Comparison of Diagnostic Values of Growth Hormone Stimulation Tests in Adolescents

Adolesanlarda Büyüme Hormonu Stimülasyon Testlerinin Tanısal Değerlerinin Karşılaştırılması

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Abstract

Objective: Growth hormone (GH) stimulation tests are used in the diagnosis of GH deficiency, therefore, various pharmacological agents are applied for this aim. Since there is no gold standard, usually two tests are performed. We aimed to investigate clinical utilities of four stimulation tests (insulin tolerance test (ITT), clonidine, L-dopa and glucagon) in adolescents with severe short stature.

Materials and Methods: Any two or three of four stimulation tests were performed to forty-three subjects (32 males, 11 females) aged 11-20 years with severe short stature. Different numbers of tests were applied to subjects (ITT to all, L-dopa to 32, clonidine to 21 and glucagon to 12 subjects). Responses to clonidine, L-dopa and glucagon tests were compared with response to ITT. In addition, sensitivity, specificity and positive predictive value (PPV) and negative predictive value (NPV) of four tests were calculated. For these analyses, patients with no response to two tests were classified as having growth hormone deficiency (GHD).

Results: There were no significant differences in the responses of the three tests when compared with ITT. Glucagon test had 100% sensitivity, specificity, PPV and NPV. Of the other three tests, the specificity (90%) and the PPV (85%) of clonidine test were highest. On the other hand, sensitivities and NPV of ITT (91% and 86% ,respectively) and L-dopa test (94% and 90%, respectively) were higher than clonidine test.

Conclusion: Glucagon, L-dopa and ITT should be preferred as the first test since NPV of these tests was high. *Turk Jem 2010; 14: 6-9*

Key words: Growth hormone deficiency, short stature, growth hormone stimulation test

Özet

Amaç: Büyüme hormonu eksikliği tanısı büyüme hormonu stimülasyon testleri ile konulmakta olup bu amaçla birçok farmakolojik ajan kullanılmaktadır. Altın standart test olmadığından genellikle iki test uygulanmaktadır. Biz de 4 ayrı stimülasyon testinin (insulin tolerans testi (ITT), klonidin, L-dopa and glukagon) ciddi boy kısalığı olan adolesanlarda klinik yararlılığını karşılaştırmayı amaçladık.

Gereç ve Yöntemler: Yaşları 11 ile 20 arasında değişen ciddi boy kısalığı olan kırk üç hastaya (32 erkek, 11 bayan) 4 stimülasyon testinden herhangi ikisi veya üçü uygulandı. Testler değişik sayılarda kullanıldı (ITT hepsine, L-dopa 32 kişiye, klonidin 21 kişiye ve glukagon 12 kişiye). L-dopa, klonidin, ve glukagon testlerine cevaplar ITT'ye cevap ile karşılaştırıldı. Ayrıca her testin sensitivitesi, spesifitesi ve pozitif ve negatif prediktivitesi hesaplandı. Bu analiz için iki stimülasyon testine yanıtsızlık büyüme hormonu yetersizliği olarak kabul edildi.

Bulgular: ITT ile diğer üç testin cevap oranları karşılaştırıldığında anlamlı bir fark bulunamadı. Glukagon testi %100 sensitivite, spesifite ve pozitif ve negatif prediktivite değerlerine sahipti. Kalan üç testten klonidin testinin spesifite (%90) ve pozitif prediktivite değeri (%85) en yüksekti. Diğer yandan L-dopa testinin sensitivitesi (%91) ve negatif prediktivite değeri (%86) ise klonidin testinden yüksekti.

Sonuç: Glukagon, L-dopa and ITT'nin negative prediktivite değerleri yüksek olduğundan ilk test olarak seçilmeleri uygun olabilir.
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Anahtar kelimeler: Büyüme hormonu yetersizliği, boy kısalığı, büyüme hormonu stimülasyon testi

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Introduction

The axis of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) must be evaluated in several conditions, i.e. severe short stature (height SD score <-3 SD), severe growth deceleration (height velocity <-2 SD) or less severe short stature (height SD score between -2 and -3 SD) and growth deceleration (height velocity <-1 SD). History of brain tumor, cranial irradiation, or other organic pituitary abnormalities and radiological evidence of abnormality of the pituitary are the other conditions that must be evaluated (1).

GH is synthesized in somatotrope cells, which are present in the anterior pituitary gland. GH plays a role in normal growth and also in the regulation of carbohydrate, protein, lipid and mineral metabolisms. In addition to direct effects of GH on peripheral tissues, its indirect effects are related with IGF-1, which is produced by liver and other tissues. The secretion of GH appears in a pulsatile form; these pulses are 8 to 12 times a day (2). Between these pulses, level of GH is low. Because of this pulsatile pattern, diagnosis of GH deficiency (GHD) requires stimulation tests (1,3,4).

GH can be stimulated by physiological or pharmacological ways. Sleep, fasting and exercise are the physiologic stimuli of the GH. Pharmacological stimuli include L-dopa, clonidine, propranolol, glucagon, arginine, GH-releasing hormone (GHRH) and insulin-induced hypoglycemia. In the diagnosis of GHD, these pharmological stimuli can be used alone or in a variety of combinations. Since no one of the GH stimulation tests have 100% specificity or 100% sensitivity, at least two tests are performed (5-7). Therefore, we investigated sensitivity, specificity, positive and negative predictivities of insulin-induced hypoglycemia, clonidine, L-dopa and glucagon tests in subjects with severe short stature.

Materials and Methods

Patients

We evaluated forty-three patients (32 males, 11 females) with short stature admitted to the Endocrinology Department at Harran University. All patients had severe short stature with height standard deviation score less than 3 SD. The patient aged between 11 and 20 years and the mean chronological age was 14.9±2.3. The bone age of the patients was at least two years delayed (12.4±2.8). Median height was 139.0 (111-158), median weight was 34.0 (23.0-58.5) and median body mass index (BMI) was 16.6 (13.3-32.5) (Table 1).

Table 1. Clinical characteristics of the patients	
Parameters	**Values**
Chronological age*	14.9±2.3
Bone age*	12.4±2.8
n (male/female)	43 (32/11)
Height (cm)+	139.0 (111-158)
Weight (kg) +	34.0 (23.0-58.5)
BMI (kg/m^2) +	16.6 (13.3-32.5)
BMI: body mass index; *values are means±SD, +values are medians (range)	

Test Procedures

L-dopa, clonidine and glucagon were applied to the patients in various combinations in addition to insulin tolerance test (ITT), which was performed to all patients. Clonidine, L-dopa and glucagon tests were applied to 21, 32 and 12 patients, respectively. Two tests were performed in 21 patients and three tests in 22 patients. Before the tests, patients fasted for a 10-hour overnight period. Then, GH stimulation tests were performed according to the following procedures at 08.30-09.00 a.m:

1) ITT: 0.10-0.15 U/kg intravenous regular human insulin was given and the target blood glucose level was less than 45 mg/dL. Glucose levels of the patients were measured every five minutes and the test was terminated when hypoglycemia occurred.

2) L-dopa test: L-dopa was given perorally accounted for by body weight; 125 mg for less than 15 kg, 250 mg for 16kg to 30 kg and 500 mg for over 30 kg.

3) Clonidine test: Clonidine was given peroral accounted for by body weight; 50 mcg for 5-15 kg, 100 mcg for 16-25 kg, 150 mcg for 26-35 kg, 200 mcg for 36-45 kg, and 250 mcg for over 46kg.

4) Glucagon test: It was administered 0.03 mg/kg (maximum 1 mg) subcutaneously.

Blood samples were collected every 15 minutes from 0 to 60 minutes and also when hypoglycemia was detected for the ITT and were taken every 30 minutes from 0 to 120 minutes for the other three tests. A value of serum GH concentration equal/over 10 ng/mL was accepted as positive response to the test.

GH Assay

Serum GH concentrations were determined in the patients by two-site chemiluminescent enzyme immunometric assay (hGH Immulite, Siemens, Llanberis, UK).

Statical Analyses

Response differences between test groups were analyzed by χ^2 test. A P value <0.05 was considered statistically significant. The results are given as mean±standard deviation. Specificity, sensitivity, positive predictive and negative predictive values were calculated for each test using the numbers of patients with true positive (TP), true negative (TN), false positive (FP) and false negative (FN) results. In these analyses, patients with no response to two tests were classified as having GHD. Patients with GHD were classified as TP or FN according to their positive or negative responses for each tests. Sensitivity was defined as the percentage of patients with GHD, who had no response to test (calculated as TP/(TP+FN)). Specificity was defined as the percentage of the subjects without GHD, who had positive response to test (calculated as TN/(TN+FP)). Positive predictive value (PPV) was defined as the possibility of GHD in the subjects with no response to test (calculated as TP/(TP+FP)). Negative predictive value (NPV) was defined as the possibility of determining an individual without GHD among the subjects with response to test (calculated as TN/(TN+FN)).

Results

ITT was administered to all 43 patients and positive response to the test (i.e GH ≥10ng/mL) was detected in 15 of them (34.6%). Hypoglycemia was occurred at a mean time of 18.7 minutes and

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the mean value of glucose was 38.9 mg/dL. L-dopa, clonidine and glucagon tests were administered to 32, 21 and 12 patients and positive responses to the tests were detected in 11 (34.4%), 12 (57.1%) and 2 (16.7%) patients, respectively (Table 2).

The comparisons of the L-dopa, clonidine and glucagon test results with the ITT response were not statically significant (Table 3). We diagnosed GHD in a patient, if the patient had no response to two tests. We found GHD in 23 patients. In determining GHD, the sensitivities of the ITT, clonidine and L-dopa tests were 91%, 72% and 94% respectively. Besides, the specificities of the tests were 65%, 90% and 66%, respectively. Sensitivity and specificity of the glucagon test were 100%. In addition PPV and NPV of the tests were analyzed; PPV and NPV of the glucagon test were 100%. PPV of the clonidine test was higher than of the other two tests, but NPV of the clonidine test was low. ITT and L-dopa had similar PPV and NPV. The results are shown in Table 4 and Table 5.

Discussion

In the evaluation of severe short stature, distinguishing GHD from other reasons for growth retardation is very important. Because of no gold standard test, at least two GH stimulation tests are required for the diagnosis of GHD (1,8,9). Although none of the

GH stimulation tests have 100% specificity and 100% sensitivity, it has been accepted that ITT should be one of the preferred stimulation tests (6,7). The protocols of stimulation tests including arginine, ITT, clonidine, L-dopa and glucagon are well-standardized. These tests should be performed by an experienced team and monitored carefully because of their important side effects (8). Shah et al. reported two deaths and one neurological damage after GH stimulation tests (10). Binde et al. reported an-8 year-old girl who had cardiac arrest after ITT (11). In this study, we evaluated forty three patients with severe short stature by using variable combinations of the stimulation tests. We performed the tests to the patients while hospitalized and all patients were observed carefully for side effect of the tests. We did not record any severe side effect.

There is no gold standard test for the diagnosis of GHD (8,12) and there is no consensus on the cut-off level. Especially, GH values below 10 ng/mL in two GH stimulation tests are accepted as GHD in most countries (7). Tillman et al. compared GH stimulation tests with IGF-1, IGF binding protein-3 (IGFBP-3) and urinary GH excretion in the diagnosis of childhood GHD. According to this study, GH stimulation test was the most useful test to exclude GHD, when cut-off point was 10 ng/ml (13). ITT is generally accepted as the first preferred stimulation test. The advantages of this test are that range between normal and severe GHD is large, and moderate hypoglycemia achieves maximal GH release. Furthermore, ACTH-adrenal axis can be evaluated at the same time (4,6,14). We also used ITT as a first test and performed it to all patients.

Clonidine is an alpha-2 adrenergic agonist agent. Clonidine increases growth hormone-releasing hormone (GHRH) and inhibits somatostatin release. It is a safe, reliable, sensitive agent in the stimulation of GH. Most important side effects of this agent

Table 2. Response rates of the tests in the patients with short stature			
Tests	**Subject**	**Response**	**Percentage (%)**
ITT	43	15	34.6
L-dopa test	32	11	34.4
Clonidine test	21	12	57.1
Glucagon test	12	2	16.7
ITT: insulin tolerance test			

Table 3. Comparison response rates of L-dopa, clonidine and glucagon tests with ITT						
Test name	**L-dopa**		**Clonidine**		**Glucagn**	
	Response	**No response**	**Response**	**No response**	**Response**	**No response**
Response to ITT	4(12.5)	7(21.9)	4 (19)	1 (4.8)	1(8.3)	2(16.7)
No response to ITT	7(21.9)	14(43.8)	8(38.1)	8(38.1)	1(8.3)	8(66.7)
Total	11(34.4)	21(65.6)	12(57.1)	9(42.9)	2(16.7)	10(83.3)
ITT: insulin tolerance test; the results were given as n (%)						

Table 4. Responses of the tests in the subjects with and without GHD								
	ITT		**L-dopa**		**Clonidine**		**Glucagon**	
	Responsive	**No responsive**	**Responsive**	**No responsive**	**Responsive**	**No responsive**	**Responsive**	**No responsive**
GHD (+)	2	21	1	16	3	8	0	10
GHD (-)	13	7	10	5	9	1	2	0
ITT: insulin tolerance test; GHD: growth hormone deficiency								

Table 5. The sensitivity, specificity and predictivity rates of the tests				
Tests	**Sensitivity (%)**	**Specificity (%)**	**Positive predictivity (%)**	**Negative predictivity (%)**
ITT	91	65	75	86
Clonidine	72	90	85	75
L-dopa	94	66	76	90
Glucagon	100	100	100	100
ITT: insulin tolerance test				

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are hypotension and drowsiness (1,15,16). L-dopa stimulates releasing of GHRH via an alpha-adrenergic mechanism. Most important side effects of L-dopa are nausea, emesis and headache (1,17). Glucagon stimulates endogenous insulin and subsequently GH secretions. ITT may create a risk in newborn and small children, and therefore, glucagon seems to be a very good option for them. Nausea and vomiting may be seen as a side effect. During the glucagon stimulation test, hypoglycemia had not be reported (1,18,19).

In our study, we estimated specificity, sensitivity, PPV and NPV of each stimulation tests according to presence of GHD. The sensitivity and the specificity of the glucagon test were 100%. The sensitivities of ITT and L-dopa test were found nearly at the same level (91% and 94%, respectively). Clonidine test was found less sensitive (72%) than ITT an L-dopa tests. However, the specificity of the clonidine test (90%) was higher than those of ITT and L-dopa test (65% and 66%, respectively). Biller et al. compared five stimulation tests (ITT, L-dopa, arginine, L-dopa+arginine and GHRH+arginine) and serum insulin-like growth factor levels in adults. According to this study, specificities of ITT and L-dopa were 96% and 100%, and sensitivities of these tests were 92% and 62% (20). We found that the sensitivities of two tests were similar to this study, but specificities of the tests were lower in our study. This discrepancy may be related with selected population (adolescent population in our study). Al-Ruhaily et al. studied patients with short stature aged 12-21 years. They compared clonidine test with ITT. The sensitivity and specificity of clonidine test were 81% and 82% and those of ITT were 65% and 59%, respectively (16). We found clonidine test less sensitive but more specific than ITT.

Conceicao et al. showed that glucagon test had 88% sensitivity and 97% specificity in adults with GHD, when cut-off point was taken as 3 ng/mL. PPV and NPV of glucagon test were 100% in their study (21). Also Gomez et Al. studied glucagon test in adults and they took 3 ng/mL as a cut-off point. They showed that sensitivity and specificity of glucagon test were 100% (22). Similar to these studies, we found specificity, sensitivity, PPV and NPV of glucagon to be 100% in adolescents.

Conclusion

In conclusion, glucagon, L-dopa and ITT have high negative predictive values, therefore, these tests, especially the glucagon test, should be firstly used in the evaluation of adolescent GHD patients. After excluding the patient with adequate GH response, the test with the highest positive predictive value (i.e. clonidine) might be used to detect the subjects with GHD.

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