CLINICAL STUDY

Can exaggerated response to a GH provocative test identify patients with partial GH insensitivity syndrome?

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Abstract

Objectives: In the majority of children with short stature, the etiology is unknown. Mutations of the GH receptor (GHR) have been reported in a few children with apparent idiopathic short stature (ISS). These patients had low IGF-I, IGF-binding protein-3 (IGFBP-3) and GH-binding protein (GHBP), but a normal or exaggerated GH response to provocative stimuli, suggestive of partial GH insensitivity (GHI). We attempted to identify children with partial GHI syndrome, based on their response to GH provocative stimuli and other parameters of the GH–IGF-I axis.

Subjects and methods: One hundred and sixty-four pre-pubertal children (97 boys, 67 girls) aged 7.2 (0.5–16.75) years were studied. All had short stature with height <3rd centile. The weight, bone age (BA) and body mass index (BMI) of the subjects, as well as the parents' heights and mid parental height (MPH) were assessed. Basal blood samples were taken for IGF-I, IGFBP-3 and GHBP. All subjects underwent a GH provocative test with either clonidine, arginine or insulin. The subjects were divided into three groups: (A) patients with peak GH concentration <18 mIU/l in two different provocative tests (GH deficiency – GHD, $n = 33$); (B) patients with peak GH between 18.2 and 39.8 mIU/l (normal response, $n = 78$); (C) patients with peak GH >40 mIU/l (exaggerated GH response, $n = 53$).

Results: No significant differences were found in age, height (standard deviation score (SDS)), parental height (SDS) and the difference between chronological age and bone age (Δ BA) between the groups. Patients with GHD were heavier ($P = 0.039$) and had significantly higher BMI (SDS) ($P = 0.001$) than the other groups. MPH (SDS) was lower in the group of exaggerated responders ($P = 0.04$) compared with the other groups. No significant differences were found between the groups for the biochemical parameters when expressed nominally or in SDS, except for IGFBP-3 (SDS), which was lower in the GHD group ($P = 0.005$). The GHBP levels were not lower in the group of exaggerated GH response to provocative stimuli. Height (SDS) correlated negatively with basal GH values in pooled data of all the subjects ($r = -0.358$, $P < 0.0001$), in normal responders ($r = -0.45$, $P < 0.0001$) and in the exaggerated responders ($r = -0.341$, $P < 0.0001$), but not in the GHD group.

Conclusion: Exaggerated GH response to provocative tests alone does not appear to be useful in identifying children with GHI.

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Introduction

The cause of short stature in children is unknown in the majority of cases. Growth hormone deficiency (GHD) defined by low peak GH response to two different GH provocative tests is diagnosed relatively rarely – diagnosis is made in 1:3500 patients (1). Laron syndrome, known also as GH insensitivity syndrome (GHI), was described initially in families of Oriental Jewish origin (2). In contrast to GHD patients, these children had elevated basal GH concentration and an

exaggerated peak GH in response to provocative tests (2, 3). GH receptor (GHR) defect was revealed to underline Laron syndrome (4). GHI patients have in addition to elevated basal GH concentration, low insulin-like growth factor-I (IGF-I), IGF-binding protein-3 (IGFBP-3) and GH-binding protein (GHBP) serum concentration (5–7). The low serum GHBP reflects the GHR defect, as it arises primarily from proteolytic cleavage of the extracellular domain of the GHR. Since the description of the human GHR gene in 1987 (8, 9), more than 30 distinct mutations have been identified in patients with

clinical Laron syndrome (10–14). Recently, heterozygosity for the GHR mutations have been reported in children with seemingly idiopathic short stature (ISS) (15–19). These children had a normal or exaggerated GH response to provocative stimuli, low IGF-I levels and low GHBP, suggesting partial GHI.

It has been speculated that GHR gene mutations may account for up to 5% of all ISS patients (18). Carlsson *et al.* have demonstrated that some short children have low levels of serum GHBP, suggesting that these children could have a defect in the GHR (21). Cumulatively, these findings suggest that GHR defect exists not only in patients with classical Laron syndrome, but it may exist in some individuals previously described as ISS (22, 23). Several attempts have been made to find biochemical parameters that can identify children in whom apparent short stature could be explained by partial GHI (24–27). In the present study we have compared three group of children with short stature, according to their response to a GH provocative test: (A) GHD, (B) children with a normal response and (C) children with exaggerated peak GH. We have hypothesized that children with exaggerated GH response to provocative stimuli may have the characteristics of partial GHI.

Subjects and methods

Subjects

One hundred and sixty-four pre-pubertal children (97 boys, 67 girls), age range 0.5–16.75 years (mean 7.2), were studied. All had short stature with height <3rd centile (28).

Anthropometry

All patients were assessed in our outpatient Pediatric Endocrine Unit for short stature. Height and weight were expressed as standard deviation scores (SDS) for chronological age (CA) (26). Bone age (BA) was assessed using the Gruelich & Pyle method (29). Body mass index (BMI) was determined by dividing weight by height² (kg/m²). BMI was expressed also by SDS for age (30), as well parental heights and mid parental height (MPH) expressed as SDS (28).

Investigation protocol

All patients underwent a GH provocative test with either clonidine (0.125 mg/m²), arginine (0.5 g/kg) or insulin (0.1 U/kg). Blood samples were taken at baseline for GH, GHBP, IGF-I and IGFBP-3 levels, and at regular intervals during the tests for GH measurements. Patients with a low GH peak (<18 mIU/l) underwent another GH provocative test using different stimuli as indicated for the diagnosis of GHD. Subjects were divided into three groups on the basis of the

peak GH level: group A – peak GH <18 mIU/l in two different provocative tests indicating GHD, according to the criteria that were adopted by the Ministry of Health in Israel; group B – peak GH between 18.2 and 39.8 mIU/l, indicating normal responders; and group C – peak GH >40 mIU/l, designated as an exaggerated GH response. Members of the last group were considered as candidates for partial GHI syndrome.

Assays

GH was measured using polyclonal RIA (Sorin, Sallugia, Italy). IGF-I was measured by RIA (DSL, TX, USA) after extraction and standardized for age and sex with the normative data provided. IGFBP-3 was measured by RIA (DSL) standardized for age and sex with the normative data provided. GHBP was measured by size exclusion chromatography (31) and expressed relative to that of an adult reference serum (% relative specific binding, %RSB) and was standardized for age by the normative data established from a total of 200 subjects in our own laboratory.

Statistical analysis

ANOVA was used to assess potential differences among groups (GHD, normal responders and exaggerated responders). When this analysis indicated statistically significant differences, the *post hoc* test was used to indicate which group differed. Linear relationships between baseline biochemical parameters were assessed using Pearson correlation. The threshold of significance was set at a *P* value ≤0.05.

The non-parametric Kruskal–Wallis test was used in cases of non-normal distributions. This study was approved by the institutional review boards and by the Ministry of Health.

Results

Population characteristics

Thirty-three patients were diagnosed as having GHD with the following etiologies: 15 patients – isolated GH deficiency; five patients – multiple pituitary hormone deficiency (MPHD); one patient – post acute lymphocytic leukemia (ALL); four patients – pre-pubertal GHD; and eight patients – biochemical GHD (low peak GH response in two different stimulus with growth rate within the normal range).

Seventy-eight patients had normal peak GH response and 53 had exaggerated peak GH response.

Clinical results

The anthropometric parameters of the three groups are presented in Table 1. No significant differences were found between the groups in age, height (standard

Table 1 Summary of the auxological parameters in the three groups (mean $\pm$ S.D.s).

	GHD (n = 33)	Normal response (n = 78)	Exaggerated response (n = 53)	All patients (n = 164)
Age (years)	8.15 $\pm$ 4.1	6.64 $\pm$ 3.4	7.4 $\pm$ 3.3	7.2 $\pm$ 3.6
Δ BA (years)	-2.17 $\pm$ 1.4	-2.2 $\pm$ 1.2	-2.39 $\pm$ 1.2	-2.26 $\pm$ 1.2
Weight (SDS)	-1.52 $\pm$ 1.22*	-1.95 $\pm$ 0.94	-2.07 $\pm$ 0.80	-1.9 $\pm$ 0.97
Height (SDS)	-2.76 $\pm$ 0.87	-2.87 $\pm$ 0.76	-2.71 $\pm$ 0.70	-2.8 $\pm$ 0.76
BMI (SDS)	1.07 $\pm$ 2.03**	0.27 $\pm$ 1.39	-0.29 $\pm$ 1.39	0.25 $\pm$ 1.60
Father height (SDS)	-0.87 $\pm$ 1.04	-1.3 $\pm$ 1.67	-1.36 $\pm$ 1.19	-1.24 $\pm$ 1.43
Mother height (SDS)	-0.88 $\pm$ 1.24	-1.24 $\pm$ 1.0	-1.5 $\pm$ 1.11	-1.26 $\pm$ 1.10
MPH (SDS)	-0.86 $\pm$ 0.84	-1.27 $\pm$ 1.10	-1.47 $\pm$ 0.97*	-1.26 $\pm$ 1.03

* $P = 0.039$, ** $P = 0.001$.

SDS, standard deviation score.

deviation score (SDS)), Δ BA and parental height (SDS). Patients with GHD were heavier ($P = 0.039$) and had significantly higher BMI (SDS) ($P = 0.001$). MPH (SDS) was lower in the group of exaggerated response ($P = 0.04$). Maternal height was also lower in this group, but the difference between the groups was not significant.

Biochemical results

Comparison of the biochemical parameters between the groups is summarized in Table 2. No significant differences were shown between the groups for any of the biochemical parameters (basal GH, IGF-I, IGF-I (SDS), IGFBP-3, GHBP (%RSB) and GHBP (SDS)), except for lower IGFBP-3 (SDS) in the GHD group ($P = 0.005$). The patients' height, expressed as SDS, was negatively correlated with basal GH values (the first sample in the GH provocative test), but not with any other auxological parameters. Basal GH was negatively correlated with height in the whole population ($r = -0.358$, $P < 0.0001$), in the normal responders ($r = -0.45$, $P < 0.0001$) and in the exaggerated response group ($r = -0.341$, $P < 0.0001$), but not in the GHD group. IGF-I was correlated positively with IGFBP-3 expressed in units and as SDS ($r = 0.578$, $P < 0.0001$; $r = 0.432$, $P < 0.0001$ respectively). This correlation was even stronger in the GHD group ($r = 0.743$, $P < 0.0001$). Basal GH in girls was

higher (3.8 ± 4.8) compared with boys (2.05 ± 2.5) ($P = 0.003$). In order to identify children suspected to have partial GHI, we have divided the patients into two sub-groups: children with GHBP lower than -2 S.D. from the mean and children with GHBP above -2 S.D. from the mean. Comparing between these two groups for the auxological and hormonal parameters indicated no difference. A similar division of the total population by the results of IGFBP-3 is presented in Table 3. Since IGFBP-3 was the only biochemical parameter that was significantly different between the groups, we have divided the patients according to the IGFBP-3 results; above or less than -2 S.D. Patients in the group with IGFBP-3 below 2 S.D. from the mean were younger, with lower peak GH and lower IGF-I. All other auxological and biochemical parameters were not different.

Discussion

It has been suggested that GHI due to mutations of the GHR gene may explain the etiology of short stature in about 5% of patients (18). An attempt to find biochemical criteria to identify children who may have GHI has been made. Attie *et al.* (25) suggested that decreased levels of GHBP with elevated 24-h GH secretion and lower IGF-I in sub-group of children with ISS may indicate that these patients may have defects in the GHR. This had been attributed to the fact that GHBP is the

Table 2 Summary of the biochemical parameters in the three groups. Values are means $\pm$ S.D.s.

	GHD	Normal response	Exaggerated response	All patients
Basal GH (mIU/l)	4.18 $\pm$ 4.26	5.94 $\pm$ 6.88	5.94 $\pm$ 9.68	5.66 $\pm$ 7.6
Peak GH (mIU/l)	7.6 $\pm$ 5.38	27.2 $\pm$ 7.04	65.8 $\pm$ 24.62	37.0 $\pm$ 26.44
IGF-I (ng/ml)	72.9 $\pm$ 102	65.4 $\pm$ 54	78.3 $\pm$ 64.4	71 $\pm$ 67.4
IGF-I (SDS)	-1.43 $\pm$ 0.63	-1.17 $\pm$ 0.67	-1.06 $\pm$ 0.79	-1.18 $\pm$ 0.71
IGFBP-3 (ng/ml)	1916.5 $\pm$ 1253	2240 $\pm$ 1137	2290 $\pm$ 722	2202 $\pm$ 1050
IGFBP-3 (SDS)	-1.99 $\pm$ 1.30*	-1.09 $\pm$ 1.21	-1.13 $\pm$ 0.87	-1.26 $\pm$ 1.16
GHBP (%)	52.6 $\pm$ 25.4	41.2 $\pm$ 17.4	43.7 $\pm$ 17.6	44.0 $\pm$ 19.2
GHBP (SDS)	-0.9 $\pm$ 1.0	-1.27 $\pm$ 0.8	-1.23 $\pm$ 1.0	-1.2 $\pm$ 0.9

* $P = 0.005$.

SDS, standard deviation score.

Table 3 Comparison between two sub-groups of children according to their levels of IGFBP-3.

	IGFBP-3 < - 2 s.d. (n = 29)	IGFBP-3 > - 2 s.d. (n = 108)
Age (years)	5.5*	7.7
Peak GH (ng/ml)	21.96*	38.6
IGF-I (SDS)	-1.71**	-1.03

* $P = 0.003$, ** $P < 0.0001$.

extracellular part of the GHR and it reflects the condition of the GHR. Cotterill *et al.* (27) demonstrated that IGF-I generation test can not be used for identifying children with GHI syndrome. Blum *et al.* (24) have used clinical and biochemical scores using the following criteria: high peak stimulated GH level, low IGF-I (SDS), and low GHBP (SDS).

In the present study we measured peak GH levels in response to GH provocative test, assuming those children with exaggerated GH response may have partial GHI. Comparing the auxological parameters in the three groups of patients according to their response to GH provocative tests, we were not able to find any significant differences in height between the groups. The finding of lower MPH (SDS) and shorter mothers in patients with exaggerated GH response may point to a major genetic component in this group. It may also indicate that maternal height is more influential on the child's height than paternal height. Comparing the biochemical parameters between the three groups, we were not able to show any unique characteristics of the group of exaggerated GH response. The only significant difference between the groups was lower IGFBP-3 (SDS) in the GHD group. This finding may point to a possible future use of IGFBP-3 in the screening of children with short stature for the diagnosis of GHD instead of using GH provocative tests. The levels of GHBP were not different between the groups and could not be used for identifying patients with partial GHI syndrome. In addition, no differences were found by dividing the patients into two sub-groups according to their levels of GHBP (SDS) (below or above -2 s.d.). Our finding is in contrast to those of Carlsson *et al.* (21), but in agreement with Attie *et al.* (26), who have shown that GHBP levels are GH independent and do not predict responses to GH therapy. These findings may be explained by the fact that patients with GHI due to mutations in the trans membrane and in the intracellular domain of the GHR have high levels of GHBP in contrast to patients with mutations in the extracellular domain which have low GHBP levels (32). The finding of negative correlation between height and basal GH values in all patients and in the normal and exaggerated response groups suggests that some degree of insensitivity to GH exists in patients with ISS.

In summary, our study has shown that GH levels in response to provocative test alone could not be used for identifying patients with GHI syndrome.

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