

Assessing the adrenal axis by the glucagon stimulation test in children with idiopathic growth hormone deficiency

Ocena osi podwzgórze–przysadka–nadnercza za pomocą testu stymulacji glukagonem u dzieci z idiopatycznym niedoborem hormonu wzrostu

¹Ach Taieb, ¹Ben Abdelkarim Asma, ¹Hasni Yosra, ¹Maaroufi Amel, ¹Kacem Maha, ¹Chaieb Molka, ²Zaouali Monia, ¹Ach Koussay

¹Department of Endocrinology, University Hospital of Farhat Hached Sousse, Tunisia

²Department of Physiology, University Hospital of Farhat Hached Sousse, Tunisia

Abstract

Approximately 30% of children with idiopathic growth hormone deficiency (IGHD) also suffer from other pituitary hormone deficiencies. Of children with IGHD, approximately 10% are unable to generate appropriate ACTH levels in response to stress. This study was prospectively designed to test the integrity of the adrenal axis in patients with an established diagnosis of IGHD using the glucagon stimulation test (GST). The study population comprised 39 patients with established childhood-onset IGHD. The diagnosis of GH deficiency was established on the basis of failure of GH to increase over 10 ng/ml after two stimulation tests. The GST was performed by intramuscular injection of 1 mg glucagon. The criteria followed to define adrenal deficiency was cortisol less than 167 ng/l in response to GST. The mean peak blood glucose level was 8.64 ± 1.71 mmol/l. Analysing the cohort using the cut-off of 167 ng/ml to define adrenal insufficiency under GST, there were 25.64% of children diagnosed: 20% among males and 35.7% among females. Subjects with GH and ACTH deficiency had a mean peak GH of 2.07 ± 1.79 ng/ml – significantly lower than GH peak of children with IGHD alone ($p < 0,001$). The frequency of children with combined somatotroph and corticotroph deficiencies with a GH peak < 3 ng/ml was 21% ($p < 0,001$). The current study identified a prevalence of adrenal insufficiency of 25.64%, which could predict greater risk for children if untreated, especially because a substantial proportion of patients do not present clinical symptoms.

Key words:

growth hormone deficiency, idiopathic growth hormone deficiency, glucagon stimulation test, adrenal insufficiency, cortisol, GH, children.

Streszczenie

Około 30% dzieci z idiopatycznym niedoborem hormonu wzrostu (IGHD) cierpi również z powodu innych niedoborów hormonów przysadki mózgowej. Około 10% dzieci z IGHD nie jest w stanie wytworzyć odpowiednich stężeń ACTH w odpowiedzi na stres. Prospektywnym celem niniejszej pracy było zbadanie integralności osi podwzgórze–przysadka–nadnercza u pacjentów z ustalonym rozpoznaniem IGHD przy użyciu testu stymulacji glukagonem (GST). Badaną populację stanowiło 39 pacjentów z rozpoznaną IGHD w wieku dziecięcym. Rozpoznanie GH-deficytu ustalono na podstawie dwukrotnej odpowiedzi deficytowej (GH poniżej 10 ng/ml) w teście stymulacji. Stymulację glukagonem przeprowadzono przez domięśniową iniekcję 1 mg glukagonu. Kryteriami stosowanymi w celu określenia niedoczynności kory nadnerczy było stężenie kortyzolu poniżej 167 ng/l w odpowiedzi na GST. Średnie szczytowe stężenie glukozy we krwi wynosiło $8,64 \pm 1,71$ mmol/l. Analizując kohortę przy użyciu wartości odcięcia 167 ng/ml w celu określenia niewydolności kory nadnerczy w ramach GST, zdiagnozowano 25,64% dzieci: 20% płci męskiej i 35,7% płci żeńskiej. Pacjenci z niedoborem GH i ACTH mieli średni szczyt GH $2,07 \pm 1,79$ ng/ml znacząco niższy niż szczyt GH u dzieci z samym IGHD ($p < 0,001$). Dzieci z łączonymi defektami somatotroficznymi i korykotropowymi ze szczytem GH < 3 ng/ml stanowiły 21% ($p < 0,001$). Stwierdzono występowanie niewydolności nadnerczy u 25,64%, co może prognozować większe ryzyko dla dzieci, jeśli pozostaną one nieleczone, zwłaszcza że znaczna część pacjentów nie ma objawów klinicznych.

Słowa kluczowe:

niedobór hormonu wzrostu, idiopatyczny niedobór hormonu wzrostu, test stymulacji glukagonem, niewydolność kory nadnerczy, kortyzol, GH, dzieci.

Introduction

Growth hormone deficiency (GHD) occurs in approximately one in 3000 to one in 4000 children and is caused by a heterogeneous group of abnormalities [1]. Most cases are classified as idiopathic GHD (IGHD), which is thought to result from abnormal hypothalamic production of GHRH or from disturbed pituitary secretion of GH [2]. Other aetiologies of GHD include central nervous system tumours, brain malformations, cerebral surgery, or radiation therapy. Growth hormone therapy has been used for years and has proven efficacy [3].

Approximately 30% of children with IGHD also suffer from other anterior pituitary hormone deficiencies [2]. Of children with IGHD, approximately 20% have insufficient pituitary TSH production, and 10% are unable to generate appropriate ACTH levels in response to stress [4, 5]. The literature suggests that it is extremely rare for children with GHD to have ACTH deficiency without concomitant TSH deficiency or an organic brain lesion [6].

However, although clinically asymptomatic [7], the hypothalamic–pituitary–adrenal axis (HPA) of these patients cannot appropriately react to stressful stimuli, with potentially life-threatening consequences. Thus, evaluation of the integrity of the HPA is essential in patients with GHD.

Although the diagnosis of overt adrenal failure is generally straightforward, identification of those asymptomatic patients with subtle dysfunction of the HPA axis is still a diagnostic challenge. The HPA axis response to insulin hypoglycaemia (ITT) is still considered the gold standard in the evaluation of suspected adrenal insufficiency [8]. Furthermore, ITT is contraindicated in infants, especially those with a history of seizures [9]. Patients with multiple pituitary hormone deficiencies experienced severe hypoglycaemia during ITT, and occasional deaths in children have been reported with the use of such tests [10]. Therefore, alternative tests to evaluate the HPA have been proposed, such as the glucagon stimulation test (GST) [11]. The GST allows the evaluation of the somatotrophic axis, such as insulin hypoglycaemia, but with fewer and less adverse effects [12]. The usefulness of the glucagon test has been evoked for a long time, but its accuracy in the evaluation of the corticotrophic axis must be determined according to the teams and methods of testing and dosing [13].

We have recently identified the cortisol cut-off under GST in a prospective cohort, so it may lead us to identify the adrenal insufficiency in this current study [14].

This study was prospectively designed to test the integrity of the adrenal axis in patients with an established diagnosis of IGHD using GST.

Material and methods

Patients

The study population comprised 39 patients with established childhood-onset IGHD. All the children were in a prepubertal state and did not have any medical history.

The diagnosis of GHD was established on the basis of failure of growth hormone (GH) to increase over 10 ng/ml after two stimulation tests (ITT and GST) [4]. In all patients, heights were under -2 standard deviations (SD), and insulin-like growth factor-1 (IGF-1) concentrations were lower than those of age-matched normal subjects.

Patients with any diagnoses that could possibly explain their GHD, such as brain tumour, cranial irradiation, hydrocephalus, Prader-Willi syndrome, chromosomal abnormality, and periventricular leukomalacia were excluded. All patients underwent MRI to exclude any pituitary abnormalities.

Evaluation of HPA by dynamic tests

The ITT and GST were performed on separate days after an overnight fast. Glucocorticoids were discontinued at least 24 h before testing. The maximum interval between the two dynamic tests was seven days. Further assessment of anterior pituitary function was done by baseline hormonal testing as well as by provocative tests as required.

The GST was performed by intramuscular injection of 1 mg glucagon. Blood samples for measurement of cortisol and glycaemia were obtained in the basal state and 30, 60, 90, 120, 150, 180, and 210 minutes after glucagon injection. All patients were asked to report side-effects associated with this test, and anti-emetics were administered at the discretion of the supervising physician.

The criterion generally followed to define adrenal deficiency during this time period was a cortisol of less than 167 ng/l in response to GST [14]. First morning cortisol and ACTH levels were not used for diagnosis due to high variability.

Assessment of hormone levels

Serum GH levels were measured using the IRMA method with a commercially available kit (Beckman Coulter) with a sensitivity of 0.1 ng/ml; intra-assay and inter-assay coefficient variations were 1.5% and 14%, respectively.

Serum cortisol levels were measured using the radioimmunoassay method with a commercially available kit (Beckman Coulter) with a sensitivity of 7 ng/ml; intra-assay and inter-assay coefficient variations were 2.8% and 5.3%, respectively.

Statistical analysis

Statistical analysis was performed using the IBM SPSS Version 23.0 (IBM Inc) program. Correlation analysis was conducted with Pearson's and Spearman's analysis for parametric and nonparametric variables, respectively. Chi-square tests were used for qualitative variables. Data were expressed as mean \pm standard deviation. A p value < 0.05 was considered as statistically significant.

Results

At evaluation the mean age of the 39 children was 9.74 ± 2.65 years (extremes: 6 years and 14 years); 25 boys (64.1%) and 14 girls (35.9%). The age of the break in the growth curve in children was 10.43 ± 2.78 years, with a mean bone age of

10.03 ±2.94 years. The mean height was 154.33 ±4.22 cm, with a standard deviation of -2 SD for 53.3% and -3 SD for 46.7%. The variabilities between genders are shown in Table I.

Cortisol evaluation

The mean peak blood glucose level was 8.64 ±1.71 mmol/l and was found most frequently (69.2%) during the 30th minute ($p < 0,001$; Table II). The mean glycaemic nadir was 3.75 ±0.92 mmol/l and was most frequently found during the 120th minute ($p < 0,001$). The mean baseline cortisol level was 97.33

±45.62 ng/ml with a peak level of 179.1 ±75.05 ng/ml, most commonly occurring (43.6%) in the 180th minute ($p < 0,001$). Analysing the cohort using the cut-off of 167 ng/ml to define adrenal insufficiency under GST, there were 25.6% of the children diagnosed: 20% among males and 35.7% among females (Table I).

Growth hormone evaluation

The mean GH peak was 4.86 ±4.09 ng/ml and was found most frequently (28.2%) during the 120th minute ($p = 0.02$). Children with a partial response (GH > 5 ng/ml) comprised 51.3%.

Table I. Clinical characteristics and comparison of tests between genders (mean ±SD values)

	Males (<i>n</i> = 25)	Females (<i>n</i> = 14)	<i>p</i> value
Clinical characteristics			
Age (years)	9.48 ±2.64	10.21 ±2.69	0.09
Height (-SD)	165 ±3.66 (-3.32 ±0.47)	149 ±9.79 (-3.75 ±0.88)	< 10 ⁻³
Body mass index	19.56 ±4.24	21.28 ±5.59	0.08
Bone age (-SD)	9.88 ±3.24 (-1.5 ±1.3)	11 ±2.60 (-1.4 ±1.1)	0.06
Glucagon stimulation test			
Peak cortisol < 167 ng/ml (%)	5 (20%)	5 (35.7%)	0.1
Basal cortisol (ng/ml)	102.80 ±43.80	87.57 ±48.81	0.04
Peak cortisol (ng/ml)	218.2 ±69.13	177.21 ±90.89	0.01
Delta cortisol increment (ng/ml)	111.4 ±59.60	89.29 ±61.73	0.05
Peak GH (ng/ml)	5.44 ±4.17	3.82 ±3.87	0.04

Table II. Mean glycaemic (mmol/l) and cortisol levels (ng/ml) and peak frequencies by time during GST in the study population

Time (minutes)	T0	T30	T60	T90	T120	T150	T180	T210
Glycaemic means (mmol/ml)	5.03 ±0.66	8.29 ±1.31	7.54 ±2.34	5.67 ±2.39	4.58 ±1.69	4.39 ±1.34	4.5 ±1.06	4.47 ±1.03
Hyperglycaemic peak frequency, <i>n</i> (%)	0	27 (69.2)	11 (28.2)	1 (2.6)	0	0	0	0
Hypoglycaemic peak frequency, <i>n</i> (%)	0	0	0	5 (12.8)	13 (33.3)	9 (23.1)	5 (12.8)	7 (17.9)
Cortisol means (ng/ml)	97.33 ±45.62	110.79 ±61.52	105.71 ±67.81	106.58 ±58.49	128.33 ±59.14	161.66 ±70.36	179.1 ±75.05	164.82 ±67.78
Cortisol peak frequency, <i>n</i> (%)	0	2 (5.1)	1 (2.6)	1 (2.6)	2 (5.1)	10 (25.6)	17 (43.6)	6 (15.4)

Table III. Frequencies of GH peak in children with IGHD only and children with combined somatotroph and corticotroph deficiencies

GH (ng/ml)	GH 9.9-5.0	GH 4.9-3.0	GH < 3.0
IGHD only, <i>n</i> (%)	17 (43.58%)	4 (10.25%)	8 (20.51%)
IGHD + adrenal insufficiency, <i>n</i> (%)	1 (2%)	1 (2%)	8 (21%)

Subjects with IGHD alone had a mean peak GH of 5.82 ± 4.24 ng/ml, and 43.58% had a GH peak > 5 ng/ml (*p* < 0,001).

Subjects with GH and ACTH deficiency had a mean peak GH of 2.07 ± 1.79 ng/ml – significantly lower than GH peak of children with IGHD alone (*p* < 0,001). The frequency of children with combined somatotroph and corticotroph deficiencies with a GH peak < 3 ng/ml was 21% (*p* < 0,001; Table III).

Discussion

As yet, few works have studied the association of adrenal insufficiency in the diagnosis of IGHD in children. Therefore, no specific recommendations exist regarding additional evaluation of anterior pituitary function in children diagnosed with IGHD. In the most common cases, the pituitary screening in short stature aims to diagnose hormonal causes of the growth break. Exploring the GHD is based on an inadequate response of the hormone to provocation by ITT or GST [6, 7].

The usefulness of the GST in the evaluation of the corticotrophic axis has been evoked for a long time [11], and its accuracy leaves no doubt in view of the many international studies that validate and recommend it [15]. Due to its mild side effects and the possibility of coupled dosing with GH, the glucagon test is a valid alternative for a contraindication to the ITT. The physiological mechanisms by which glucagon stimulates the cortisol secretion are still poorly explained [12, 16]. Glycaemic fluctuation, which stimulates the synthesis of cortisol during the late fall in glycaemia, is among the most frequently mentioned hypotheses [17]. The cortisol peak after glucagon was observed at the 180th min, and blood the glucose peak recorded in our patients was at 30 minutes after glucagon followed by a decline in glucose concentrations at 120 minutes, as in several other studies [18-20].

Some studies have documented that a significant percentage of children with IGHD also have other anterior pituitary hormone deficiencies [2]. In adults, gonadotropin deficiency occurs as the second most common hormonal deficiency [21]. In our study, adrenal insufficiency was found in 25.6% of our cohort, which is similar to the results found by Tenenbaum *et al.* [22]. One of the most important studies reviewed 1366 children with IGHD; 11% had ACTH deficiency [4]. Overall, 33% of the children were deficient in one or more hormones other than GH. In previous studies made by Rona and Maghnie *et al.*, the prevalence of ACTH deficiency was between 6 and 26% [5, 23]. Exploring the adrenal axis in the diagnosis of GHD

appears to be important, knowing that later on, in adulthood, those patients are at risk of developing other pituitary abnormalities [24]. A recent publication that examined a small number of adults with a history of childhood-onset IGHD found that 44% of those retested had a subnormal cortisol response to ITT [25]. This emphasises the interest of using a reliable test in assessing the adrenal axis in childhood.

In the understanding of this association between adrenal and somatotroph deficiencies, genetics plays a fundamental role, knowing that radiologic imaging may be normal. Most cases of IGHD and hypopituitarism are still considered sporadic, and genetic causes are being identified with increasing frequency. The developmental cascade that leads to normal pituitary development and function use some regulatory genes leading to the differentiation of the corticotroph cell line, which occurs fairly early during development, whereas somatotrophs differentiate near the end of pituitary development [26]. Mutations in the genes encoding the *HESX1*, *PROP1*, and *PIT1* developmental transcription factors are associated with combined pituitary hormone deficiency diseases [27]. This sustains the hypothesis that ACTH deficiency is unlikely in the setting of normal pituitary anatomy with a normal TSH secretion [28].

In our study, we tried to explore the variability of gender and age on cortisol secretions. Our findings were that male patients had a higher cortisol peak and increment compared to female patients. Those findings are similar to other works aiming to study cortisol responses to GST with a comparison by gender and sex. In the same study, they demonstrated an inverse relation between age and cortisol response, suggesting that the use of a single age threshold across the entire paediatric age group is inappropriate, and that age and gender should be factors to be considered when interpreting the results of stimulation tests of the HPA axis in children [22]. Sexual dimorphism has been reported previously with respect to cortisol production and metabolism in adolescents [29] and older adults [30], with most adult studies showing higher levels in males as demonstrated in our cohort. However, we still need larger cohorts to consider interpretation of cortisol secretion considering gender and age.

Conclusions

Children with IGHD display a significant risk of developing additional pituitary deficiencies at the onset of diagnosis. The absence of structural pituitary abnormalities does not exclude

the occurrence of additional pituitary deficits. Adrenal insufficiency can silently evolve years after the onset of IGHD if the correct cortisol cut-off is not determined. The current study identified a prevalence of adrenal insufficiency of 25.64%, which could predict greater risk for children if untreated, especially because a substantial proportion of patients do not present clinical symptoms. Based on the above considerations, our adrenally insufficient patients in whom ITT was contraindicated

could be safely identified with the GST, which allows the concomitant exploration of both adrenal and somatotroph axis. Longer duration of follow-up is associated with a higher risk of additional pituitary deficits, suggesting that hypopituitarism is a dynamic condition where new deficiencies can appear years after the initial diagnosis. Thus, it remains important to repeatedly look for additional pituitary hormone deficiencies.

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