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A comparison of L-dopa and clonidine growth hormone stimulation tests in children with short stature

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Abstract

Aim: The purpose of this study was to compare the results of L-dopa and clonidine GH stimulation tests in children with short stature and to identify which of these should be primarily selected.

Materials and Methods: The records of 68 patients aged between 2.5 and 16.6 years presenting to the pediatric endocrinology clinic with short stature and undergoing GH stimulation tests between September 2016 and February 2021 were evaluated retrospectively. Cases with GH levels <10 ng/ml following the first GH stimulation test then underwent the other GH stimulation test. Thirty-four (50%) of the cases in the study began with the clonidine test and the other 34 (50%) with the L-dopa test.

Results: Thirty-five patients (51.5%) were girls and 33 (48.5%) were boys. The clonidine test results were also low in 35 of the 44 patients with peak GH levels <10 ng/ml at the L-dopa test. L-dopa test results were similarly low in 35 of the 37 patients with clonidine test results <10 ng/ml. Chi-square analysis revealed a statistically significant difference between the groups in terms of L-dopa and clonidine measurements ($p< 0.001$). A cut-off point of 8.9 ng/ml was determined for 50% sensitivity and 100% specificity for the L-dopa test, and a cut-off point of 9.76 for 88% sensitivity and 94% specificity for the clonidine test.

Conclusion: GH stimulation tests performed to investigate GHD are laborious and time-consuming. The first stimulation test to be applied to differentiate GHD from ISS must therefore be well selected. The clonidine stimulation test, with higher sensitivity than but similar specificity to the L-dopa test, can be employed as the first test.

Introduction

Short stature is an important problem frequently encountered in pediatric clinics, and one which causes anxiety in both children and parents [1]. The majority of cases presenting with short stature represent the idiopathic variant, and pathological short stature constitutes a smaller proportion of children presenting with that complaint. Short stature more than two standard deviations below standard community values for age and gender, with no systemic, nutritional, chromosomal, or endocrine cause, is known as idiopathic short stature (ISS) [2,3]. Growth hormone deficiency (GHD), one form of pathological short stature, is an important cause of endocrine short stature seen in one in every 3,500-4,000 live births [4]. Early detection of GHD can contribute to patients receiving prompt growth hormone therapy and thus achieving target heights [5]. No benefit has been shown from investigating basal growth hormone levels when deficiency is suspected. Growth hormone release is pulsatile, and daytime levels are low. Growth hormone stimulation tests are therefore needed in cases requiring growth hormone level investigation [6]. The purpose of this study was to compare the advantages and disadvantages, sensitivity, and specificity of the L-dopa and clonidine stimulation tests used in the differential diagnosis of ISS and GHD.

Material and Methods

Clinical and laboratory data were retrieved through a retrospective examination of the files of 68 patients presenting to the pediatric endocrinology clinic with short stature between September 2016 and February 2021 and undergoing growth hormone stimulation tests following clinical and laboratory evaluation. Patients with other systemic and hormonal disorders affecting growth hormone levels or with syndromic short stature were excluded from the

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study. Anthropometric and measurements and biochemical values in terms of systemic diseases were retrieved from patients' old records. Pubertal examinations were based on Tanner staging [7]. Thirty-four (50%) of the cases commenced with the clonidine test, and the other 34 (50%) with the L-dopa test. Examination of our clinic records showed that 130 patients started with the L-dopa test as the first growth hormone test, and 34 with the clonidine test. Our intention was to compare the data for the two groups. In order to ensure that the numbers of individuals in the groups were balanced, 34 members of the L-dopa group were randomly selected. Values for the selected and non-selected groups were compared using the T-test for independent groups (such as age, bone age, birth weight, height, BMI-SDS, IGF1, and IGF BP 3) to check whether a biased sample would emerge. The findings showed no significant difference between the groups (p> 05). Growth hormone stimulation tests began between 08:00 and 09:00 after minimum 8-h fasting. Intravenous access was established before the tests commenced. Following collection of the first blood specimen, tablets containing 125 mcg dopamine was given to patients weighing less than 15 kg, tablets containing 250 mcg dopamine to patients weighing 15-30 kg, and tablets containing 500 mcg to children weighing more than 30 kg for the L-dopa tests, while tablets containing 150 mcg/m2 clonidine were given for the clonidine tests. Blood samples were collected for growth hormone level measurement at 30, 60, 90, and 120 min for both tests, and the fact that sex steroids had not been administered before the tests was confirmed from the old records. Peak growth hormone levels <10 ng/ml following growth hormone stimulation tests were regarded as representing insufficient response [8]. Cases with growth hormone levels <10 ng/ml following the first test then underwent the other stimulation test. Cases with peak growth hormone levels <10 ng/ml after both tests constituted the GHD group, and those with peak growth hormone levels >10 ng/ml from either test constituted the ISS group. Blood specimens were collected at 08.00 following 10-h fasting and were used for the examination of serum growth hormone levels, insulin-like growth factor-1 (IGF-1), and insulin-like growth factor-binding protein-3 (IGFBP-3) levels. Serum IGF-1 and IGFBP-3 levels were measured using the solid-phase, enzyme-labeled chemiluminescent immunometric method, and growth hormone levels were determined using the chemiluminescence method on a Siemens Immulite 2000XPi device, with commercial kits.

Statistical analysis

Statistical power analysis was performed to determine the sample size based on data obtained from other studies in the literature. A minimum sample number of 34 individuals each was calculated with a 5% margin of error, and 80% effect size, for 90% power. Descriptive statistics are presented as frequency and percentage values for categorical variables and as mean plus standard deviation for continuous variables. The variables were investigated using visual (histograms and probability plots) and analytical (Kolmogorov-Smirnov) methods to determine whether or not they were normally distributed. Differences between distributions of different variables in the groups were examined using chi-square analysis, and cross tables are presented. Chi-square analysis was used to compare categorical data. The independent samples t test was applied in the comparison of mean variable values between the groups. ROC analysis was performed to examine the sensitivity and specificity of the tests used in the research. Data analysis was performed on SPPS 25 software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY, USA). p values <0.05 were regarded as significant for all analyses.

Table 1. Distribution of L-Dopa and Clonidine Groups by Gender, Puberty and Diagnosis

Variables		L-Dopa	Clonidine	Total
Gender	Girl	18 (52.9)	17 (50.0)	35 (51.5)
	Male	16 (47.1)	17 (50.0)	33 (48.5)
Puberty	Prepubertal	16 (47.1)	17 (50.0)	33 (48.5)
	Pubertal	18 (52.9)	17 (50.0)	35 (51.5)
Diagnosis	GH deficiency	17 (50.0)	18 (52.9)	35 (51.5)
	Idiopathic short stature	17 (50.0)	16 (47.1)	33 (48.5)
Total		34 (100.0)	34 (100.0)	68 (100.0)

Results

The study population consisted of 68 patients ranging in age between 2.5 and 16.6 years, 35 (51.5%) girls and 33 (48.5%) boys. GHD was diagnosed in 35 (51.5%) cases and ISS in 33 (48.5%). Thirty-three (48.5%) cases were prepubertal and 35 (51.5%) were pubertal. Three separate chi-square analyses were applied in order to determine whether the two groups were similar in terms of diagnosis, gender, and puberty. The results showed no significant differences in these three variables (p> 0.05) (Table 1). Two separate independent samples t test analyses were applied to determine whether there was any significant variation in terms of age, bone age, birth weight, height, body mass index standard deviation score (BMI-SDS), and IGF1, or IGF BP 3 values between the GHD and ISS groups. The results showed a significant difference between the groups in terms of BMI-SDS values (p <0.05), but no significant difference in terms of the other variables (p >0.05) (Table-2). The L-dopa test was applied to 53 patients, as the first test in 34 of these. Peak growth hormone response was insufficient in 27 (79.4%) of the group in which L-dopa was employed as the first test. Consistent with the L-dopa test, clonidine test results were <10 ng/ml in 18 (66.7%) of these individuals. In total, clonidine test results were also low in 35 of the 44 patients with peak growth hormone response <10 ng/ml at the L-dopa test. The clonidine test was applied to 61 patients, as the first test in 34 of these. Peak growth hormone response was insufficient in 17 (50%) of the group in which clonidine was employed as the first test. Consistent with the clonidine test results, the L-dopa test results were <10 ng/ml in 15 (88.2%) of these individuals. In total, L-dopa test results were also low in 35 of the 37 patients with clonidine test results <10 ng/ml.

Table 2. Calendar Age, Bone Age, Birth Weight, Height, Body Mass Index, IGF1 and IGF BP 3 Values in Groups with Growth Hormone Deficiency and Idiopathic Short Stature

Variables	Diagnosis	n	Mean	SD	t^*	p
Calendar Age	GH deficiency	35	11.44	3.07	.392	.696
	Idiopathic short stature	33	11.13	3.43		
Bone age	GH deficiency	35	10.38	3.44	.606	.547
	Idiopathic short stature	33	9.86	3.57		
Birth weight	GH deficiency	35	2870.00	724.89	.067	.947
	Idiopathic short stature	33	2859.70	514.51		
Height SDS	GH deficiency	35	-2.97	.83	.568	.572
	Idiopathic short stature	33	-3.09	.85		
BMI SDS	GH deficiency	35	-.37	1.50	2.349	.022
	Idiopathic short stature	33	-1.08	.88		
IGF1	GH deficiency	35	176.90	96.05	.020	.984
	Idiopathic short stature	33	176.43	103.44		
IGFBP3	GH deficiency	35	4171.37	1479.06	.543	.589
	Idiopathic short stature	33	3987.79	1297.25		

IGF1: İnsulin like growth factor 1; IGFBP3: İnsulin like growth factor binding protein 3; SDS: standart deviation score; BMI: body mass index; GH: Growth hormone; SD: Standard deviation; *Student t testi

Chi-square analysis revealed significant variation between distribution rates of the individuals in the groups ($\chi^2(2) = 84.59$, $p < 001$). Detailed information is shown in Table 3. Nausea and vomiting developed in 10 (29.4%) patients undergoing the L-dopa stimulation test. The symptoms resolved without treatment being required in seven of these cases, and after a few hours of intravenous fluid therapy in the other three. Hypotension requiring brief intravenous fluid therapy developed in one patient (2.9%) undergoing the clonidine test, and drowsiness in nine (26.4%). The sensitivity and specificity values of the L-dopa and clonidine tests and appropriate cut-off points for these values were also investigated. The L-dopa test cut-off point for 50% sensitivity and 100% specificity was 8.93. The cut-off point for 88% sensitivity and 94% specificity on the clonidine test was 9.76 (Table 4).

Discussion

Short stature is defined as a mean height under at least two standard deviations according to age and gender or a height below the third percentile according to standard growth curves. Although most short stature is a variant of normal, it may also be due to an underlying systemic disease or endocrine disorder [9]. GHD represents the most important group among the endocrine causes of short stature. One study reported an endocrine cause in 26% of children presenting due to short stature, with GHD being the most common endocrine cause (45.2%) [10]. Growth hormone therapy contributes to the achievement of normal height in adulthood in children with GHD, and the accurate identification of children with GHD is therefore highly important [11]. Growth hormone is released in a pulsatile manner, higher at night than in the daytime. Random serum growth hormone values are not a useful guide in the diagnosis of GHD. Stimulation tests are therefore required in order to confirm a diagnosis of GHD (6). Since false positive results can be obtained in growth hormone stimulation tests, at least two stimulation tests are recommended in patients with suspected GHD. Peak growth hormone levels <10 ng/ml with both these different tests are defined as GHD [8]. Various growth hormone stimulation tests have been used to identify GHD in children, including the insulin tolerance test, clonidine, glucagon, levodopa, arginine, and growth hormone releasing hormone [8,12]. Although the insulin tolerance test is regarded as the gold standard for GHD in adults, there is no consensus regarding the first choice test for GHD in children. Clinical application of the insulin tolerance test is difficult since it can lead to severe hypoglycemia, and rarely death [13,14]. The L-dopa and clonidine stimulation tests, which are safer than the insulin tolerance test, were therefore used to diagnose GHD in the present study. However, various side-effects can also be seen in children after these two tests [15]. Gastrointestinal problems such as nausea and vomiting can develop with the L-dopa test, and drowsiness and hypotension with the clonidine test [16-19]. Nausea and vomiting developed in 10 (29.4%) patients undergoing the L-dopa stimulation test. Three patients with severe symptoms were given a few hours' intravenous fluid therapy. The other seven patients' symptoms resolved without treatment. Drowsiness developed in nine (26.4%) patients with the clonidine test, and hypotension necessitating short-term intravenous fluid therapy in one (2.9%). Drug-related side-effects developing following stimulation tests can cause anxiety in children and adults, which can prevent the performance of a second test. In

Table 3. Distribution of Participants according to L-Dopa and Clonidine Values

		L Dopa				
		< 10	≥ 10	No Measurement*	Total	p**
Clonidine	< 10	35 (51.5%)	2 (2.9%)	0	37 (54.4%)	
	≥ 10	9 (13.2%)	0	15 (22.1%)	24 (35.3%)	<0.001
	No Measurement*	0	7 (10.3%)	0	7 (10.3%)	
	Total	44 (64.7%)	9 (13.2%)	15 (22.1%)	68 (100%)	

* Participants who did not receive a second test because the value obtained from the first test was ≥ 10 ng/ml. ** Chi-Square test

Table 4. Specificity and Sensitivity Values for L-Dopa and Clonidine

Variables	Sensitivity	Specificity	Threshold	AUC	*p*
L-Dopa	50	100	8.93	.78	.006
Clonidine	88	94	9.76	.98	.000

AUC: Area under the ROC Curve

addition, the first stimulation test applied in the diagnosis of GHD has low sensitivity, thus imposing the burden of a second test on the patient. The selection of the first stimulation test is therefore highly important. Petriczko et al. examined the prognostic significance of the L-dopa and clonidine tests in cases of GHD and reported that the L-dopa test was superior to the clonidine test in showing pituitary growth hormone reserves [20]. However, in contrast to that research, the purpose of the present study was to determine which of these two tests should primarily be employed for differentiating between GHD and ISS. When the groups undergoing L-dopa or clonidine as the first stimulation test were compared, the rate of confirmation the GHD diagnosis of the second test in the group with a growth hormone level of <10 ng/ml detected with clonidine was higher than in the group in which L-dopa was employed as the first test. Additionally, the clonidine test exhibited greater sensitivity than the L-dopa test, while specificity levels were similar between the two. The results of this study show that the clonidine stimulation test is superior to the L-dopa test in determining GHD.

Limitations

The most important limitation of our study is the inability to fully evaluate the two different stimulation tests used to show growth hormone deficiency in terms of superiority, since the gold standard growth hormone stimulation test in children is not available. The lack of priming with sex steroids before the GH stimulation test in peripubertal children is another limitation of this study.

Conclusions

In conclusion, growth hormone stimulation tests performed for the purpose of determining GHD are laborious and time-consuming. Side-effects also give rise to anxiety in children and their families. The first test to be employed for differentiating GHD from ISS must therefore be carefully selected. The application of the clonidine stimulation test, which has higher sensitivity than and similar specificity to the L-dopa test in demonstrating GHD, as the first test may provide a number of advantages for both the child, the family and the clinician.

Ethical approval

Ethics committee approval was obtained from Adıyaman University Institutional Review Board with the decision number of 2020/3-5.

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