Clonidine Stimulation Test: Is Single Best Time Point, Convenient Yet Efficacious?

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Abstract

Context: To find a single time point during clonidine stimulation test (CST), with highest diagnostic value to rule out growth hormone deficiency (GHD). **Settings and Design:** This is a retrospective study of 79 CSTs carried out in a tertiary care center in India. **Materials and Methods:** A cohort of 79 children with unexplained short stature was divided into two groups: GHD and non-GHD. Any one stimulated growth hormone (GH) level >10 ng/mL was used to rule out GHD. Diagnostic accuracy of not only single time points but also time points in pairs was calculated. **Statistical Analysis:** The data were analyzed using SPSS statistical software 22.0. Descriptive statistics were used for analyzing demographic data. Mode for time to peak GH was calculated in each group. The specificity and false positive rates at each time point as well as combined time points were determined. **Results:** Assaying a single sample at 60 min after clonidine resulted in 20.5% false positive tests with specificity of 79.5%. Addition of the 90 min sample increased specificity to 92.3%. **Conclusion:** The 60 min sample after clonidine stimulation was the best single sample to rule out GH deficiency. Combined sampling at 60 min + 90 min is economical and less cumbersome, with minimal compromise on the specificity.

Keywords: Clonidine stimulation test, growth hormone deficiency, short stature, growth hormone

INTRODUCTION

Short stature is one of the commonest problems encountered in pediatric endocrine clinics. Growth hormone deficiency (GHD) is one of the important endocrine causes of short stature. Growth hormone (GH) stimulatory tests are mandatory to rule out GHD, considering the pulsatile nature of GH secretion. Clonidine stimulation test (CST) is the most commonly used provocative test, to rule out GHD. Conventionally, CST involves collection of multiple (5–6) samples, making it cumbersome and expensive.[1] In the resource-limited setting, this could be a major obstacle. The references and cut offs that are in use for CST are based on foreign studies. The time to stimulated peak GH has been studied in very few Indian centers, which were based on small sample size.[2] Therefore, we undertook this retrospective study to determine whether peak GH could occur in any single sample during CST with sufficient sensitivity and specificity, making the CST efficacious, yet convenient and cost-effective.

The primary aim of this study was to determine the diagnostic value of sample collected at each time point in CST for ruling out GHD. We also wanted to determine the most frequent time to peak GH in our cohort.

MATERIALS AND METHODS

We reviewed retrospective data of CST performed at our center on 79 patients after Institutional Ethics Committee approval. Our criteria to investigate any child were: (1) severe short stature, defined as a height more than 3 standard deviation (SD) below the mean; (2) height more than 1.5 SD below the mid-parental height; (3) height more than 2 SD below the mean; and (4) a height velocity over 1 year more than 1 SD

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below the mean for chronological age, or a decrease in height SD of more than 0.5 over 1 year in children over 2 years of age.[1] Other causes of short stature were ruled out before subjecting them to CST.

Inclusion criteria
1. Patient presenting with unexplained short stature (criteria as described earlier)
2. Age between 2 and 18 years
3. Patients with follow-up data for at least 6 months showing height velocity <25th centile for that age.

Exclusion criteria
1. Age <2 years and >18 years
2. Patients with known chronic systemic illness
3. Patients who did not have at least 6 months follow-up
4. CST with less than five samples
5. Height velocity within normal range for the age.

Patients with other anterior pituitary deficiencies were appropriately replaced prior to the testing. Prepubertal girls >10 years and boys >11 years were primed with ethinyl estradiol (50 μg for three consecutive days) and testosterone (100 mg IM single dose), respectively.[3] Tablet clonidine was administered at the dose of 150 μg/m² and GH was estimated basally (0 min) and at 30, 45, 60, and 90 minutes thereafter.[4] CST did not extend beyond 90 min as this is not included in our routine protocol for CST.

GH estimation was done by IMMULITE chemiluminescent immunometric assay with an intraassay coefficient of variation (CV) of 5.3–6.5% and interassay CV of 5.5–6.2%.

Insulin hypoglycemia test (IHT), although a gold standard for the diagnosis of GHD, is associated with morbidity. The diagnosis of GHD in our cohort was based on history, physical examination, auxological data, insulin-like growth factor (IGF1) levels, CST, and imaging. GH Research Society Consensus Guidelines recommends two GH provocation tests in suspected isolated GHD.[1] We, being a charitable institute, find it difficult to perform the second test due to financial constraints.

Based on the auxological data, those children with unexplained short stature with low height velocity (<25th centile for the age), low IGF1 levels, with all five values of clonidine-stimulated GH <10 ng/mL were considered to have GHD.[1] Those patients with any single value of clonidine-stimulated GH >10 ng/mL were included in non-GHD group. The diagnostic value in the form of specificity and false positive rates was determined at each time point.

Statistical analysis
The data were analyzed using SPSS Version 13 and OpenEpi software Version 2.3. Descriptive statistics were used for analyzing demographic data. Means were calculated for peak GH values for whole cohort and GHD and non-GHD groups as well. Mode for time to peak GH was calculated in each group. The specificity and false positive rates at each time point as well as combined time points were determined.

RESULTS
The data of 79 unexplained short stature patients (40 GHD and 39 non-GHD) from old records satisfying the inclusion and exclusion criteria were analyzed. Out of 40 patients with CST suggestive of GHD, 24 were classified as isolated growth hormone deficiency (IGHD), 13 had multiple pituitary hormone deficiency (congenital), 2 had acquired causes of hypopituitarism (one with tuberculous meningitis with hydrocephalus, 1 with arachnoid cyst with hydrocephalus), and 1 patient had septo optic dysplasia. The remaining 39 patients who showed adequate GH response to CST were diagnosed as idiopathic short stature.

The bone age standard deviation score (SDS) in our cohort was −4.2 ± 1.65 (mean ± SD). Subgroup analysis showed that the bone age SDS in GHD and non-GHD groups were −5.17 ± 1.42 and − 3.18 ± 1.2, respectively. The difference between the bone age SDS of the two groups was statistically significant ($P = 0.0001$).

The chronological age in 79 subjects was 9.9 ± 2.9 (mean ± SD) years, height age was 5.9 ± 2.2 years, and the bone age was 5.9 ± 2.4 years. The difference between the bone age and chronological age was statistically significant ($P = 0.0001$). The difference between the height age and chronological age was statistically significant ($P = 0.0001$). However, the difference between the bone age and height age was not statistically significant ($P = 1.0$). In GHD patients for chronological age of 9.36 ± 3.4 years, corresponding bone age was 4.5 ± 2.1 years and the height age was 5.13 ± 2.4 years. The mean bone age was delayed as compared to height age. However, the difference between the bone age and height age was not statistically significant ($P = 0.21$). In non-GHD patients for chronological age of 10.6 ± 2.3 years, corresponding bone age was 7.5 ± 1.7 years and the height age was 6.9 ± 1.7 years. The mean bone age was delayed as compared to height age. However, the difference between the bone age and height age was not statistically significant ($P = 0.12$).

There were 15 patients with multiple hormonal axis involvement (as a part of multiple/combined pituitary hormone deficiency) out of which all had thyroid axis involvement, 7 (46.66%) had cortisol axis involvement and 3 had gonadal axis involvement. However, we could assess the gonadal functions only in 3 patients as other patients were prepubertal.

On magnetic resonance imaging (MRI) pituitary of 40 GHD patients, hypoplastic pituitary was seen in 25 (62.5%) patients, ectopic posterior pituitary in 1 (2.5%) patient, hydrocephalus in 2 (5%) patients, pituitary stalk interruption in 1 (2.5%), empty sella in 1 (2.5%), and septo-optic dysplasia in 1 (2.5%) patient, and 9 (22.5%) patients had normal MRI pituitary. Out of 24 patients of IGHD, 14 (58.3%) had hypoplastic/small anterior pituitary, 9 (37.5%) patients had normal MRI pituitary, and 1 (4.2%) patient had empty sella.
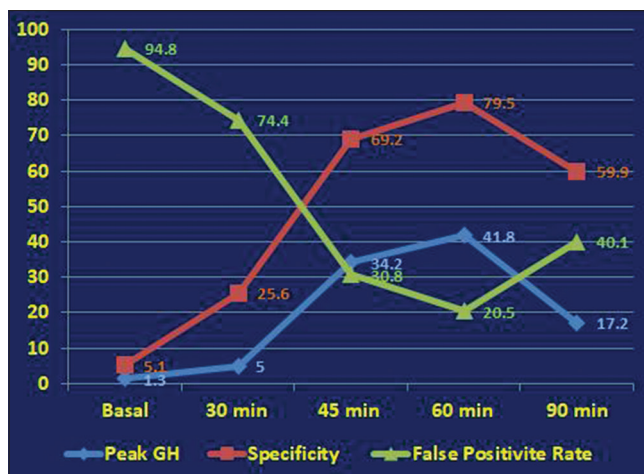
Graph 1: Peak GH, Specificity and False positive rate at each time point

Graph 2: Peak GH Specificity and False positive rates at combined time points

The specificity of CST at 0, 30, 45, 60, and 90 min was 5.1, 25.6, 69.2, 79.5, and 59.9%, respectively. The false positive rate at 0, 30, 45, 60, and 90 min was 94.8, 74.4, 30.8, 20.5, and 40.1%, respectively [Table 1 and Graph 1]. Thus, the 60 min sample had the highest specificity (79.5%) and the lowest false positive rate (20.5%).

The most frequent time to peak GH was 60 min (41.8%) [Graph 1]. However, stimulated GH of >10 ng/mL (with or without peak) was present in almost 80% non-GHD patients at 60 min, helping to rule out GHD. Other samples had low specificity to rule out GHD when considered singly.

As none of the single time point samples gave us high specificity with low false positive rate, we decided to analyze the combined time points. We analyzed the specificity and false positivity with combined (30 + 60 min, 30 + 90 min, 45 + 60 min, 45 + 90 min, and 60 + 90 min) time points. The highest specificity (92.3%) with lowest false (7.7%) positive rate was seen with 60 + 90 min time points together [Table 2 and Graph 2]. Other combinations were less specific and had greater false positive rates as compared to 60 + 90 min combination.

DISCUSSION

GH is secreted from the pituitary gland in pulses. Therefore, a single reading of GH is rarely of diagnostic utility. Multiple sampling (24 h GH sampling) is required to establish the diagnosis of disorders of GH secretion. However, performing 24 h GH sampling is cumbersome and costly. Therefore, to establish the diagnosis of GHD, many stimulatory tests have been formulated. Zodik *et al.* compared the reproducibility of 24 h integrated concentration of GH versus GH response to provocative test; however, they found that the reproducibility of 24 h integrated concentration of GH was better than provocative test.[5]

Despite lower reproducibility as compared to 24 h integrated concentration of GH, many GH releasing agents (e.g., clonidine, glucagon, hypoglycemia, etc.) have been studied for stimulatory testing till date, from the point of view of convenience and cost

Table 1: Diagnostic value of CST at various time points

Time (min)	Number of patients with peak GH *n* (%)	Specificity at a given time	False positive at a given time *n* (%)
Basal	1 (1.3)	5.1	37 (94.8)
30	4 (5.0)	25.6	29 (74.4)
45	27 (34.2)	69.2	12 (30.8)
60	33 (41.8)	79.5	8 (20.5)
90	14 (17.2)	59.9	16 (40.1)

Table 2: Diagnostic value of CST at combined time points

Combined time points (min)	Number of patients with peak GH *n* (%)	Specificity at a given time	False positive at a given time *n* (%)
30+60	37 (49.36)	87.2	5 (12.8)
30+90	18 (22.8)	66.7	13 (33.3)
45+60	60 (75.9)	89.7	4 (10.3)
45+90	41 (51.9)	87.2	5 (12.8)
60+90	47 (59.5)	92.3	3 (7.7)

effectiveness. Lal *et al.*[6] studied the effect of intravenous clonidine on pituitary functions in normal men to establish that noradrenergic mechanism takes part in modulating the GH secretion. Since then many studies based on stimulatory effects of clonidine on GH secretion have been conducted. At present oral clonidine is one of the most widely used agents to carry out GH stimulation test in pediatric population. However, the GH response to clonidine in the pediatric population is not very uniform, with few subjects peaking early and few peaking late, making it necessary to assess multiple samples post clonidine stimulation.

Although interpretation of all five samples of CST with auxological data would be the ideal situation, cost and convenience is an important factor in real-life situation. Gillis *et al.* reported that the peak in CST indicating GH sufficiency ("sufficient tests") (85.15%) tended to occur more often at typical times (60, 90 min) as compared to those

indicating GHD (68%).[7] Therefore, the aim of this study was to assess whether minimizing the sampling points could reduce the cost while maintaining the efficacy and sensitivity of the test.

In our study we have found that 60 min sample after clonidine stimulation was the best single sample to rule out GHD. It resulted in specificity of 79.5%, which was comparable to previous studies. It diagnosed 8 of 39 non-GHD patients as GHD when considered as a single test, i.e., a false positive rate of 20.5%. Moreover, addition of 90 min sample to the 60 min sample increased the specificity to 92.3%, making it one of the most convenient, short test with no major compromise on its diagnostic accuracy. These results were consistent with the results of previous study by Muster *et al.* (*n* = 87), where peak GH occurred at 60 min in 47.1% and at 90 min in 33.3% subjects in CST. The combined 60 + 90 min test had a specificity of 95.7%, which is similar to our study. Thus, they concluded that CST can be shortened without compromising on its diagnostic accuracy in a major way.[8] Similarly according to Christoforidis *et al.* the highest average GH level was at 60 min followed by 90 min (23.42%). Moreover, 60 min was also the most frequent peak time (53.80%).[9] In contrast to above studies, Morris *et al.* concluded that 90 min sample is to be preferred for screening GHD by CST; as normal GH response was demonstrated in 29 out of 30 patients at 90 min.[10]

In a study by Gil-Ad *et al.* (*n* = 25), oral administration of clonidine was found to be sensitive and reliable to test for GH reserve in pediatric follow-up. Moreover, they also suggested that clonidine being a more potent stimulator (than insulin hypoglycemia) of GH secretion, it can differentiate between complete and partial GHD.[11]

In the study by Menon *et al.* (*n* = 20), Indian cohort of 20 short children was studied, which included 15 children with idiopathic short stature and 5 with GHD. This study evaluated the effect of high dose clonidine test (150 $\mu g/m^2$ single oral dose of clonidine) versus low dose clonidine test (25 μg single oral dose of clonidine), which were carried out along with IHT. They observed that high-dose clonidine was more potent stimulator of GH secretion than low-dose clonidine and insulin hypoglycemia. There was decrease in both the BP and cortisol levels after oral administration of clonidine; however, it did not correlate with the dose of clonidine. They also observed that the GH peaks between 60 and 90 min in response to clonidine in the cohort of Indian children and advised a single sample drawn between 60 and 90 min, as a screening test.[2] In keeping with these results, we also found that drawing two samples at 60 and 90 min gives high specificity with low false positive rates.

The limitations of this study were small sample size (*n* = 79), retrospective analysis, and biochemical diagnosis of GHD being based on results of single stimulation test. Moreover, we were unable to compare the results against a gold standard test as mentioned before. Performing a gold standard test would have improved the quality of the study.

Conclusion

Assaying a single sample at 60 min after clonidine resulted in 20.5% false positive tests with specificity of 79.5%. Addition of 90 min sample to 60 min sample increased specificity of the test to 92.3%. Thus, the combined sampling at 60 min + 90 min during a CST is economical and less cumbersome without compromising majorly on the specificity of CST.

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Conflicts of interest
There are no conflicts of interest.

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