HIGH AND LOW DOSE CLONIDINE TESTS FOR THE DIAGNOSIS OF GROWTH HORMONE DEFICIENCY

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ABSTRACT

The growth hormone (GH) response to three provocative stimuli was assessed in 15 short normal children (ISS) and 5 children with GH deficiency (GHD). The pharmacologic agents used were insulin 0.1 unit/kg TV (IHT), oral clonidine 0.15 mg/m^2 (HDCT) and 25 μg irrespective of age and weight (LDCT). Blood glucose and GHwere measured at 0, 30, 60, 75, 90 and 120 minutes; and BP and serum cortisol levels were measured during clonidine tests. A steep rise in GH levels were found between 60 and 90 minutes during HDCT and LDCT. The peak GH levels were 29.5 ±5.7 ng/ml in HDCT compared to 17.9±5.7ng/ml during IHT and 6.7±2.9 ng/ml during LDCT ($p < 0.01$). The peak GH levels above 7 ng/ml were seen in 60% children with ISS during LDCT. No significant adverse effects were noticed during HDCT and LDCT except transient drowsiness. The peak GH levels were not related to the fall in BP or cortisol levels. LDCT appears to be a reasonably effective stimulator of GH release. It can be used for screening children with reduced growth velocity as an outpatient procedure. Those with an abnormal response will need a more definite testing such as HDCT.

Key words: *Clonidine stimulation test, Growth hormone, Growth hormone deficiency, Insulin-hypoglycemia test.*

The diagnosis of growth hormone deficiency (GHD) before 1963 was based primarily on clinical features. The development of a radioimmunoassay (RIA) for growth hormone (GH) led to the establishment of laboratory criteria for diagnosis. Because of the pulsatile nature of GH secretion, a physiological or pharmacologic stimulus must be applied to distinguish normal from GHD subjects. Insulin hypoglycemia test (IHT) is reliable and has the advantage of assessing hypothalamic-pituitary-adrenal axis in addition to GH secretion. IHT has some risk albeit small, for hypoglycemic seizures and produces subnormal results in upto 25% of children tested(l-3). High dose clonidine test (HDCT) has been evaluated in large series and results suggest that a single dose of 0.15 mg/m^2 clonidine is as effective and perhaps a better diagnostic tool compared to IHT. The side effects are minimal (drowsiness and hypotension) and less serious. The ability of HDCT to induce GH secretion in normal children and constitutional short stature is reported as over 90%(2,4-8).

Most GH stimulation tests last between 90 and 120 minutes, requiring frequent blood sampling and have unpredictable side effects. Smaller doses of clonidine are reported to release GH and a low dose clonidine test (LDCT) with 25 μg orally, irrespective of age and weight has been used

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as a screening procedure in initial evaluation of short stature(9).

The aim of this study was to assess the reliability of LDCT as a screening procedure for GHD and to compare its utility with HDCT and IHT to stimulate GH secretion.

Material and Methods

Fifteen short normal children (idiopathic short stature, ISS) aged 4 to 14 years (10 boys, 5 girls) referred to the Pediatric Endocrinology Clinic of the All-India Institute of Medical Sciences were the subjects of this study. These were children with height for age less than 5th centile of the Indian standards. Patients with identifiable genetic, psychosocial and systemic (including endocrine) causes of short stature were excluded. In addition 5 children (4 boys, 1 girl) proved by insulin-hypoglycemia and levodopa tests to have GHD (hypopituitarism) and multiple pituitary hormone deficiencies (MPHD) due to removal of craniopharyngioma, were also included.

GH tests were performed on resting patients after an overnight fast on consecutive days. The tests were, fully explained and informed consent was obtained from parents and older children. The test procedure was as follows:

1. *Insulin hypoglycemia test (IHT)(6):* After an overnight fast, regular insulin was injected IV at a dose of 0.1 unit/kg. Samples for measurement of blood glucose and GH were collected at 0, 30, 60, 75, 90 and 120 minutes. Test was considered valid if blood glucose fell to a nadir less than half the fasting level or less than 2.2 mmol/L. The test was repeated with insulin 0.15 units/kg IV if hypoglycemia did not occur.

2. *High dose clonidine test (HDCT)(10):* After an overnight fast, patient was placed on recumbent position. An intravenous canula was inserted into cubital vein and a mercury sphygmomanometer placed in the other arm. After 30 minutes of complete rest, clonidine was given orally at a dose of 0.15 mg/m^2. Blood samples for determination of GH, glucose and cortisol were drawn at 0, 30, 60, 75, 90 and 120 minutes. Blood pressure was measured half hourly till it returned to normal.

3. *Low dose clonidine test (LDCT)* (9): Single oral dose of 25 µg of clonidine was given irrespective of age and weight. Blood samples collected at 0, 30, 60, 75, 90 and 120 minutes after the dose were evaluated for GH, glucose and cortisol. Blood pressure was recorded every 30 minutes till it returned to normal.

Blood glucose was measured by a dextrostix-reflectance meter (Glucometer-II, Miles India Ltd). Radioimmunoassay in serum was done using commercially available GH and cortisol kits (BARC, Bombay). The lower limit of measurement of the GH assay was 0.5ng/ml. Measurements less than that was assigned that value. Samples were measured in duplicate and results were expressed as the mean of the duplicate values. The intra- and interassay coefficients of variation were 4.0% and 5.7%, respectively. A peak GH level of 7 ng/ml or greater was considered an adequate GH secretory response(6). Statistical analysis was done using Students 't' test and linear regression analysis.

Results

The salient clinical profile and growth data of the two groups of children are given in *Table I.* In general, children with GHD had lower growth velocity compared with the group of children with idiopathic short stature (ISS) and growth retardation was also noticed at an earlier age. The lag between

TABLE I-*Clinical Characteristics of Patients with Short Stature*

Characteristics	GHD	ISS
Age, CA (yr)	11.3±2.2	9.7±2.6
Sex ratio (M : F)	4:1	2:1
Growth velocity (cm/yr)	2.8±1.5	3.6±1.8
Truncal obesity (%)	20	20
IQ <80 (%)	-	20
Birth asphyxia (%)	-	13.3
Age at which growth retardation was noticed (yr)	2.75±1.7	5.5±2.2
Height SD score	-3.92±1.17	-2.02±0.95
Height age, HA (yr)	6.2±1.8	6.9±2.1
Bone age, BA (TW2-RUS, yr)	6.5±2.3	8.3±2.6
CA-BA (yr)	4.8 ± 1.1	1.6±1.2
HA-BA (yr)	0.25±0.85	-1.29±1.0

GHD-Growth hormone deficiency, ISS-Idiopathic short stature.

TABLE II-*Response to Stimulation Tests in Children with Short Stature (Mean SD)*

Parameters	IHT	HDCT	LDCT
Peak GH levels (ng/ml)			
GHD	2.8±1.2	2.9 ±1.1	1.6±0.5
ISS	17.9±5.7	29.5±5.7	6.7±2.9
Serum cortisol levels (µlg/ dl)			
Basal	-	13.5 ±6.8	12.9±6.1
90 min	-	7.5 ±2.1	8.1 ±2.2
Systolic blood pressure (mm Hg)			
Basal	-	99.7±4.7	102.3 ±5.3
Lowest recorded	-	86.2±7.9	90.1±5.4

chronologic and bone age was 1.6 ±1.2 yrs in ISS compared to 4.8 ±1.07 yrs in GHD children.

The GH levels did not rise significantly above the baseline concentration in children with GHD. The peak GH levels were 2.8 ± 1.2 ng/ml on IHT, 2.9 ±1.1 ng/ml on HDCT and 1.68±0.5 ng/ml on LDCT *(Table II)*. All the children in the ISS group had steep rise in serum GH with a peak between 60-90 minutes in response to HDCT. The mean peak serum GH level on HDCT was 29.5±5.7

ng/ml as compared to 17.9 ±5.7 ng/ml with IHT ($p<0.01$). In three children with elevated basal GH levels (11-163 ng/ml), there was further moderate increase in GH levels on HDCT. Two patients had GH levels less than 7ng/ml on IHT; however on HDCT they responded with peak GH levels more than 21 ng/ml.

The low dose clonidine test produced peak GH levels of only 6.7 ±2.9 ng/ml. The peak GH response was seen between 60 and 90 minutes with LDCT. Six of the children with ISS had peak GH levels less than 7 ng/ml.

Following HDCT all children became browsy for a period of 1-3 hours whereas LDCT produced a transient drowsiness in only 4. The systolic blood pressure (BP) decreased from mean baseline levels of 99.7±4.7 and 102.3±5.3 mmHg to 86.2±7.9 and 90.1±5.4 mmHg in HDCT and LDCT groups, respectively. Serum cortisol values decreased from mean ± SD baseline of 13.5±6.8 to 7.512.1 µg/dl at 90 minutes during HDCT whereas during LDCT the cortisol levels decreased from baseline of 12.9±6.1 to 8.1 ±2.2 µg/dl at 90 minutes. The differences were not statistically significant. The fall in BP and cortisol levels did not correlate with the dose of clonidine used. There was no correlation between the peak level of GH and the decrease in BP or cortisol levels. There was no fall in blood glucose with HDCT and LDCT.

Following IHT, 14 children had symptomatic hypoglycemia characterized by lethargy, sweating, apprehension, headache and tachycardia, but none developed convulsions. This test had to be repeated in 3 of the 20 children with a higher dose of insulin (0.15 units/kg) as the initial dose did not produce significant fall in blood sugar.

The observed GH response on clonidine did not show any correlation with sex, chronological and bone age, height velocity and height velocity standard deviation score of the children studied. All these children were prepubertal (PHI, Gl stage of Tanner).

Discussion

Short stature represents a major reason for referral of children to pediatric endoc-rinologists. If no identifiable systemic, genetic or psychosocial factor is found, sophisticated tests such as radioimtnunoas-says are required to detect a possible endocrine cause. With the availability of biosyn-thetic GH, assessment of the GH status of these children is imperative for early therapeutic intervention.

The laboratory criteria for diagnosis of GHD are well defined. GH measurements at timely intervals after the onset of sleep or following exercise have not been useful due to the pulsatile nature of GH secretion. Among the pharmacologic tests for GH release, IHT is generally relied upon and is still the investigation of choice preferred by many(11). Its main disadvantages are hypoglycemic seizures and occasional false subnormal responses. Use of glucagon or arginine is limited because of expense and nonavailability. These procedures have other disadvantages. Some of the newer GH stimulation tests are safe, reliable and inexpensive and can be performed as an outpatient procedure.

Oral administration of an alpha-2-adrenergic agonist, clonidine was shown to stimulate GH release in normal subjects(12) and was later tried successfully as a test for GHD(10). It provoked a significantly higher mean plasma GH response and gave fewer subnormal results compared to IHT(5). The

Health Services Human Growth Hormone Committee of UK in a multi-centre trial observed that IHT and oral clonidine test are equally effective stimuli of GH secretion, clonidine being safer(6). The effectiveness, reliability and tolerance of oral clonidine were reported in a number of studies(2,4,7,8).

Clonidine is believed to stimulate endogenous secretion of GH-releasing hormone, GHRH(13). Recent studies suggest that the alpha-2-adrenergic agonism acts by inhibiting the hypothalamic release of somatostatin rather than stimulating GHRH(14,15). Experimental observations suggest that clonidine-induced GH secretion is modulated by a chain of events involving primary stimulation of GHRH release resulting in increased GH secretion which finally enhances somatostatin release by feedback, ultimately normalizing the system(16).

Pharmacological stimuli such as HDCT, IHT and arginine may not reflect the actual status of endogenous GH secretion(17,18). As with IHT, normal controls may not respond adequately to either clonidine or GHRH. The GH secretory response to clonidine is modified by factors including age, sex, puberty, skeletal maturity and height velocity. The state of endogenous secretion GH can also be measured by intermittent or continuous sampling over 24 hrs. Zadik *et al.* compared the reproducibility of GH response to pharmacologic stimuli such as clonidine, insulin and arginine with 24-hour integrated concentration (24 Hr-IC) and observed maximum correlation of 24 HR-IC with clonidine test(18).

We observed HDCT to be more potent stimulus for GH secretion than LDCT and IHT. HDCT produced adequate GH release in all short normal children in this study, whereas subnormal levels were seen in two and six children with IHT and LDCT, respectively. Peak GH levels were also significantly higher. The side effects of clonidine were transient and less unpleasant than insulin.

Most GH stimulation tests last between 90 and 180 minutes, require frequent blood sampling and monitoring of patients. A simple and effective outpatient screening test is desirable. Smaller doses of clonidine also effectively release GH(9,19). Lanes *et al.* observed that 100 µg of oral clonidine induced rapid and sustained GH release in 90% of healthy short children but a lower dose of 50 µg was less effective and had higher failure rate with no decrease in side-effects(20). On the contrary, Laron *et al.(9)* demonstrated that a lower dose of 25 µg of oral clonidine was an effective stimulator of GH release and could be used as an outpatient screening test with a single blood sample collection between 75 and 90 minutes. In the present study LDCT evoked a rise in GH level above 7 ng/ml in 60% of short normal children between 60 and 90 minutes with no side effects.

LDCT appears to be a reasonably effective stimulator of GH release and hence may be used as a screening test in the initial evaluation of short stature as an outpatient procedure. This should be followed by a more definite pharmacological test such as HDCT if the response is abnormal.

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