Growth Hormone & IGF Research 23 (2013) 165–169

Contents lists available at ScienceDirect

Growth Hormone & IGF Research

journal homepage: www.elsevier.com/locate/ghir

Comparison of two growth hormone stimulation tests and their cut-off limits in healthy adults at an outpatient clinic

Helene M. Markkanen [a], Tuula Pekkarinen [b,*], Matti J. Välimäki [c], Henrik Alfthan [a], Esa Hämäläinen [a], Ulf-Håkan Stenman [a]

[a] Department of Clinical Chemistry, Helsinki University Central Hospital, FI-00029 HUS, Finland
[b] Division of Endocrinology, Department of Medicine, Helsinki University Central Hospital, Vantaa, FI-00029 HUS, Finland
[c] Division of Endocrinology, Department of Medicine, Helsinki University Central Hospital, FI-00029 HUS, Helsinki, Finland

ARTICLE INFO

Article history:
Received 13 March 2013
Received in revised form 20 May 2013
Accepted 11 June 2013
Available online 6 July 2013

Keywords:
Growth hormone deficiency
Adults
ITT
GHRH + ARG
Diagnosis

ABSTRACT

Objective: A peak GH less than 3 μg/L to insulin tolerance test (ITT) is commonly used as a threshold indicating severe adult GH deficiency (GHD). This cut-off is based on results obtained by polyclonal radioimmunoassays preferably under standard conditions at hospital. Our aim was to evaluate the validity of this cut-off limit using two currently used immunometric GH assays and to compare GH responses in the ITT and the GH releasing hormone + arginine (GHRH + ARG) test in healthy adults at our outpatient endocrine unit.
Design: ITT was performed on 73 subjects and the GHRH + ARG test on those 28 who showed insufficient response to the ITT.
Methods: GH was measured by an immunofluorometric and immunochemiluminometric assay.
Results: GH peak above 3 μg/L was observed in 56% of the healthy volunteers with adequate hypoglycemia in the ITT. Among the 28 subjects with a peak GH below 3 μg/L, only two overweight men had a GH peak response below the commonly used cut-off limit of 9.1 μg/L in the GHRH + ARG test.
Conclusions: Lean healthy adults could erroneously be classified as GH deficient by the ITT while their results in the GHRH + ARG test were normal. The GH results are highly dependent on the immunoassay used, but false positive results in the ITT are often obtained even if lower cutoff limits determined on the basis on the calibration of the GH assay are used. Confounding factors seemed to blunt the GH response to the ITT more than to the GHRH + ARG test at our outpatient clinic.

1. Introduction

The clinical diagnosis of adult growth hormone deficiency (GHD) is demanding because symptoms and pathophysiologic features are nonspecific. Correct diagnosis, however, is important since treatment with recombinant GH in severe GHD is expensive but can significantly improve body composition, bone health, cardiovascular risk factors and quality of life [1,2]. Therefore, it is essential that the diagnostic tests and laboratory methods used to identify patients with severe GHD are reliable.

The insulin tolerance test (ITT) has been considered the gold standard for diagnosis of adult GHD in patients with an appropriate clinical history. According to consensus guidelines, the cut-off limit for severe GHD justifying treatment is defined as a GH peak response in the ITT less than 3 μg/L, while healthy subjects have been reported to respond to ITT with peak GH exceeding 5 μg/L [1–3]. These cut-off values were initially defined on the basis of results determined by polyclonal competitive radioimmunoassays (RIAs) calibrated against the WHO International Reference Preparation (IRP) 80/505 [1,4,5]. Presently used sandwich assays give up to 50% lower GH results than the earlier used polyclonal RIAs [6,7]. However, even results with different sandwich assays vary [7,8].

The ITT is problematic. It is unpleasant and contraindicated in patients with epilepsy, cardiovascular disease and age over 60 years [1]. The test conditions used in the ITT, e.g. the dose of insulin and the timing of sampling vary in different studies, and there are concerns about poor reproducibility of ITT [9,10]. Previously, mild physical activity like hospital admission on the morning of the test has been shown to blunt the GH response to hypoglycemia among healthy adults and thus reduce the specificity of the ITT [11]. Therefore, it has been suggested that the ITT condition should be standardized as overnight fast in hospital [11]. Whether external factors are equally important with other provocative tests is not known.

The GH releasing hormone + arginine (GHRH + ARG) test is an alternative provocative test of GH secretion [1,2,12–14]. With appropriate cut-off limits the GHRH + ARG test is considered highly sensitive and specific, and it is safe and well tolerated [6,12]. With this test,

* Corresponding author at: Division of Endocrinology, Department of Medicine, Helsinki University Central Hospital, Sairaalakatu 1, Vantaa, PL 900, FI-00029 HUS, Finland. Tel.: +358 407512146.
E-mail address: tuula.pekkarinen@hus.fi (T. Pekkarinen).

1096-6374/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ghir.2013.06.002

cut-off of 9.1 μg/L is widely used [12–14]. Body mass index (BMI), waist circumference and age dependent cut-off values have recently also been introduced [15,16]. The GH Research Society statement suggests cut-off levels for the diagnosis of GHD for BMI <25 kg/m^2, a peak GH <11 μg/L; for BMI 25–30 kg/m^2, a peak GH <8 μg/L, and for BMI >30 kg/m^2, a GH peak <4 μg/L [1].

The objective of our study was to validate the GH cut-off limit for severe adult GHD with the ITT at our outpatient endocrine clinic. We determined peak GH after ITT in healthy subjects with two different immunometric GH assays, the AutoDELFIA (Perkin-Elmer Wallac) and Immulite 2000 (previously DPC, now Siemens). Participants with a pathological ITT were subjected to the GHRH + ARG.

2. Subjects

This study is a part of our prospective study aiming to improve routine practices and to validate the cut-off limits for growth hormone stimulation tests. The ITT and GHRH + ARG tests were performed at the Division of Endocrinology, Department of Medicine, Helsinki University Central Hospital (Helsinki, Finland). Seventy-three apparently healthy adults (37 males and 36 females) were recruited among medical students, hospital personnel and their relatives. No one used medications and none of the women received estrogen replacement. The study was approved by the Ethical Committee of Helsinki University Hospital, and all subjects gave written informed consent.

3. Methods

Standing height was measured and body weight was determined using calibrated balance beam scale. BMI was calculated as weight (kg) divided by the square of the height in meters. Of the 36 women 32 were menstruating: they were studied during the late follicular phase of the menstrual cycle (days 7–11). The subjects arrived at the hospital after an overnight fast, and they were asked to refrain from strenuous exercise on the morning of the tests. After a short clinical examination, intravenous (iv) cannulas were inserted in each arm for blood sampling and infusions, respectively, and the test was started at 7.30–10.30 a.m. The time interval between ITT and GHRH + ARG test was generally at least seven days but in five subjects it was between three to six days.

The ITT was performed by giving an iv bolus injection of regular human insulin, Actrapid (Novo-Nordisk, Copenhagen, Denmark) at a dose of 0.1–0.15 U/kg at time 0 (15–30 min after insertion of the cannula). Plasma glucose of 2.2 mmol/L or less with hypoglycemic symptoms was the target. Additional insulin boluses were administered if needed to reach this level if the clinician believed it to be safe. When the target was reached, the subjects ate a snack and/or were given iv glucose. The test was terminated earlier if the clinician judged it unsafe to continue due to the symptoms. Blood samples for GH measurements were drawn at 0, 30, 60, 90 and 120 min.

The GHRH + ARG test was performed according to Ghigo et al. [12]. The GHRH (1–29) (Geref; Serono, Rome, Italy) was given at a dose of 1 μg/kg as an iv bolus at time 0, and arginine (L-arginine monohydrochloride, Braun, Melsungen, Germany) at a dose of 0.5 g/kg (up to a maximal dose of 30 g) was infused from 0 to 30 min. Blood samples were taken at −15, 0, 15, 30, 45, 60, 75 and 90 min. Serum was separated by centrifugation and the samples were kept frozen for 4–6 weeks at 20 °C and then at −80 °C until analyzed.

The consensus cut-off limits of 5 μg/L and 3 μg/L have been established using RIAs with polyclonal antisera. These assays give approximately two fold higher concentrations than the AutoDELFIA assay [7]. We therefore used cut-off limit of 2.5 and 1.5 μg/L as well.

3.1. Assays and cut-off limits

All serum samples were assayed for GH by the AutoDELFIA (Wallac, Turku, Finland). Samples from 58 out of the 73 subjects were also analyzed by the Immulite 2000. The AutoDELFIA hGH is an immunofluorometric sandwich assay (IFMA) using two monoclonal antibodies recognizing 22-kDa GH. The Immulite 2000 (by Diagnostic Products Corporation, Los Angeles, CA during this study, since 2007 by Siemens) is an immunochemiluminometric sandwich assay (ICMA) using a monoclonal capture antibody and a polyclonal tracer antibody. The assay mainly recognizes 22-kDa GH and to a lesser extent 20-kDa hGH. Both assays were calibrated against the 1st IRP of hGH 80/505, in which 1 mg equals to 2.6 IU of hGH.

The analytical sensitivity of AutoDELFIA and Immulite 2000 was similar (0.01 μg/L). The lowest reported results were 0.04 μg/L for AutoDELFIA and 0.05 μg/L for Immulite 2000. Values below the lowest reported result were assigned a value half of that. The interassay coefficients of variation (CV) for the AutoDELFIA assay was 3.9% at 3.3 μg/L, 4.8% at 9.7 μg/L and 3.2% at 21.4 μg/L. For the Immulite 2000 assay the interassay CV was 3.0% at 2.2 μg/L, 4.9% at 4.9 μg/L and 4.8% at 12.5 μg/L.

Blood glucose was measured using a bed-side glucometer (One Touch Ultra, Life Scan). According to the manufacturer's announcement imprecision of the One Touch Ultra is 15%. Part of the samples were also analyzed by a hexokinase/glucose-6-phosphate dehydrogenase assay (Roche Diagnostics, Gluco-quant glucose/hexokinase, cat. no. 1876899) using a Hitachi Modular PP (Hitachi Ltd., Tokyo, Japan) automatic analyzer. The interassay coefficients of variation (Hitachi) were 2.3% at a glucose level of 4.7 mmol/L and 2.1% at the level of 14.9 mmol/L. The serum cortisol concentration was measured in samples of ten subjects by the Immuno-1 assay (Bayer Diagnostic).

4. Statistical analysis

The results are expressed as median values with a range. Spearman and Pearson analyses were used for correlations involving normally and non-normally distributed values, respectively. The Mann–Whitney U test was used to compare results between men and women. We performed statistical analyses with SPSS (Statistical Package for the Social Sciences). All tests were performed two-sided, and a P value of less than 0.05 was considered as statistically significant.

5. Results

5.1. ITT

The median age of the 73 healthy subjects was 35.0 years (range 20.2–57.5) and median BMI 23.6 kg/m^2 (range 19.7–30.3). Of the 73 subjects, 71% had BMI <25 kg/m^2, 27% had BMI 25–30 kg/m^2, and one man was mildly obese (BMI 30.3 kg/m^2). All subjects considered the ITT unpleasant due to the hypoglycemic symptoms, but no one experienced severe complications. Adequate symptomatic hypoglycemia (≤2.2 mmol/L) was achieved in 63 of the 73 subjects, while in ten subjects with hypoglycemic symptoms the nadir glucose was 2.3–2.8 mmol/L. Distribution of peak GH in the ITT with different cut-offs and assays is shown in Table 1. Using the AutoDELFIA assay and the consensus cut-off limits of 5 μg/L and 3 μg/L, 40% (25/63) and 56% (35/63) of the appropriately hypoglycemic subjects were correctly classified as GH sufficient, respectively, and with the cut-offs of 2.5 μg/L and 1.5 μg/L, 59% (37/63) and 70% (44/63), respectively. Subanalysis of lean subjects with BMI <25 kg/m^2 (n = 46) showed similar results: using cut-off limits of 5 μg/L and 3 μg/L, 46% and 59% of the appropriately hypoglycemic subjects were correctly classified, and with cut-offs of 2.5 μg/L and 1.5 μg/L, 63% and 72%, respectively. Furthermore, including only younger subjects under 35 years old with BMI <25 kg/m^2 (n = 24), 46%, 67%, 71% and 75% were classified as GH sufficient, respectively. The correlation between the AutoDELFIA and Immulite 2000 assays was excellent (r = 0.998, P < 0.001), but the GH results with Immulite 2000 were