NOTE

Continuing efforts to standardize measured serum growth hormone values in Japan

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Abstract. Determination of serum growth hormone (GH) levels is mandatory for diagnosis of GH deficiency and excess. In the present study, we, the Study Committee for GH and Its Related Factors, The Foundation for Growth Science, Japan measured GH values in serum samples using all the commercially available kits in Japan. Significant discrepancies in the GH values were observed among the kits in spite of using the unified recombinant human GH-based standards. To deal with the discrepancies, we established a formula using a linear structural relationship model and were able to standardize the GH values. We propose to use the formula to diagnose GH deficiency and excess in Japan.

Key words: Growth hormone (GH), Immunoassay, Assay kit, WHO IS, Somatropin

DETERMINATION of growth hormone (GH) levels in serum samples is essential for diagnosis of GH deficiency and excess [1-3]. In clinical practice, GH levels have been determined by immunological methods including radioimmunoassay and enzyme immunoassay. In Japan, several reagents to measure serum GH (GH assay kits) have been commercially available. Until 2005, measured GH values in individual sera varied significantly among GH assay kits. To deal with the variations, we as the Study Committee for GH and Its Related Factors, The Foundation for Growth Science, Japan collaborated with the Study Group for Hypothalamo-Pituitary Disorders of the Ministry of Health, Labour and Welfare (MHLW), Japan and the suppliers of GH assay kits, and provided the formulas to adjust the measured values to their average. Since the majority of discrepancies in the measured values were attributed to the unequal potencies of the in-house GH standards of the suppliers, the unified recombinant human GH (rhGH)-based standards were introduced to all GH assay kits in 2005 [4-6]. As the result, the discrepancies in measured values among them became

less prominent, and the cutoff value in the GH stimulation tests was set to 6 ng/mL rather than previous 10 ng/mL since the measured GH values became 60% of those obtained before the introduction of rhGH-based standards [5]. Recently, however, the considerable variations were noticed again by the annual nationwide control survey for immunoassays [7-10], so that additional studies became required. Here, we describe the results of the recent studies.

Materials and Methods

Serum samples were collected after informed consent was obtained from each subject and were kept at -70 °C. Approval by the Institutional Review Boards was acquired when necessary. 2nd WHO IS somatropin (recombinant human GH) (code: 98/574) was purchased from the National Institute for Biological Standards and Control (NIBSC). GH assay kits used in this study are shown in Table 1.

In 2012, we determined GH levels in 100 sera from healthy adult volunteers using 5 GH assay kits listed in Table 1. To standardize measured values, equations were calculated using a linear structural relationship model as reported previously [6]. In addition, we serially diluted the IS somatropin (NIBSC 98/574) solution with the phosphate buffered saline and determined GH content in the diluted materials using the TFB, Roche

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Table 1 GH assay kits available in Japan

GH assay kit (abbreviations)	Assay method	Supplier
GH Kit Daiichi (TFB)	IRMA	TFB, Inc.
ECLusys hGH (Roche)	ECLIA	Roche Diagnostics, Inc.
Access hGH (Beckman)	CLEIA	Beckman Coulter, Inc.
IMMULYZE GH II (Siemens)	CLEIA	Siemens HCD, Inc.
E Test TOSOH II HGH (Tosoh)	IEMA	Tosoh Co., Ltd.

IRMA, immunoradiometric assay;
 ECLIA, electrochemiluminescence immunoassay;
 CLEIA, chemiluminescent enzyme immunoassay;
 IEMA, immunoenzymometric assay.

and Beckman kits. Data of the diluted IS somatropin solution were analyzed with a linear regression model.

In 2015, since the TFB kit was no longer available, we determined GH levels in 103 sera using the 4 GH assay kits other than the TFB kit. Forty sera were the stored samples since 2012, and the remaining 63 were newly collected from short children. To validate the formula established in 2012, we calculated equations using the data transformed with this formula and the linear structural relationship model.

Results

Study in 2012

In Fig. 1 measured GH levels in individual sera with the 5 GH assay kits were plotted against their averages. Their correlation between the kits was acceptable, but significant discrepancies in the slopes were observed among the kits. The TFB kit tended to give higher values, while the Beckman kit lower values. To adjust the measured values to their average, equations were calculated using a linear structural relationship model (Table 2).

In Fig. 2 measured GH content in the diluted IS somatropin solution with the TFB, Roche and Beckman kits is plotted against the expected values from the dilution factors. The measured content was in good agreement with the expected values.

Based on these results, the following formula was recommended: $Y=1.4X$. Here, Y denotes standardized GH values, and X denotes measured GH values when the Beckman kit is used.

Study in 2015

In Fig. 3 measured GH levels with the 4 GH assay kits were plotted against their averages. The Beckman

Fig. 1 Relationship between mean and individual GH values measured by commercially available kits in the year 2012.**Table 2** Equations calculated using a linear structural relationship model to adjust the measured GH values (X) in 2012 to their averages (Y)

GH assay kit	Equation	Correlation coefficient
TFB	$Y=0.83X-0.06$	0.997
Roche	$Y=0.97X+0.03$	0.999
Beckman	$Y=1.41X+0.07$	0.997
Siemens	$Y=1.03X-0.04$	0.999
Tosoh	$Y=1.01X$	0.999

Fig. 2 Relationship between the expected and measured GH values in serially diluted IS somatropin solutions.

kit tended to result in lower values. To verify the formula established in 2012, we transformed measured GH values with this formula. In Fig. 4, transformed GH values were plotted against their averages. Equations were calculated using the transformed data and the linear structural relationship model (Table 3). The same formula as that established in 2012 was recommended.

Discussion

In the present studies, we measured GH levels using commercially available GH kits in Japan, and observed discrepancies in the measured values among the kits. To evaluate the potencies of GH standards in the kits, we quantified GH content in the diluted IS somatotropin solutions using the kits. The obtained content was the same as the expected values. Therefore, it is concluded that the potencies of GH standards in the kits are equivalent and that the discrepancies in the measured values are due to other causes than inequality of the standards.

Immunoassays for GH inherently cannot avoid some variability in the determined values because of the molecular heterogeneity in circulating GH, the presence of circulating GH binding protein and matrix effects [11, 12]. Therefore, it is preferable to establish reference ranges and cut off values in GH stimulation and suppression tests for each assay. However, such an ideal approach is practically impossible especially in children. One of the practical approaches is to standardize the values determined by various GH assay kits as we have performed.

In Japan, almost all the cost for GH treatment of children with GH deficiency is supported by social insurance and by a grant for special chronic children's disease from MHWL [4]. Nowadays, the cost for GH treatment of adults with severe GH deficiency is also supported in part by a grant for intractable diseases from MHWL. Thus, nationwide criteria for diagnosis of GH deficiency need to be established. The Study Group for Hypothalamo-Pituitary Disorders of MHWL has been making efforts to establish the criteria, including cutoff values in GH stimulation tests. To equalize interpretations of individual GH stimulation tests, we have been working on formulas to standardize GH values measured by different GH assay kits. We have proposed a new formula in 2012, which is approved by MHLW. In 2015, we have reconfirmed that the formula is still appropriate. Therefore, we propose to

Fig. 3 Relationship between mean and individual GH values measured by commercially available kits in the year 2015.

Fig. 4 Relationship between mean and standardized GH values measured in the year 2015.

Table 3 Equations calculated using a linear structural relationship model to adjust the transformed GH values (X) in 2015 to their averages (Y)

GH assay kit	Equation	Correlation coefficient
Roche	$Y=0.98X-0.20$	0.997
Beckman	$Y=0.97X+0.33$	0.994
Siemens	$Y=1.00X+0.02$	0.997
Tosoh	$Y=1.03X-0.12$	0.997

keep using the same formula in the meanwhile. It is noteworthy that some of the Y-interceptions are apart from 0 in the equations of 2015 (Table 3). Therefore, we should pay attentions when we interpret the standardized values in the lower range of GH.

Internationally, Clemmons has published the consensus statement on the standardization and evaluation of GH assays [12]. Collaborators have been working on the harmonization of GH measurements, which might include identification of a commutable matrix reference material and evaluation of liquid chromatography mass spectrometry as a reference measurement procedure [13].

In conclusion, we have established a practical formula using a linear structural relationship model to standardize the measured GH values. Further efforts are needed to harmonize GH measurements.

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Disclosures

None of the authors have any conflicts of interest associated with this research.

References

1. Growth Hormone Research Society (2000) Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. *J Clin Endocrinol Metab* 85: 3990-3993.
2. Growth Hormone Research Society; Pituitary Society (2004) Biochemical assessment and long-term monitoring in patients with acromegaly: statement from a joint consensus conference of the Growth Hormone Research Society and the Pituitary Society. *J Clin Endocrinol Metab* 89: 3099-3102.
3. Ho KK; 2007 GH Deficiency Consensus Workshop Participants (2007) Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: a statement of the GH Research Society in association with the European Society for Pediatric Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society, and Endocrine Society of Australia. *Eur J Endocrinol* 157: 695-700.
4. Tanaka T, Takano K, Hanew K, Nishi Y, Igarashi Y, *et al.* (1998) Registration system for growth hormone (GH) treatment with standardized immunoreactive GH values in Japan. *Endocr J* 45: 459-465.
5. Tanaka T, Tachibana K, Shimatsu A, Katsumata N, Tsushima T, *et al.* (2005) A nationwide attempt to standardize growth hormone assays. *Horm Res* 64 (Suppl 2): 6-11.
6. Saito T, Tachibana K, Shimatsu A, Katsumata N, Hizuka N, *et al.* (2006) Standardization of blood growth hormone levels measured by different kits using a linear structural relationship. *Clin Pediatr Endocrinol* 15: 79-84.
7. Subcommittee for Radioisotope *in vitro* Test, Medical Science and Pharmaceutical Committee, Japan Radioisotope Association, Immunoassay Research Society Japan (2006) A summary report on the 27th quality control survey for immunoassay in Japan, 2005. *Radioisotopes* 55: 599-649 (In Japanese).
8. Subcommittee for Radioisotope *in vitro* Test, Medical Science and Pharmaceutical Committee, Japan Radioisotope Association, Immunoassay Research Society Japan (2007) A summary report on the 28th quality control survey for immunoassay in Japan, 2006. *Radioisotopes* 56: 637-686 (In Japanese).
9. Subcommittee for Radioisotope *in vitro* Test, Medical Science and Pharmaceutical Committee, Japan Radioisotope Association, Immunoassay Research Society Japan (2008) A summary report on the 29th quality control survey for immunoassay in Japan, 2007. *Radioisotopes* 57: 617-668 (In Japanese).
10. Subcommittee for Radioisotope *in vitro* Test, Medical Science and Pharmaceutical Committee, Japan Radioisotope Association, Immunoassay Research Society Japan (2009) A summary report on the 30th quality control survey for immunoassay in Japan, 2008. *Radioisotopes* 58: 655-708 (In Japanese).
11. Bidlingmaier M, Freda PU (2010) Measurement of human growth hormone by immunoassays: current status, unsolved problems and clinical consequences. *Growth Horm IGF Res* 20: 19-25.
12. Clemmons DR (2011) Consensus statement on the standardization and evaluation of growth hormone and insulin-like growth factor assays. *Clin Chem* 57: 555-559.
13. Wieringa GE, Sturgeon CM, Trainer PJ (2014) The harmonisation of growth hormone measurements: taking the next steps. *Clin Chim Acta* 432: 68-71.