A Comparison of L-Dopa and Clonidine Growth Hormone Stimulation Tests in Children with Short Stature

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Abstract

Background: Growth hormone (GH) release is pulsatile, and daytime GH levels are low. GH stimulation tests are therefore needed in cases requiring GH level investigation. The purpose of this study was to compare the results of L-dopa and clonidine GH stimulation tests applied in children with short stature and to identify which of these tests should be primarily selected. 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/dl following the first GH stimulation test then underwent the other GH stimulation test. Thirty-four (50%) of the cases in the study consisted of individuals beginning with the clonidine test, while the other 34 (50%) started with the L-dopa test. Results: Seventeen (50%) individuals in whom clonidine was employed in the first test had low GH responses, while a low GH response was determined at the second, L-dopa test, in 15 (88.2%) of these individuals, significant variation being observed between the groups (p< 0.001). 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.

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Keywords : Growth hormone stimulation test, Clonidine, L-Dopa

WHAT İS ALREADY KNOWN? The first test to be employed for differentiating GHD from ISS must carefully selecte

INTRODUCTION

Short stature is an important problem frequently encountered in pediatric clinics, and one which causes anxiety in children and parents.[1] The majority of children presenting with short stature represent the idiopathic variant, and pathological short stature constitute 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.[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 3500-4000 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 superiorities, sensitivity, and specificity of the L-dopa and clonidine stimulation tests used in the differential diagnosis of idiopathic short stature (ISS) and GHD.

METHODS

Clinical and laboratory data were elicited 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 disorder affecting growth hormone levels or with syndromic short stature were excluded from the study. Anthropometric and measurements and biochemical values in terms of systemic diseases were recorded from patients' old records. Pubertal examinations were evaluated based on Tanner staging.[7] Thirty-four (50%) of the cases in the study consisted of individuals commencing with the clonidine test, while the other 34 (50%) started with the L-dopa test. The 34-member group undergoing the L-dopa test as the first test was randomly selected from among 164 cases.

Growth hormone stimulation tests began between 08:00 and 09:00 after fasting of at least 8 h. Intravenous access was established before the tests commenced. Following collection of the first blood specimen, a tablet containing 125 µg dopamine was given to patients weighing less than 15 kg, containing 250 µg dopamine to patients weighing 15-30 kg, and containing 500 µg to children weighing more than 30 kg for the L-dopa tests, while tablets containing 150 mcg/m^2clonidine 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 growth hormone stimulation 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/dl following the first test then underwent the other stimulation test. Cases with peak growth hormone levels <10 ng/dL after both tests constituted the GHD group, and those with peak growth hormone levels >10 ng/dL 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 GH levels, IGF-I, and 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.

The study protocol was approved by the local ethics committee (Adıyaman University Non-Interventional Clinical Research Ethics Committee. No. 2020/3-5, dated 21.04.2020).

Statistical Analysis

Descriptive statistics are presented as frequency and percentage values for categorical variables and as mean plus standard deviation for continuous variables. Differences between distributions of different variables in the groups were examined using chi-square analysis, and cross tables are presented. The independent samples t test was applied in the comparison of mean variable values by 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.

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, 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).

Peak GH response was insufficient in 27 (79.41%) patients undergoing L-dopa was applied as the first test. Clonidine test results were also low, consistent with the L-dopa test, in 18 (66.7%) of these. Growth hormone response was insufficient in 17 (50%) of the patients undergoing the clonidine test as the first test. A sufficient growth response was also achieved at the L-dopa test performed as the second test in 15 (88.2%) individuals. Chi-square analysis was performed to examine whether there was any significant variation in distribution rates in the groups, and significant variation was determined in distributions by groups ($p<0.001$) (Table 3).

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. The accurate identification of children with GHD is therefore highly important.[11] Growth hormone is released in a pulsatile manner, and release is 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 negative results are obtained in between 10% and 20% of 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 sedation and hypotension with the clonidine test.[16-19] 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 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 on differentiating 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 significantly higher than in the group in which L-dopa was employed as the first test. However, 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.

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 then 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.

DISCLOSURES

The authors declare they have no conflict of interest

AUTHOR CONTRIBUTIONS

Semih Bolu—Study conception and design, Collection of data, analysis and interpretation of results, drafting the manuscript, study conception and design, Collection of data, analysis and interpretation of results, drafting the manuscript, critical revision of manuscript.

Abdulvahit Aşık—Collection of data, study conception and design, drafting of manuscript, analysis and interpretation of data.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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REFERENCES

1. Esen İ, Demirel F, Tepe D, Demir B. Characterıstıcs Of Patıents Who Referred To A Pedıatrıc Endocrınology Outpatıent Clınıc. Turkish J. Pediatr. Dis. 2011;5:133-8
2. Ranke MB. Towards a consensus on the definition of idiopathic short stature. Horm Res 1996;45:64-6.
3. Wit JM, Ranke MB, Kelnar CJH. ESPE classification of paediatric endocrine diagnosis. Horm Res 2007;68:1-120
4. Murray PG, Dattani MT, Clayton PE. Controversies in the diagnosis and management of growth hormone deficiency in childhood and adolescence. Arch Dis Child. 2016;101(1):96-100.

5. Antoniazzi F, Cavarzere P, Gaudino R. Growth hormone and early treatment. Minerva Endocrinol. 2015;40:129-143
6. Ghigo E, Bellone J, Aimaretti G, et al. Reliability of provocative tests to assess growth hormone secretory status. Study in 472 normally growing children. J Clin Endocrinol Metab. 1996;81:3323-3327
7. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 1976;51:170-79
8. Hilczer M, Smyczynska J, Lewinski A. Limitations of clinical utility of growth hormone stimulating tests in diagnosing children with short stature. Endocr Regul 2006;40:69-75
9. Grimberg A, Lifshitz F. Worrisome growth. In: Lifshitz F, ed. Pediatric Endocrinology. 5th ed. New York: Infroma Heathcare USA; 2007:1-50
10. Hussein A, Farghaly H, Askar E, et al. Etiological factors of short stature in children and adolescents: experience at a tertiary care hospital in Egypt. Ther Adv Endocrinol Metab. 2017;8(5):75-80
11. Growth Hormone Research S. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. J Clin Endocrinol Metab 2000;85:3990-3
12. Hanew K, Utsumi A. The role of endogenous GHRH in arginine-, insulin-, clonidine- and l-dopa-induced GH release in normal subjects. Eur J Endocrinol. 2002;146:197-202.
13. Biller BM, Samuels MH, Zagar A, et al. Sensitivity and specificity of six tests for the diagnosis of adult GH deficiency. J Clin Endocrinol Metab. 2002;87:2067-2079
14. Shalet SM, Toogood A, Rahim A, Brennan BM. The diagnosis of growth hormone deficiency in children and adults. Endocr Rev. 1998;19:203-223
15. Moon JS, Lee SY, Nam CM, et al. 2007 Korean National Growth Charts: review of developmental process and an outlook. Korean J Pediatr. 2008;51:1-25
16. Richmond EJ, Rogol AD. Growth hormone deficiency in children. Pituitary 2008;11:115-120
17. Lanes R, Recker B, Fort P, Lifshitz F. Low-dose oral clonidine. A simple and reliable growth hormone screening test for children. American Journal of Diseases of Children 1985;139:87-88.
18. Al-Ruhaily AD, Malabu UH. Growth hormon deficiency sort stature in a third world adult endocrine clinic: usefulness of clonidine test in its diagnosis Indian J Med Sci. 2008;62(4):149-56.
19. Collu R, Leboeuf G, Letarte J, Ducharme JR. Stimulation of Growth Hormone Secretion by Levodopa-Peopranolol in Children and Adolescents. Pediatrics 1975;56:262-266
20. Petriczko E, Szmit-Domagalska J, Horodnicka-Józwa A, Walczak M. Prognostic value of the L-Dopa and clonidine GH stimulation tests in patients with endogenous growth hormone deficiency - retrospective analysis after discontinuation of treatment - own experience. Pediatr Endocrinol Diabetes Metab. 2009;15(1):13-9.

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