 \mu\text{g/L}$, $n=32$), group I (st) ($0.6 \mu\text{g/L} \leq \text{nadir GH} < 1.5 \mu\text{g/L}$, $n=10$) and group P (st) (nadir GH $\geq 1.5 \mu\text{g/L}$, $n=8$) (Fig. 1). We compared the rates of serum IGF-I normalization and changes in glucose metabolism under these

Fig. 1 Japanese criteria for evaluating treatment outcomes based on nadir GH levels during OGTT.

two classification schemes. For postoperative evaluation, OGTT results one week after TSS were a strong predictive marker of disease activity, although IGF-I levels fluctuated in the early postoperative period [3]. It is recommended that for postoperative assessment, serum IGF-I should be measured repeatedly until 12 weeks after TSS [3]. In this study, nadir GH during OGTT approximately 12 (range: 6–28) days after TSS and serum IGF-I levels three months after TSS were analyzed.

Hormone assays

Through August 2005, serum GH levels were measured using an immunoradiometric assay (IRMA) kit (Daiichi Radioisotope Laboratories, Ltd., Tokyo, Japan). An immunoenzymometric assay (IEMA) kit was used from September 2005 onward. Serum IGF-I levels were measured using an immunoradiometric assay kit (Daiichi Radioisotope Laboratories, Ltd., Tokyo, Japan). The standard deviation scores (SDS) for serum IGF-I levels were adjusted for sex and age, according to the constructed reference [4].

Statistics

All data are expressed as means $\pm$ SD. Spearman's rank test was used to assess associations between post-surgical nadir GH during OGTT and IGF-I SDS. A chi-square test was used to compare rates of IGF-I normalization among the different nadir GH cut-off criteria. All statistical analyses were performed using Stat View R 5.0 (SAS Institute Inc., USA), with statistical significance established at $p < 0.05$.

Results

Post-surgical nadir GH levels during OGTT were significantly correlated with IGF-I SD scores 3 months after TSS (Fig. 2). Among the 6 patients whose post-surgical nadir GH levels ranged between 0.6 and 1 $\mu\text{g/L}$ (classified in group C but in group I (st) when GH assay standardization was taken into account, plotted in the gray area of Fig. 2), 5 had normal serum IGF-I levels 3 months and 1 year (from -1.67 to 1.69 SDS) after TSS, while persistent elevation of serum IGF-I was observed in one patient (IGF-I SDS 3 months after TSS was 3.81).

Eighty-nine percent (34/38) of the patients in group C had normal IGF-I levels 3 months after TSS. The rate of IGF-I normalization in group C was not significantly different than in group C (st) (91%, 29/32) (Table 1). One year after TSS, IGF-I SDS in 2 patients who had elevated IGF-I 3 months after TSS in group C(st) went from 3.54 to 1.73 (nadir GH: 0.33) and from 2.57 to 2.10 SDS (nadir GH: 0.19) without any additional therapy. However, all patients in group P (nadir GH levels greater than 2.5 $\mu\text{g/L}$) had persistently elevated IGF-I levels. When nadir GH levels were lowered by 40% (from ≥ 2.5 to $\geq 1.5 \mu\text{g/L}$, defined as group P (st)), all patients still had persistently elevated IGF-I levels. The IGF-I normalization rate was 33% for group I and 70% for group I (st). There was no significant difference between these rates. Two patients in group I had post-surgical nadir GHs during OGTT between 1.5 and 2.5 $\mu\text{g/L}$ and were categorized in group P (st). Dopamine agonist treatment was started within one month after surgery in one of these patients, as this patient had persistent disease activity after TSS (post-

Fig. 2 Relationships between nadir GH during OGTT and IGF-I SDS 3 months after TSS. Open circles: females, closed circles: males, C and C(st): cut-off levels of nadir GH for cured, I and I(st): cut-off levels of nadir GH for inadequately controlled. Data from 2 patients (1 from group I and 1 from group P) were omitted because they received dopamine agonist treatment 3 months after TSS.

glucose nadir GH: 1.55 $\mu\text{g/L}$, IGF-I SDS 2 weeks after TSS: 6.76). Post-glucose nadir GH in another patient was 1.62 $\mu\text{g/L}$. This patient had clearly elevated IGF-I 3 months after TSS (3.21 SD).

Before TSS, 40 of the 50 patients had impaired glucose tolerance or frank diabetes mellitus (DM), 30 patients in group C, 5 in group I and 5 in group P. Among the 30 patients with abnormal glucose metabolism in group C, 15 who had impaired glucose tolerance (IGT) improved to have normal glucose tolerance (NGT) and 6 patients who had DM improved (one to NGT, 2 to IGT, and 3 patients developed lower hypoglycemic drug requirements) after TSS (Fig. 3(b)). The

Table 1 Rates of IGF-I normalization: comparison of present and newly proposed criteria

Criterion	Cured	Inadequately Controlled	Poorly Controlled
Group (N)	group C (38)	group I (6)	group P (6)
Nadir GH cut-off	GH < 1.0	$1.0 \leq \text{GH} < 2.5$	$2.5 \leq \text{GH}$
Rate of IGF-I normalization	89% (34/38)	33% (2/6)	0% (0/6)
Group (N)	group C (st) (32)	group I (st) (10)	group P (st) (8)
Nadir GH cut-off	GH < 0.6	$0.6 \leq \text{GH} < 1.5$	$1.5 \leq \text{GH}$
(taking GH standardization into consideration)			
Rate of IGF-I normalization	91% (29/32)	70% (7/10)	0% (0/8)

Fig. 3 Changes in glucose metabolism before and after TSS. Comparison of the newly proposed (a) and present criteria (b) is shown. Circles: cured (group C(st) in (a), group C in (b)), Squares: inadequately controlled (group I(st) in (a), group I in (b)), Triangles: poorly controlled (group P(st) in (a), group P in (b)). *: improved (hypoglycemic drug or insulin treatment decreased after TSS) In the columns of DM, open markers indicate patients with a history of DM less than 5years and gray markers indicate patients with a history of DM $\geq$ 5years.

Table 2 Rates of improvement in glucose metabolism: comparison of present and newly proposed criteria

Criterion	Cured	Inadequately Controlled	Poorly Controlled
Group (N)	group C (38)	group I (6)	group P (6)
Nadir GH cut-off	GH<1.0	$1.0 \leq \text{GH} < 2.5$	$2.5 \leq \text{GH}$
Glucose metabolism status (N/I/D*)	(8/21/9)	(1/3/2)	(1/0/5)
Rate of improvement after TSS	70%	60%	40%
	(21/30)**	(3/5)	(2/5)
Group (N)	group C (st) (32)	group I (st) (10)	group P (st) (8)
Nadir GH cut-off	GH<0.6	$0.6 \leq \text{GH} < 1.5$	$1.5 \leq \text{GH}$
(taking GH standardization into consideration)			
Glucose metabolism status (N/I/D*)	(7/18/7)	(1/5/4)	(2/1/5)
	68%	78%	33%
	(17/25)**	(7/9)	(2/6)

*: N: normal glucose tolerance, I: impaired glucose tolerance, D: diabetes melitus

** : Number of patients with improved glucose metabolism /number of patients with IGT or DM

improvement rate of glucose metabolism in group C was 70% (21/30) (Table 2). The improvement rate in group C (st) (68%) was not different from the rate in group C. Improved glucose metabolism was observed even in patients from group P and P(st), and the improvement rates were 40 and 33% in these groups, respectively. Among 16 patients with DM before TSS, six patients had the history of DM more than five years. After TSS, glucose metabolism improved in all patients, while it improved in three of ten patients with the shorter history (less than five years) of DM.

Discussion

In this study, we observed a significant positive correlation between post-surgical nadir GH levels during OGTT and IGF-I levels in the first three months after TSS, suggesting that lower nadir GH levels in the early postoperative period are a good predictive marker for subsequent lower IGF-I levels. These findings are consistent with previous reports [5, 6], although Freda *et al.* reported that these 2 parameters were not correlated in healthy subjects or in patients with cured acromegaly whose nadir GH was less than 0.14 $\mu\text{g/L}$ when

measured by immunoradiometry [7].

Since 2000, the treatment goal for acromegaly has been established by international consensus as a nadir GH during OGTT of less than 1 $\mu\text{g/L}$, with normal IGF-I levels [1]. It should be noted that uniform GH assay methods have not yet been defined. These criteria specify only that “the GH assay should have adequate sensitivity (at least 0.5 $\mu\text{g/L}$), established validity, specificity, reliability and uniform reproducibility”. Therefore, the criteria have been interpreted as a general guideline [8]. In this study, 5 of 6 patients whose nadir GH ranged between 0.6 and 1.0 $\mu\text{g/L}$, which is classified as inadequately controlled when taking GH standardization into account, had normal IGF-I levels 3 and 12 months after TSS. Ronchi *et al.* observed that the long-term disease recurrence rate was only 2% among patients with normal IGF-I in the first 3 months after TSS [6]. Given these findings, our results suggest that the current Japanese remission criteria with a nadir GH cut-off still, and even after the GH standardization, adequately assess clinical disease activity in most patients with acromegaly. Additional therapy may not be required for most of our patients whose nadir GH ranged between 0.6 and 1.0 $\mu\text{g/L}$. Still, careful long-term follow up is required in these patients, because serum IGF-I levels in 26% of patients whose post-surgical nadir GH ranged between 0.15 and 1.2 $\mu\text{g/L}$ later had IGF-I levels above the upper limit of the normal range during a 3-year observation period [9]. Furthermore, glucose-suppressed GH levels of 1.0 $\mu\text{g/L}$ are markedly higher than those in healthy subjects and recently, a nadir GH of less than 0.4 $\mu\text{g/L}$ measured using ultrasensitive assays was proposed as a new criterion for cured acromegaly [7, 10].

Regarding cut-off levels of nadir GH in the inade-

quately and poorly controlled groups, all patients whose nadir GH was more than 1.5 $\mu\text{g/L}$ had elevated IGF-I levels in this study. As there was a linear regression between GH values measured according to the previous standards and to the rhGH standard [2], 2.5 $\mu\text{g/L}$ of GH, which was cut-off level of nadir GH in the inadequately and poorly controlled group equivalents almost 4.2 $\mu\text{g/L}$ of pre-standardized GH. Therefore, there is a possibility that patients who were considered to have a clearly persistent disease might be included in the inadequately controlled group post-GH standardization.

Abnormal glucose metabolism is one of the most frequent complications of acromegaly. We compared rates of glucose metabolism improvement after TSS in groups C, I, and P. Arita *et al.* reported that prevalence of diabetes mellitus decreased from 44.6 to 21.5% after surgery even in 68 patients with acromegaly who were not determined to be cured by TSS [11]. Consistent with these results, we observed that post-surgical glucose metabolism improved in some patients in the poorly controlled group, and that the prevalence of improvement in glucose metabolism among the 3 groups did not differ when the nadir GH cut-off was lowered by 40% to reflect post-standardization criteria. Moreover, diabetes even lasted for more than 5 years improved after TSS.

In conclusion, the current Japanese remission criteria for acromegaly still reflect post-surgical disease activity well in most patients. However, this study only investigated changes in GH and IGF-I levels and glucose metabolism over a short postoperative period. Long-term observation is needed to confirm whether the current criteria can predict long-term disease activity accurately in patients with acromegaly.

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