ORIGINAL ARTICLE

Assessment of insulin-like growth factor-I serum concentration as a screening procedure in diagnosing children with short stature

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Abstract

OBJECTIVES: Insulin-like growth factor-I (IGF-I) secretion is growth hormone (GH) dependent. However the data on using IGF-I assessment as a screening procedure in diagnosing GH deficiency are not consistent. The goal of the study was an analysis of the relations between GH secretion in stimulating tests and plasma IGF-I concentration.

PATIENTS & METHODS: The analysis comprised 540 children with short stature in whom two standard GH stimulating tests (GHST) were performed, together with an assessment of plasma IGF-I concentration. The relationships between GH peak in both tests and IGF-I secretion were analysed.

RESULTS: There was no correlation either between GH peaks in different tests or between GH and IGF-I secretion in particular patients. Moreover, both the mean IGF-I concentration was similar in the patients with normal and subnormal results of GHST and the mean GH peak in GHST presented similar in the groups of children with normal and decreased IGF-I secretion.

CONCLUSIONS: Assessment of IGF-I secretion fails to be a screening procedure for the results of GHST. The lack of correlation between the results of two GHST should be taken into account when evaluating the significance of GHST and IGF-I assessment in diagnosing GH deficiency.

INTRODUCTION

The most frequent hormonal disorder leading to short stature is growth hormone (GH) deficiency (GHD) of different origin. The main peripheral mediators of both anabolic and mitogenic GH activities are insulin-like growth factors (IGFs), including the most important of them, insulin-like growth factor-I (IGF-I).

Undoubtedly, in children, GHD is the primary indication to long-term growth-promoting GH replacement therapy. However, some questions concerning the definition and diagnosis of GHD still exist. In 1995, Rosenfeld *et al*. [20] described in detail particular limitations in GHD diagnostics and pointed at the impossibility to establish the final diagnosis in some cases. It is well known that the standard element of diagnosing GHD is an assessment of GH secretion in stimulating tests. Additional diagnostic procedure is measurement of IGF-I and its binding protein-3 (IGFBP-3) serum concentrations, as well as assessment of other IGFs and IGFBPs secretion, if possible. Owing to the relatively high stability of IGF-I concentration, for the assessment of IGF-I secretion, a single morning blood sample is sufficient with no need for performing stimulating tests [10]. The obtained results should be interpreted with respect to patient's age and sex, in relation to adequate normative data. In patients with low IGF-I serum concentration, the possible reasons of that situation – unrelated to GHD – as liver diseases, hypothyrosis, malnutrition and/or malabsorption syndrome should be excluded [10,22]. Since, after exclusion of the above-mentioned diseases, IGF-I concentration reflects GH secretion, Moore *et al*. in 1982 [13] and Rasat in 1996 [16] proposed IGF-I serum concentration measurement as a screening procedure in diagnosing GHD. Similarly, Rosenfeld [19,18,17] postulated that IGF-I serum concentration assessment should be first step in diagnosing children with short stature. However, another studies pointed to the limited importance of IGF-I assessment in diagnosing GHD [22,9,15,21,23,7,2]. In 1999, Mitchell *et al*. [12] denied the possibility of predicting GH peak in stimulating tests on the basis of IGF-I secretion. It seems worth to underline, that – in all the quoted studies – the GH stimulating tests were considered as a "gold standard" in the assessment of GH secretion. On the other hand, the data exist that in children with normal results of GH stimulating tests, the more severe deficit of height correlated with lower IGF-I secretion [8]. Moreover, taking into account all the doubts, concerning the credibility of the results of GH stimulating tests, Badaru and Wilson [1] stated that decreased IGF-I secretion is no less reliable for confirmation GHD than decreased GH peak in stimulating tests. Similarly, Loche *et al*. [11] stressed that GHD diagnosis should not be based solely on the assessment of GH secretion in stimulating tests.

Taking into account the variety of reports, concerning the significance of IGF-I secretion measurement in GHD diagnostics, the goal of the study was an analysis of the relations between GH secretion in stimulating tests and serum IGF-I concentration. The obtained results should allow us to assess the clinical usefulness of IGF-I measurement as a screening procedure in diagnosing GHD in short children.

PATIENTS AND METHODS

The analysis comprised 540 children (373 boys and 167 girls), aged 11.7±3.2 years (mean±SD, range: 3.0–17.5 years) with short stature, diagnosed in our Department. The study was approved by the local Ethics Committee in Polish Mother's Memorial Hospital – Research Institute (Lodz, Poland).

The height of patients qualified to diagnostics was below 3rd centile for age and sex, with respect to current centile charts for Polish children [14], their height velocity (HV) was below 4 cm/year and bone age (BA) was delayed. All the children with disorders of nutrition, other diseases of gastrointestinal tract, as well as those with any chronic diseases and/or congenital defects of heart, kidneys or other organs that may disturb growing (by the effect on either GH or – particularly – IGF-I secretion) were excluded from the study. For every patient, height age (HA) was calculated as the age of child of the same height, growing on the level of 50th centile.

For the assessment of GH secretion, 2 standard stimulating tests – one with clonidine and another one with either insulin or glucagon (*i.e*. with hypoglycemia as a factor stimulating GH secretion) were performed in all the patients. Clonidine was administered orally in a dose of 0.75 mg/m^2, the blood samples were collected every 30 minutes from 0 to 120 minute of test. Insulin was administered in a dose of 0.1 IU/kg, *i.v*., the blood samples for GH measurement were collected in the same time points as in the test with clonidine, together with the assessment of glucose concentration to confirm hypoglcemia. Glucagon was administered in a dose of 30 μg/kg (not exceeding 1 mg), blood samples were collected for GH measurement were collected in 0, 90, 120, 150 and 180 minute of the test, glucose level was assessed every 30 minutes from 0 to 180 minute of the test. The tests with insulin and glucagon were repeated if there were no sufficient fluctuations of glucose concentration.

Serum GH concentration was measured by the two-site chemiluminescent enzyme immunometric assay (hGH IMMULITE, DPC) for the quantitative measurement of human GH, calibrated to WHO IRP 80/505 standard, with the analytical sensitivity up to 0.01 ng/ml, the calibration range up to 40 ng/ml, the sensitivity of 0.01 ng/ml, the intra-assay coefficient of variation (CV) of 5.3–6.5% and the inter-assay CV of 5.5–6.2%. The diagnosis of GHD was established in case of GH peak below 10 ng/ml in both tests, performed in the patient. Next, the patients with GHD were classified as having severe GHD (sGHD) when GH peak in both tests was below 5 ng/ml, while those with GH peak between 5 and 10 ng/ml – as ones with partial GHD (pGHD).

Serum IGF-I concentration was assessed in single blood sample, collected in 0 minute of 1st GH stimulating test, by a solid-phase, enzyme-labelled chemiluminescent immunometric assay, (IMMULITE, DPC), calibrated to WHO NIBSC 1st IRR 87/518, with analytical sensitivity 20 ng/ml, the calibration range up to 1 600 ng/ml, the intra-assay CV 3.1–4.3% and the inter-assay CV 5.8–8.4%. For every patient, IGF-I concentration was expressed as SDS for age and sex (IGF-I SDS).

Radiography of non-dominant hand and wrist was performed in all the children; patients' BA was assessed according to Greulich-Pyle stadards [3].

Compatibility of distribution of particular variable with normal distribution was assesses with Shapiro-Wilk's test. Some variables were transformed to obtain the normally-distributed form as follows:

- GH peak in both stimulating tests was expressed as logarithm of the highest GH level in both tests,
- serum IGF-I concentration was expressed as IGF-I SDS for age and sex.

For the variables that could not be transformed into normally-distributed forms, the non-parametric tests, either for 2 independent samples (U Mann-Whitney's test) or for dependent samples (Wilcoxon's test), were used in further analysis, where necessary.

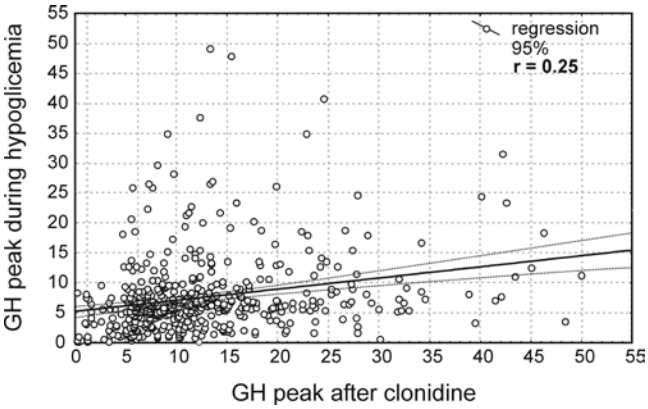

Figure 1. Correlation between GH peak in 2 stimulating tests, performed in particular patients.

Table 1. The number of patients with either decreased or normal GH peak in particular stimulating tests

		GH peak after clonidine		
		<10 ng/ml	≥10 ng/mL	total
GH peak after insulin/glucagon	<10 ng/ml	234	202	436
	≥10 ng/ml	32	72	104
	total	266	274	540

RESULTS

The comparison of GH peak in both stimulating tests, performed in particular patients, showed hardly weak correlation (r=0.025, p<0.05) between GH peak in the 1st and 2nd test (see Figure 1).

On the basis of GH peak in 2 stimulating tests, GHD was diagnosed in 234 patients (43.3%). The detailed data, concerning the number of children with either decreased or normal GH peak in particular tests was presented in Table 1.

Special attention was paid to the discrepancies between the results of 2 tests, performed in the same patient, *i.e.* normal (in some cases even high) GH peak in one of the tests, while decreased in another one. For example, among 85 patients with high GH peak in the test with clonidine (over 20 ng/ml), in 15 children GH peak in the 2nd test was very low (below 5 ng/ml). The above-mentioned data indicate a lack of correlation between the results of different GH stimulating tests, performed in the same patient. That phenomenon seems to be an important difficulty in searching the common screening procedure, allowing predict GH response in different stimulating tests.

The mean value of IGF-I SDS in the examined group of 540 children with short stature was 0.12±1.03. In 25 patients, IGF-I SDS peak was below –2.0. The cut-off for normal and decreased IGF-I secretion was the value IGF-I SDS n the level of –1.0. However, IGF-I SDS below –1.0 was found in only 92 patients (17.0%) of the examined group. Thus, the incidence of decreased IGF-I secretion was significantly lower (p<0.05) than the incidence of decreased GH peak in 2 stimulating tests (43.3%). In 240 patients (44.4%), IGF-I SDS was below 0, so the incidence of IGF-I SDS below 0 was similar to the incidence of GHD, diagnosed on the basis of the results of GH stimulating tests.

As the similar incidence of decreased GH peak in stimulating tests and IGF-I SDS below 0 was observed, it seems very important to assess the concordance between the results of both procedures. It should be underlined, that, if both examinations are reliable, the diagnosis of GHD in the patients with decreased GH peak in stimulating tests should be confirmed by decreased IGF-I serum concentration. For that reason, next analysis concerned the relations between GH peak in stimulating tests and IGF-I SDS. Only a weak correlation (r=0.23, p<0.05) was found between IGF-I SDS and GH peak in any of GH stimulating tests performed. Moreover, there was no correlation between IGF-I SDS and either GH peak in the test with clonidine (r=0.08, p<0.05) or the highest GH peak in both tests (r=0.12, p<0.05). The transformation of GH peak into logarithms did not improve the values of correlation coefficients between GH and IGF-I secretion. Similarly, calculating IGF-I SDS for either BA or HA and comparing the obtained values with both GH peak in stimulating tests and the logarithm of GH peak did not improve significantly the values of correlation coefficients between particular variables (see Table 2). In any of the analysed situations, the obtained correlation

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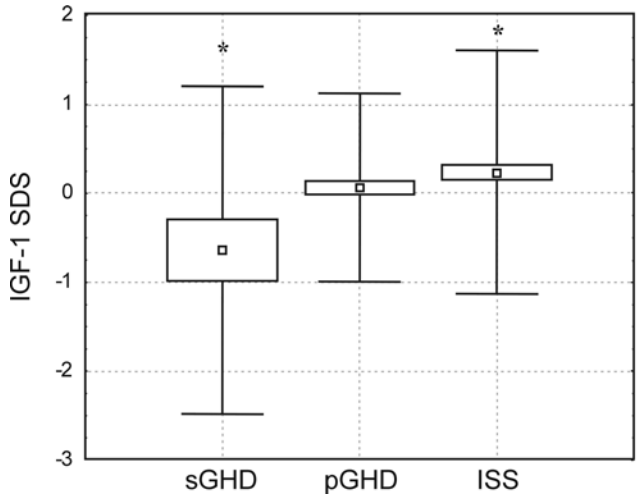

Figure 2. Serum IGF-I concentration in the patients with sGHD, pGHD and ISS; *p<0.05.

Table 2. Correlation coefficients between GH peak in stimulating tests and IGF-I SDS for CA, HA and BA

TEST		IGF-I SDS		
		for CA	for HA	for BA
log GH peak [ng/ml]	clonidine	0.17	0.26	0.28
	Insulin/ glucagon	0.26	0.33	0.30
	GH peak in both tests	0.17	0.32	0.34

presented too weak to make use of IGF-I assessment in screening for GHD, *i.e.* for identifying the patients, in whom decreased GH secretion in stimulation test is highly probable.

Moreover, among 233 patients, diagnosed as GH-deficient on the ground of decreased GH secretion in both stimulating tests, there were only 38 children (16.5%) with IGF-I SDS below –1.0, and 118 children (50.6%) with IGF-I SDS below 0. On the other hand, among 307 patients with normal GH peak, IGF-I SDS was below –1.0 in 54 children (17.5%) and below 0 in 122 children (39.7%). Thus, the difference between the incidence of decreased IGF-I secretion between the patients with normal and decreased GH secretion in stimulating tests was insignificant.

Next, all GH-deficient patients were classified into the subgroups with severe GHD (sGHS, n=44) and with partial GHD (pGHD, n=189). Further analysis comprised 3 groups of patients: sGHD, pGHD and ISS (idiopathic short stature). The analysis of IGF-I secretion in those groups showed that IGF-I SDS was –0.49±1.58 in sGHD, 0.02±1.11 in pGHD, and 0.27±1.38 in ISS, being significantly lower (p<0.05) in sGHD than in ISS, while another differences among the groups were insignificant (see Figure 2).

Finally all the patients were divided into the groups according to IGF-I secretion: Group A – IGF-I below the median value for age and sex (IGF-I SDS below 0, n=240), Group B – IGF-I over the median value (IGF-I SDS over 0, n=300). Growth hormone secretion was very similar in both groups (13.4±9.0 ng/ml in the Group A vs. 13.7±8.8 ng/ml in the Group B).

Our results indicate clearly that measurement of IGF-I serum concentration cannot be a screening procedure for preliminary identification the patients with decreased GH secretion in stimulating tests.

DISCUSSION

The results of our study indicate some doubts in interpretation both the results of GH stimulating tests and IGF-I assessment. It was found that the incidence of decreased IGF-I secretion is very similar in the patients with normal and decreased GH peak in stimulating tests. Moreover, the results of GH stimulating tests presented similar in the groups of patients with decreased and normal IGF-I serum concentration. It should be recalled that – in all the patients – other diseases leading to decreased IGF-I production despite normal GH secretion were excluded.

The algorithm of diagnosing GHD requires performing two different GH stimulating tests in every patient. This procedure is necessary due to the low credibility of the result of single test, as the low reproducibility of test results was documented [20]. The same problem was a subject of previous studies of our group. Hilczer at al. [4] pointed at a relatively high incidence of falsely decreased GH secretion in both stimulating tests, performed in the same, in fact not GH-deficient patients may lead to overdiagnosing GHD. It seems possible particularly in the patients with normal IGF-I secretion despite decreased GH peak in stimulating tests.

Furthermore, in some patients diagnosed as GH-deficient in childhood, GH secretion in stimulated tests repeated at final height may be normal, even with respect to "paediatric" criteria. For instance, in more than 70% of our patients GH peak in stimulated tests, performed after completion growth-promoting therapy, was normal (*i.e.* over 10 ng/ml) [5]. The above observation constitutes background for diagnosing – so-called – "transient" GHD. However, Van den Broeck *et al.* [24] stated that normalisation of the results of GH stimulating tests might be explained by either the real increase of GH secretion or the poor reproducibility of the results of tests. The authors lean towards the latter possibility, questioning the phenomenon of "transient" GHD.

In 2002, Loche *et al.* [11] reported normal GH secretion in most of the patients with a normal hypothalamic-pituitary in magnetic resonance in stimulating tests, with previously established diagnosis of GHD, when the same tests were repeated after 3–6 months. The authors

explained their observation by the possibility of early normalisation of GH secretion. Similarly, Hilczer *et al.* [6] observed normal GH secretion in stimulating tests in 60% of the patients, diagnosed previously as having idiopathic GH-deficient on the basis of the same tests. It seems particularly important that the reverse situation, *i.e.*, decreased GH secretion in stimulating tests repeated in the patients with previously normal results of stimulating tests, was observed in 37.5% of cases. Moreover, despite the divergences in the results of GH stimulating tests, IGF-I secretion remained quite stable, indicating the poor reproducibility of test results and speaking against the possibility of real normalisation (or deterioration) of GH secretion.

The main goal of searching for screening procedures is to avoid performing the onerous, expensive, and – sometimes – even risky GH stimulating tests in the patients in whom GHD may be excluded on the ground of less complicated examinations. The results of our study, similarly to other studies quoted above, point at the impossibility of using IGF-I assessment as a screening procedure allowing to predict the results of GH stimulating tests. It seems, however, that there is no evidence that the results of GH stimulating tests are more reliable than the assessment of IGF-I secretion, but some data suggests that the converse situation is possible. Such a possibility was supported by Badaru et Wilson [1] who stated that in diagnosing GHD, assessment of IGF-I secretion is no less reliable than the results of GH stimulating tests. The results of both current and previous studies of our research group [4,6] seem to confirm this conception.

Thus, although the measurement of IGF-I serum concentration might not be a screening procedure for GH secretion in simulating tests – especially in the patients with excluded organic abnormalities of hypothalamic-pituitary region – IGF-I assessment should not be recognised less reliable than GH stimulating tests. Even so, it seems that the studies on the effectiveness of GH therapy in the patients with decreased IGF-I secretion despite normal results of GH stimulating tests are necessary to resolve that problem.

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