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Diagnostic Value of Provocative Test by Insulin Combined with Clonidine for Growth Hormone Deficiency in Children

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Abstract

Objective: To evaluate the diagnostic value of provocative test by insulin combined with clonidine for growth hormone deficiency (GHD) during childhood

Methods: Eighty children underwent a provocative test with insulin(0.075U/Kg, intravenous) combined with clonidine (4μg/kg, orally). Among them, 40 children underwent clonidine provocative test, 40 children underwent insulin tolerance test (ITT) in another day.

Findings: The specificity of ITT+clonidine test (74%, 88%) was remarkably higher than that of ITT (48%) or clonidine test (65%). ITT+clonidine test had a better accuracy (75%, 85%) than that of ITT (63%) or clonidine test (73%)

Conclusion: We conclude that the combined clonidine+insulin test is a feasible, reliable, convenient, time saving, and safe tool for evaluation of the growth hormone (GH) axes than the clonidine test or ITT.

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Key Words: Clonidine; Insulin; Growth Hormone; Growth Hormone Deficiency; Short Stature

Introduction

Short stature is a common reason for pediatric endocrine evaluation. When the other cases--including genetic short stature, constitutional delay of growth and puberty, hypothyroidism, Turner syndrome, and chronic disease such as celiac disease--are excluded, the growth hormone deficiency (GHD) need to be considered. The prevalence of GHD is estimated at approximately 1: 4000 to 1: 10 000.GHD can be idiopathic or organic, familial or sporadic, with recognizable genetic defect or linked to a neuroendocrine dysfunction of GH secretion. There are, however, still many cases of GHD where the etiology is not defined, so-called idiopathic.

The efficient diagnostic assessment of growth hormone (GH) secretion is important in children with GHD or growth failure, because GHD is treatable already. However, the diagnosis of GHD remains difficult[1]. Various laboratory methods were used to diagnosis the GHD, GH provocative tests play a critical role in the diagnosis of GHD among those[2], although the results of provocative GH testing are dependent on the assay used, the pubertal and nutritional status of the child and the GH secretion pattern prior to testing[3,4]. A variety of provocative tests have been devised that rapidly increase the level of GH in normal children. The most common provocative agents include insulin, glucagon, clonidine, arginine, and L-dopa. The clonidine test was first described in children in

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1979. which was aα-adrenergic agonist ,lower blood pressure, act probably through stimulation of growth hormone-releasing hormone (GHRH) release. The insulin-induced hypoglycaemia is the oldest test that has been described to evaluate GH function, based on the pituitary responsiveness to hypoglycaemia, Insulin-induced hypoglycaemia suppresses the somatostatin tone and stimulates the α-adrenergic receptors which has been recommended by the Growth Hormone Research Society as the standard test for the diagnosis of GHD in adults.

It traditionally requires demonstration of absent or low levels of GH in response to stimulation. The inadequacy is that it need more blood samples for GH determination and was expensive. So many methods were employed to simplify it without cause much more false-negative or false positive responses. Li etal[5] reported that the diagnostic value of pyridostigmine (PD)+ levodopa (L-dopa) test was better than that of insulin tolerance test (ITT) or arginine (ARG)test, it was convenient and safe for short children. It has been agreed that GHRH+arginine, GHRH+ growth hormone-releasing peptide (GHRP), and glucagon stimulation tests are also now well validated in adults[6]. However there were few reports about the provocative test of ITT+clonidine. We have, therefore, performed a comparative study of ITT + clonidine test vs ITT or clonidine test, to evaluate the diagnostic value of ITT+clonidine for GHD during childhood.

Subjects and Methods

Patients

Eighty children (mean age,10.7±3.4 years; 58 boys) referred to the department of pediatrics of the Third Hospital of Hebei Medical University (Shijiazhuang, China) from June 2010 to June 2011 were evaluated for short stature or growth retardation. A clinical diagnosis of GHD or non GHD was made in each case by the specialized physicians, who had long-standing experience in the diagnosis and treatment of GHD.

Thirty one patients (median 11.4 years, 4.5~ 16.9 years, 22 boys) were diagnosed clinically as isolated idiopathic GHD (group I), on the basis of 1) the presence of more than one typical phenotypic feature: frontal bossing, immature face, midfacial hypoplasia, truncal adiposity, hypogenitalism in a male, and high-pitched voice; 2) appropriate auxological characteristics: height <-3 standard deviation (SD) below mean or height less than -1.5 SD below mid-parental height, height velocity below the 25th percentile for age (>6 months follow-up), and a bone age delayed more than 2 years;3) the exclusion of other endocrinopathies and chronic diseases such as constitutional delay of growth and puberty, hypothyroidism, Turner syndrome, and celiac disease[7]. The control group (group II or Non GHD) consisted of 49 patients (median 10.2 years, 3.4 ~15.3 years, 36 boys), who were referred to our pediatric clinic for evaluation of short stature with no other evidence of pituitary pathology, in whom GHD was not suspected , but needed to be excluded formally ,included constitutional delay in growth and puberty (10 patients), familial short stature(12 patients) and idiopathic short stature(27 patients) Forty children in all patients, underwent clonidine provocative test while the rest 40 children underwent ITT in the first day, all children underwent a combined provocative test of clonidine+ ITT on the second day.Height SD score was calculated from the Chinese standards[8], Body mass index (BMI) was calculated as weight (kilograms)/height (meters 2). Bone age(BA) was estimated by the method of Greulich and Pyle[9]. The study protocol was approved by the Ethics Committee of Hebei Medical University, and informed consent was obtained from the patient and from the parents.

Testing protocol

All tests were carried out in the morning (08:30-09:00) following an overnight fast and 30 min after an indwelling catheter was placed in a forearm vein for slow infusion of isotonic saline. The ITT test was performed with intravenous injection of short-acting human insulin (Novo Nordisk, Bagsvaerd, Denmark) 0.075 U/kg. Sampling for glucose levels were carried out every 15min, and for GH every 30min, for 120min. The test was considered adequate for GH reserve assessment if hypoglycemia of 2.8 mmol/L or less than half of the basis level. The clonidine stimulation test was performed with a single oral

dose of 4μg/kg. Sampling for GH levels was carried out every 30min for 120min.

The combined test was performed with intravenous injection of short-acting human insulin 0.075 U/kg, and simultaneously a single oral dose of clonidine (Double- Crane pharmaceutical corporation, Beijing, China), 4μg/kg. Blood samples were withdrawn every 15min for glucose levels and at 0, 30, 60, 90 and 120min for GH determination. Pass levels were defined as a peak serum GH level of 10 ng/ml. All test were carefully observed by special doctor, 50% glucose was prepared in case severe hypoglycemia.

Assays

Blood samples were immediately separated and kept frozen at -20.8°C until assayed. The serum GH concentrations were determined with a commercially available solid phase chemilu-minescent enzyme immunoassay employing an Immulite automated analyzer (Unicel DxI 800 Access Immunoassay system, Beckman Coulter Inc, California, USA). The detection limit was 0.002 ng/ml for GH. Serum glucose was measured by the GOD- PAP (Boehringer Manheim GmbH, Manheim, Germany) enzymatic colorimetric test on a Hitachi 717/911 device (Hitachi, Osaka, Japan) with typical interassay coefficients of variation of 0.7 ± 3%.

Statistics

We have used clinical assessment of GH status to define groups I (GHD) and II (non-GHD). So test performance is based on this classification. Sensitivity was defined as the number of true positive results (below the cut-off point) divided by the total number of results in group I. Specificity was defined as the number of true negative results (above the cut-off point) divided by the total number of results in group II. Efficiency was defined as the number of correct results divided by the total number of tests in both groups. All values were expressed as a percentage. The data were expressed as mean± SD if normally distributed, or as median and ranges if the data were skewed Student's t test and the Kruska - Wallis test were used to compare data between group Statistical analyses were performed with the SPSS 17.0 for Windows (SPSS Inc)and Excel (Microsoft Corporation, Redmond, WA, USA). P value less than 0.05 was taken as significant.

Findings

Patient characteristics:

Patients with group I (isolated idiopathic GHD) could not be distinguished from those in group II (NGHD)by gender (71%vs73%), age (mean±SD, 11.44±3.59 vs. 10.18±3.19yr), body mass index (17.32±2.46 vs. 17.13±5.10) But the bone age delay (-2.62±1.21 vs. -0.68±0.86 yr) has significant difference .

Test performance

In the combined GH stimulation tests, the mean peak GH concentrations in group I were significantly less than the concentration in group II (mean±SD, 6.41 ± 4.47 vs. 15.61±6.53μg/L), but there was a broad range of responses in both groups (0.01~18.2μg / L vs. 5.77~29.13μg/L). The mean peak GH concentration of the combined test in group I has not significant different from the ITT or clonidine test, but the mean peak GH concentration of the combined test in group II was significantly more than the ITT or clonidine test (Table 1).

Table 1: Growth hormone response to combined ITT + clonidine test compare with the ITT or clonidine test (N=40)

Test	group I (n=40) Mean (SD)	group II (n=40) Mean (SD)
ITT	5.88 (4.52)	11.03 (5.24)
ITT + clonidine	6.32 (4.54)	14.70 (6.89)
Clonidine	5.56 (4.72)	13.16 (5.62)
ITT + clonidine	6.66 (4.72)	16.58 (6.56)

ITT: Insulin Tolerance Test; SD: Standard Deviation

Table 2: Sensitivity, specificity, and efficiency (expressed as number of patients and as percentage of patients) of each test at the given cut-off point for all patients

Test	Cut-off point	Sensitivity No. (%)	Specificity No. (%)	Efficiency No. (%)
ITT + clonidine	5 ng/mL	13.31 (42)	49.49 (100)	62.80 (78)
	7.5 ng/mL	20.31 (64)	44.49 (90)	64.80 (80)
	10 ng/mL	24.31 (77)	38.49 (77)	62.80 (78)
ITT + clonidine	10 ng/mL	13.17 (76)	17.23 (74)	30.40 (75)
ITT	10 ng/mL	13.17 (76)	11.23 (48)	25.40 (63)
ITT + clonidine	10 ng/mL	11.14 (79)	23.26 (88)	34.40 (85)
clonidine	10 ng/mL	11.14 (79)	17.26 (65)	29.40 (73)

ITT: Insulin Tolerance Test

The efficiency, sensitivity, and specificity of the combined provocative test:

The efficiency, sensitivity, and specificity of all tests at defined cut-off points are shown in Table 2. The specificity of ITT+clonidine test (74%, 88%) was remarkably higher than that of ITT (48%) or clonidine test (65%), whereas the sensitivity (76%,79%) was similar to ITT (76%) or clonidine test (79%). ITT+clonidine test had a better accuracy (75%,85%) than that of ITT (63%) or clonidine test (73%). The ITT+clonidine test with a cut-off value of 7.5 ng/mL was the most efficient (Table 2).

GH peak time distribution of the combined provocative test:

The percentage of GH peak concentration at 0,30,60,90 and 120min respectively was 6%, 16%, 48%, 23% and 6%(GHD group); 20%, 20%, 45%, 31% and 2%(group II), 4%, 19%, 46%, 28% and 4%(all patients). The percentage of GH peak concentration mainly appear at 60-90min.

Adverse reactions:

One (1.25%) patient was observed with hypoglycaemic reaction such as dizziness, palpitation, sweating. No other adverse reactions were observerd.

Discussion

Since human pituitary GH became available for treatment in 1960, the diagnosis of GHD has been the subject of many debates and controversies[10,11]. However our ability to make a definitive diagnosis of GHD remains limited. We need more effective means of achieving a correct diagnosis particularly when considering the long term implications of a diagnosis of GHD and the cost of GH therapy.

Tools for the diagnosis of GHD include auxology, radiographic assessment of bone age, measurement of insulin-like growth factor I (IGF-I) and IGF-binding protein 3 (IGFBP-3), provocative GH test, cranial MRI, and, in certain cases, genetic test. Clinical presentation and auxology are the most important factors in the diagnosis of GHD. Both IGF-I and IGFBP-3 are reflective of circulating GH, and both vary relatively little through the course of the day and thus can be measured easily as a screening test for GHD. However age-specific norms are needed to interpret both IGF-I and IGFBP-3.[12] Right now the genetic testing is not performed routinely in the diagnosis of GHD, but it may play a larger role in diagnostic algorithms in the coming years[13]. Although the results of provocative GH testing are dependent on the assay used, the pubertal and nutritional status of the child, and the GH secretion pattern prior to testing, and it is poorly reproducible [2], provocative GH testing continues to play a primary role in the diagnosis of GHD[1].

To evaluate a test, it is necessary to use a gold standard. In the case of GHD, this has proved difficult to define .So we have chosen a specific approach in this study in which initial clinical evaluation (based on history, examination , and growth parameters) has been used as the gold standard[14]. The clonidine test was first described in children in 1979[15]. This α-adrenergic agonist acts probably through stimulation of growth hormone-releasing hormone (GHRH) release .It lowers blood pressure and can induce mild

somnolence. The clonidine test is very useful for paediatric practice[16]. The insulin tolerance test is the oldest test that has been described to evaluate GH function, based on the pituitary responsiveness to hypoglycaemia. Insulin-induced hypoglycaemia suppresses the somatostatin tone and stimulates the α-adrenergic receptors. Because the mechanisms of action of clonidine test and ITT are different, when combined clonidine test with ITT, it maybe improve the provocation efficacy, as reports of combined arginine and insulin tolerance test[17].

Using the now accepted cut-off value of 10 ng/ml, the sensitivity, specificity, efficiency of the combined test in our study was 77%, 77%, 78%, which was similar with the VALLO TILLMANN's report[18] There were 7 patients (23%)in GHD group whose mean peak GH concentration were above the cut-off point. That maybe caused by the GH resistance or over excitation of the ITT + clonidine simultaneous. To reduce this case futher standard tests were needed. There were 9 patients (23%) in group II whose mean peak GH concentration were below the cut-off point. The reason may be that[2]: First part of the normal children before puberty, the peak GH concentration below the diagnostic criteria for GHD (<10 ug / L); Second, the subjects age, sex, sex hormone levels and metabolic status caused a transient lack of GH secretion, or other reasons causes temporary insufficient GH secretion; Third there has just been a pulse of growth hormone and the pool is low, the subsequent response will be attenuated. Then the growth and development indicators are needed to be measured regularly for this group patients. Serum GH levels are need reassessment when further growth retardation appearing. When using a cut-off level of 7.5 ng/mL in combined ITT+clonidine test, the efficient was the best.

Our study shows that the efficacy and specificity of the combined clonidine +insulin test were better than the clonidine test or ITT, while in the GHD group ,the mean peak GH concentration and sensitivity were similar with that of the clonidine test or ITT, it will reduce the false-positive results and will not cause much more false-negative results.

In our study only 1 patient was observed with hypoglycaemic reaction such as dizziness, palpitation, sweating. no other adverse reactions were observed. It shows that it quite safe with carefully monitor throughout the test.

Study Limitations: Although GHRH, glucagon and GHRP have better diagnosis value in GHD and safe, but they are not easily got especially in our county. The provocative agents, such as clonidine, insulin, arginine, are easily found. Those drugs are still the mainly provocative agents right now. It will very effectively to combine the ITT+ clonidine test result with the serum IGF-I and IGFBP-3 to diagnosis GHD .But because of the economy of patients and absence of standard value of serum IGF-I and IGFBP-3 of age-specific, we did not mesure serum IGF-I and IGFBP-3.

Conclusion

We conclude that the combined clonidine+insulin test is a feasible, reliable, convenient, time saving, and safe tool for evaluation of the GH axes than the clonidine test or ITT.

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Conflict of Interest: None

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