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A Survey of Growth Hormone Stimulation Test Efficacy for Workup of Short Stature in a Referral Laboratory

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ABSTRACT

Objective: To assess growth hormone (GH) stimulation test efficacy in short stature workup for establishing GH deficiency.
Study Design: Cross-sectional survey.
Place and Duration of Study: Diagnostic Endocrine Section, Department of Chemical Pathology & Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi Pakistan, from Aug 2020 to Jan 2021.
Methodology: The study was conducted on 129 individuals aged 2-16 years. History, axiological data and biochemical parameters were assessed to establish GH deficiency for short stature workup.
Results: Out of 129 individuals, 76(59%) boys and 53(41%) girls reported GH stimulation tests. 81(62.3%) children were in <3rd percentile, 29(22.3%) in <5th percentile, 9(6.9%) in <10th percentile and 5(3.8%) in <25th percentile. Among the group with bone age difference >2 years, 34(82.9%) fell in <3rd percentile. GH Stimulation test post-Levo Dopa was performed in 102(78.46%) patients. 49(37.4%) patients responded inadequately to the GH stimulation test. The adequate response to the GH Stimulation test improved as the percentile declined from the 50th to the 3rd percentile.
Conclusion: GH stimulation test results can only partially rely upon though their importance remains in combination with other short-stature workup parameters for ruling out growth hormone deficiency.
Key words: Growth hormone stimulation tests, Short stature, Height percentile.

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INTRODUCTION

Short stature is always a great concern for parents due to psychosocial concerns, reporting in pediatric clinics.[1] Prevalence of Growth hormone deficiency (GHD) is approximately 1:4,000 to 1:10,000 in different populations.[2] Most of the time, parents keep looking for a cause to address short stature due to a lack of appropriate workup and guidance. GHD contributes to a very small fraction of short stature causes, and even after going through growth hormone-relevant tests, there are many unanswered queries. This is because of unstructured short-stature workup requiring an appropriate dedicated diagnostic setup. Moreover, Available diagnostic testing lacks the re-quired sensitivity due to variations in GH Assay and poor reproducibility that affect GHD diagnosis. Diagnostic modalities and protocols for establishing GH deficiency vary in different countries.[3,4]

Accuracy of auxologic measurement using standard height parameters and formulas is necessary before starting the necessary workup along with detailed history and physical examination.[5,6] Height standard deviation score (SDS) is considered for evaluating height velocity but relying on standard height cut-offs alone is inadequate for establishing the diag-nosis.[7] Constitutional delay, short familial stature (FSS), delayed puberty, and falsely raised height due to early onset of puberty are some of the common variables which need to be addressed before making any diagnosis.[8] Therefore, apart from biochemical and radiological investigations role of genetic workup has also been introduced for supporting short-stature workup.[9]

This study demonstrates the need for structured short-stature workup incorporating all causes for early detection of growth disorders. Growth hormone stimulation test requirement and utility in the workup of short stature need to be assessed in our study. There has yet to be a previous study in the country addressing this issue. This will require awareness of the subject and establishing diagnostic workup clinics.

METHODOLOGY

The cross-sectional survey was conducted at the Diagnostic Endocrine section, Department of Chemical Pathology & Endocrinology, Armed Force Institute of Patholgy, Rawalpindi Pakistan, from August 2020 to January 2021, after approval of the Institutional Review Board (Cons-CHP-3/READ-IRB/20/349) Data of short stature workup were extracted from Diagnostic Endocrine Data Base and Laboratory Information

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Management System (LIMS) AFIP, Rawalpindi.

Inclusion Criteria: Patients 2 to 16 years reporting for GH Stimulation Test as part of short stature workup were included in the study.

Exclusion Criteria: None.

Patients were divided according to their age groups as pre-schooler (2-6yrs), middle year children (6-12yrs) and adolescents (12-<16yrs). The significant diff-erence in bone age is considered as >2yrs based on the difference between bone age to chronological age.[10] Detailed history was taken for failure to thrive, developmental delay, mental retardation, birth asphyxia, jaundice, hypoglycemia, delayed puberty and consanguinity. Demographic data include age in years and gender (male/female). Anthropometric data included; height in (cm) of the child along with percentile, the father's & mother's height in cm, mid-parental height and weight in (kg) along with percentile.

Laboratory investigations data record include haemoglobin (Hb), calcium(mmol/L), phosphorus (m mol/L), Alkaline phosphatase(ALP, U/L), thyroid stimulating hormone (TSH, mIU/l), cortisol(nmol/L), GH(mIU/L), Insulin-like Growth Factor-1 (IGF-1,nmol /L), Insulin Growth Factor Binding Protein-3 (IGFBP-3, nmol/L) and Anti tissue transglutaminase antibody (Anti-TTG Ab) before GH stimulation test was performed. GH stimulation test (Post levo Dopa and post-exercise) and Insulin tolerance test (ITT) were performed as per the advice of the paediatrician. Informed consent from parents was taken before the test. Blood samples of subjects were drawn aseptically in plain serum tubes for GH levels pre and post-stimulation, which were analysed on Immulite 2000 Immunoassay Analyzer utilising immuno-chemiluminescence assay. An adequate response is considered as post-stimulation GH levels of ≥20mIU/l. Patients were divided into two groups (Adequate and inadequate response) and compared using growth hormone parameters (IGF-1, IGFBP-3 and growth hormone stimulation tests).

Statistical Package for the Social Sciences (SPSS)-version 23.0 was used for the data analysis. Quantitative variables were expressed as mean±SD and qualitative variables were expressed as frequency and percentages. Chi-square test was applied to find out the association. Independent sample t-test was applied to find the mean differences among the groups. The *p*-value lower than or up to 0.05 was considered as significant.

RESULTS

Out of 129 individuals, 76(59%) boys and 53(41%) girls reported GH stimulation tests. The patient characteristics of the study are shown in Table-1. 70 (53.4%) parents of patients had given a history of consanguineous marriages, with a higher percentage found in boys (55.3%) as against girls (52%). 48(37%) patients gave a history of failure to thrive, while 20(15.5%) had developmental delays. 6(7.4%) patients' Anti-TTG were positive among 81 who had Anti TTG records. 76(59%) boys and 53(41%) girls reported GH stimulation tests. 81(62.3%) children were in <3rd percentile, 29(22.3%) in <5th percentile, 9(6.9%) in <10th percentile and 5(3.8%) in <25th percentile. Most of the boys [n=57(75%)] reported when their height were in <3 percentile as against girls who fell in <3rd percentile [n=24(44%) and <5th percentile [n=24 (44%)] equally. Twenty-six (20.0%) patients had both IGF-1 and IGFBP-3 results, 09(7.0%) had IGF-1 and 04(3.1%) patients had IGFBP-3 results available before the GH stimulation test.

Table-I: Characteristic of Study Population based on Gender Distribution (n=129)

Parameters	Female n=53 (%)	Male n=76(%)	Total n=129	*p*= 0.05
Age Group (years)				
Pre School (2-6 yrs)	17(31.5%)	22(28.9%)	39(30.0%)	0.950
Middle yr school (6-12)	27(50%)	39(51.3%)	66(50.8%)	
Adolescent (12-16 yrs)	10(18.5%)	15(19.7%)	25(19.2%)	
History of Consanguinity	28(52%)	42(55.3%)	70(53.4%)	<0.001
Bone Age Diff >2 years	15(40.5%)	26(43.3%)	41(42.3%)	0.78
Height Percentile Groups				
<3rd	24(44.4%)	57(75.0%)	81(61.8%)	0.000
<5th	24(44.4%)	05(6.6%)	29(22.1%)	
<10th	02(3.7%)	07(9.2%)	09(6.9%)	
<25th	03(5.6%)	02(2.6%)	05(3.8%)	
Response to GH stimulation test				
Adequate	36(66.7%)	40(52.6%)	76(58%)	0.001
Partial	01(1.9%)	04(5.3%)	05(3.8%)	
Inadequate	17(31.5%)	32(42.1%)	49(37.4%)	

Yrs, years; GH, growth hormone

Post Levo Dopa GH Stimulation test was performed in 101(78.29%) patients, followed by the GH stimulation test post-exercise in 31(24.0%). In comparison, ten patients (7.8%) had Insulin Tolerance Test (ITT).[11] patients underwent both Levo dopa and Post-exercise stimulation test, out of which 2(18%) showed an inadequate response in both tests, 7(63%) showing adequate response in both tests whereas 2(18%) patients showed an adequate response in levo dopa test but the inadequate response in GH Post-exercise.

53(65.4%) show adequate response to GH stimulation tests. An adequate response to the GH Stimulation test declined as the percentile declined from the 3rd to the 50th percentile. Among 27(34.6%) patients showing an inadequate response, 15(55.5%) patients' parents had consanguineous marriages. Response of GH stimulation test post levo dopa is more pronounced (33.72 nmol/L±13.8) than GH stimulation post-exercise (23.83 nmol/L±3.0). Response of GH biochemical parameters post GH Stimulation test are shown in Table-II.

Table-II: Response of GH Biochemical Parameters post GH Stimulation test (n=129)

Parameters	GH Stimulation Test Response		
	Adequate	Inadequate	*p*-value
	Mean±SD		
Growth Hormone (nmol/L)			
Post Levo dopa	33.4±18.78	8.02±4.8	<0.001
Post Exercise	23.83±3.0	13.32±4.5	0.109
Post Insulin Tolerance Test	35.85±7.4	6.16±2.6	-

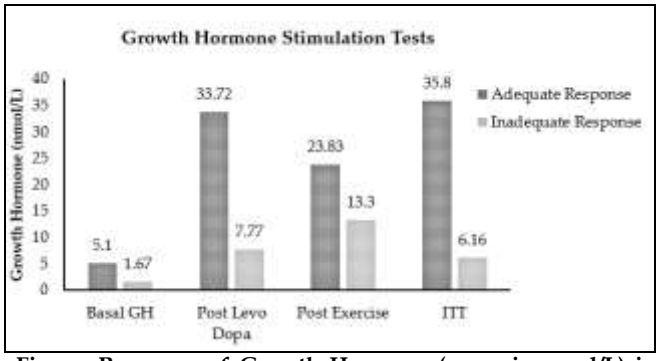

Figure: Response of Growth Hormone (mean in nmol/L) in Growth Hormone Stimulationtests(n=129)

DISCUSSION

Early diagnosis of GH deficiency helps initiate treatment time for a better outcome. Most of the time, the cause cannot be established, and the child is labelled as having a constitutional delay or with short familial stature. Early GH workup has been observed for the female group in our study compared to the male group. Most of the boys reported when their height was <3rd percentile as against girls who were mostly in their 5th percentile. More than half of our patient's parents gave a history of consanguineous marriages, and among them, 31.25% also have an inadequate response to GH stimulation test as against 85.1% in a Jordanian study by Zayed *et al*.[9]

Bone age determination is one of the reliable methods for the workup of GH deficiency. However, the Greulich-Pyle (GP) method was mostly used for assessing skeletal maturity, which is reliable but based on Western data, which does not include corporate ethnicity and area-wise data.[10] In our study, patients with a significant bone age difference (>2yrs) are mostly in <3rd percentile and 51.2% also showed an inadequate response to GH stimulation tests which authenticates the importance of bone age before GH stimulation tests.

IGF-1 and IGFBP-3 tests were underutilised in our patients as GH stimulation tests were advised without prior evaluation of these investigations. These tests have good specificity but poor sensitivity in GHD.[11] Provocative growth hormone testing reproducibility is poor because of pubertal status and body composition. Mostly, as advised by the paediatrician, only one growth hormone stimulation test is being used in our setup, contradictory to at least two GH stimulation tests for making a diagnosis. ITT, considered a gold standard GH stimulation test for GH deficiency, is rarely used nowadays, which was used previously by paediatricians considering the risk of hypoglycemia in patients. This practice is similar to data collected by Yavaş Abalı *et al*.[12] Body mass index and short fasting also raise GH response.[13] No patients were sex primed in our setup, as against many studies where sex priming before the GH stimulation test is advised. There was a lack of consensus on it as it falsely failed the GH stimulation test as observed by Martínez *et al*.[14] The cut-off value utilised in most studies was 7ng/ml (21mIU/l) as in our setup.[15]

Some mutations have been identified in the routine workup, though not utilised in idiopathic GHD. Genetic causes can be considered in a specific group of children whose phenotypic assessment suggest increased chances of genetic causes. Chances of genetic causes are more with a history of consanguinity.[16] Role of a new agonist for GH release like Macimorelin needs to be established in children also.[17,18]

There is a need for establishing a consensus statement for diagnostic workup followed by recombinant GH treatment at the national level to address this neglected area. No local growth charts were available for short stature evaluation. Growth hormone stimulation tests need to be standardised. Need for establishing cut-off for GH at different pubertal ages.

CONCLUSION

Growth hormone stimulation test has varied responses concerning history, auxologic and biochemical parameters. However, the importance of the GH test remains in combination with other short-stature workup parameters for ruling out growth hormone deficiency.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MUM & MA: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

ZHH & SIK: Data acquisition, data analysis, drafting the manuscript, approval of the final version to be published.

AY & HH: Conception, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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