International Journal of Contemporary Pediatrics
*Thomas HC et al. Int J Contemp Pediatr. 2021 Feb;8(2):327-332*
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: https://dx.doi.org/10.18203/2349-3291.ijcp20210123

**Original Research Article**

# The usefulness of serum IGF-1 and serum IGFBP-3 for the diagnosis of growth hormone deficiency in comparison to clonidine stimulation test: a prospective cohort study

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**Received:** 02 December 2020
**Accepted:** 08 January 2021

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## ABSTRACT

**Background:** Growth Hormone Deficiency is conventionally diagnosed by low peak Growth Hormone levels to provocative testing. Serum IGF-1 and IGFBP-3 are under the influence of GH and reflect the endogenous GH secretion. Owing to the absence of a circadian rhythm, it is possible to take individual measurements of IGF-1 and IGFBP-3 at any time of the day for evaluation of GH status instead of subjecting the individual to cumbersome provocative tests. Objectives of this study were to compare IGF-1 and IGFBP-3 assays with clonidine stimulation test in children of different age group with short stature.
**Methods:** 90 children with short stature were included in the study. Samples for basal GH, IGF-1 and IGFBP-3 were obtained and afterwards all children were subjected to clonidine stimulation test. The diagnostic value of the tests were analysed in terms of sensitivity, specificity, predictive value and accuracy in younger and older age groups.
**Results:** 40% of the study population was diagnosed to have GHD. IGF-1 had high sensitivity in both age groups. But in the younger age group IGFBP-3 was more specific. Both the tests had same specificity in the older age group. Combining the two tests helped to improve diagnostic value in all the age groups.
**Conclusions:** Measurements of IGF-1 and IGFBP-3 have shown comparable diagnostic performance with growth hormone stimulation tests and are valuable for patient`s convenience and ease of performance and can be useful in the workup of growth hormone deficiency.

**Keywords:** Growth hormone deficiency, IGF-1, IGFBP-3, Short stature

## INTRODUCTION

Short stature is a common problem in children globally, mainly in developing countries.[1] Short stature is defined as height below 3rd percentile for age and sex or height 2SD below sex adjusted mid parental height.[2] There are different causes of short stature. But the most common causes, beyond the first two years of life are Familial Short Stature (FSS) and Constitutional Growth Delay (CGD). Other causes of short stature can be endocrinological or non endocrinological. Common endocrinological causes of short stature include hypothyroidism, hypopituitarism (isolated GHD and multiple anterior pituitary hormone deficiencies), hypercortisolism and classical Laron syndrome etc. All chronic diseases can cause short stature, such as renal disease, malignancy, chronic pulmonary disease, Cystic Fibrosis, cardiac disease etc.[3]

### *Growth hormone deficiency.*

Growth hormone deficiency (GHD) is one of the most important endocrine and treatable causes of short stature.[4] Growth hormone is a polypeptide hormone, secreted by

the somatotrophs of the pituitary gland. The rate of occurrence of GHD has been estimated as one per 4000 persons.[5]

***IGFs and IGFBPs***

The insulin-like growth factors (IGF-I and IGF-II etc.) are single chain polypeptide hormones sharing 50% homology with insulin. IGF-I is produced in liver and in local tissues in response to GH stimulation. Insulin-like growth factors constitute a family of GH-dependent peptides that mediate many of the anabolic and mitogenic actions of growth hormone. IGF-I is the major mediator of GH's effect on postnatal growth. However, serum IGF-I levels, are influenced by age, degree of sexual maturation, and nutritional status.

The IGFs circulate bound to the IGF binding proteins (IGFBPs). There are six classical high affinity IGFBPs.[6] Serum levels of IGFBP is reduced in patients with GH deficiency or GH insensitivity, conditions in which assays for serum IGFBP-3 have important diagnostic value .Serum levels of IGF-I and IGFBP-3 reflect the endogenous GH secretion in healthy children and do not exhibit diurnal variation, which makes them potential candidates for screening of GHD.[7]

***Diagnostic dilemmas***

The diagnosis and treatment of GH deficiency (GHD) during childhood and adolescence is a subject of controversy. The assessment of growth hormone deficiency includes direct and indirect methods. Direct methods measures serum Growth hormone after stimulation and indirect methods include IGF-1, IGF-2 and insulin like growth factor binding protein-3 (IGFBP-3).[8]

Traditionally, the diagnosis of growth hormone (GH) deficiency has been based upon measurement of serum concentrations of GH following either physiological or pharmacological stimulation. The disadvantages of growth hormone provocative tests include assay related problems, the arbitrary definitions of subnormal responses, age and puberty related variability of results, need of sex steroid priming, and lack of normative data.[9]

There are reports of many normally growing children showing growth hormone deficiency in standard provocative test and repeated tests on the same children may yield divergent results.[10] The advantages of using indirect methods (measurement of IGF-1 or IGFBP-3) are that no pharmacologic stimulation or hospitalization is required and the tests are relatively inexpensive.[11]

In view of all these controversies, more research needs to be conducted to develop a robust diagnostic criterion for GHD. Early initiation of therapy could better the chances of achieving final adult height.[12] So, early diagnosis of growth hormone deficiency is of utmost importance.

Many regional studies in India have shown high prevalence of growth hormone deficiency among children and adolescent. But the lack of simple diagnostic methods might lead to under diagnosis of growth hormone deficiency.

The diagnosis and treatment of GHD are hurdled with various challenges, restricting the availability of growth hormone therapy to only a very limited group of children in India.

The objective of the study was to evaluate the diagnostic value of serum IGF-1 and serum IGFBP-3 for the diagnosis of growth hormone deficiency in comparison to clonidine stimulation test in children of different age group.

## METHODS

This prospective cohort study was carried out in a tertiary care hospital in south India over a period of 22 months from September 2015 to June 2017. 90 children under the age of 18 years with short stature were included in the study after getting an informed consent from the parents.

Approval from institutional ethics committee was obtained. Children with major medical illness, those with contraindications for clonidine stimulation test, syndromic children, girls with bone age>14 years and boys with bone age>16 years were excluded from the study.

Demographic profile and baseline clinical and laboratory data were recorded in a performa. Detailed clinical and physical examination of the patient with special emphasis to the auxology was done. Baseline blood investigations were done to rule out chronic infections, liver disease, chronic renal disease, thyroid disorder and rickets.

It was followed by MRI brain to visualise structural anomalies of the pituitary. Boys and girls above eight years of age with tanner stage less than 2 were primed with sex steroids either inj.testosterone or oral estrogen.[13]

The patients were admitted and advised overnight fasting. In the morning blood sample was taken for 0 growth hormone, serum IGF-1, and serum IGFBP-3. After that clonidine was given at the dose of 0.15 mg/m$^2$.

Serial blood samples were taken for growth hormone at 30, 60, 90, 120. During the procedure child was closely monitored for hypotension and excessive drowsiness. All samples were analysed by chemiluminescence immunoassay.

***Statistical methods***

The collected data were coded and entered into Microsoft Excel to make a spread sheet and was analysed using SPSS for windows version 21. All variables were

summarised as percentages. Graphical summarisations of the data were also done by way of bar diagrams and Pie diagrams. For categorical measurements, the comparisons of proportions were done by Pearson Chi-square test or Fishers exact test as appropriate.

The diagnostic value of IGF-1 and IGFBP-3 will be assessed by using variables like sensitivity, specificity, predictive value and test accuracy (with special emphasise to sensitivity and specificity).

Sample is again divided in to less than 10 years and more than 10 years for assessing the diagnostic values in each age group. A ROC curve was also made to assess the diagnostic value of IGF-1 and IGFBP-3.

## RESULTS

A total of 90 children with short stature were enrolled in the study, comprising 55 males and 35 females. 26 children were less than 10 years of age. Most of the study population had normal birth weight (88.9%) and normal BMI (77%). 15 patients had abnormal MRI features like anterior pituitary hypoplasia or truncated pituitary stalk. Four out of the 90 patients had significantly low serum cortisol and low level of TSH. They were considered to have multiple pituitary hormone deficiency (MPHD).

Clonidine stimulation test was positive in 36 out of the 90 patients (40%). These patients were considered as growth hormone deficient. In the age group less than ten years 36% patients had growth hormone deficiency and in the age group of more than ten years 42% patients had growth hormone deficiency.

**Table 1: Diagnostic value in terms of sensitivity and specificity of IGF-1 and IGFBP-3.**

| Test | Study group | Sensitivity (%) | Specificity (%) |
|---|---|---|---|
| **IGF-1** | Total population | 86.1 | 87 |
| | ≤10 years | 100 | 76.5 |
| | >10 years | 81.5 | 91.9 |
| **IGFBP-3** | Total population | 61.1 | 94.4 |
| | ≤10 years | 88.9 | 100 |
| | >10 years | 51.5 | 91.9 |
| **Two test combined IGF-1 or IGFBP-3** | Total population | 86.1 | 83.3 |
| | ≤10 years | 100 | 76.5 |
| | >10 years | 81.5 | 86.5 |
| **Two test combined IGF-1 and IGFBP-3** | Total population | 61.1 | 98.1 |
| | ≤10 years | 88.9 | 100 |
| | >10 years | 51.5 | 97.3 |

**Table 2: Diagnostic value in terms of predictive value of IGF-1 and IGFBP-3.**

| Test | Study group | PPV (%) | NPV (%) |
|---|---|---|---|
| **IGF-1** | Total population | 81.6 | 90.4 |
| | ≤10 years | 62.2 | 100 |
| | >10 years | 88 | 87.2 |
| **IGFBP-3** | Total population | 88 | 78.5 |
| | ≤10 years | 100 | 94.4 |
| | >10 years | 82.4 | 72.3 |
| **Two test combined IGF-1 or IGFBP-3** | Total population | 77.5 | 90 |
| | ≤10 years | 69.2 | 100 |
| | >10 years | 81.5 | 86.5 |
| **Two test combined IGF-1 and IGFBP-3** | Total population | 95.7 | 79.1 |
| | ≤10 years | 100 | 94.4 |
| | >10 years | 93.3 | 73.5 |

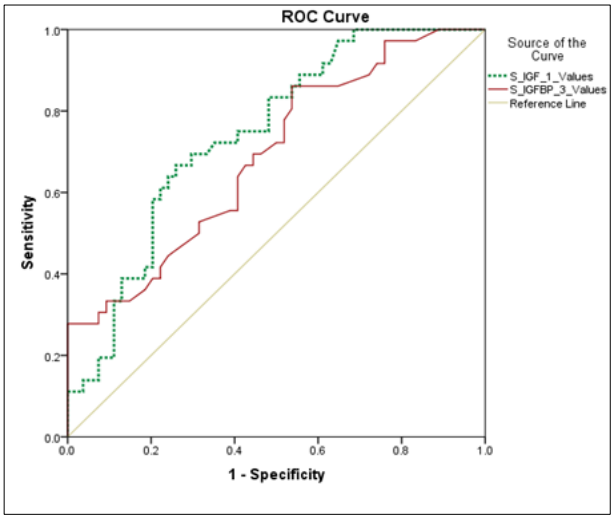


**Figure 1: ROC to represent the diagnostic value of IGF-1 and IGFBP-3.**

**Table 3: Diagnostic value in terms of accuracy of IGF-1 and IGFBP-3.**

| Test | Study group | Accuracy |
|---|---|---|
| **IGF-1** | Total population | 86.6 |
| | ≤10 years | 84.6 |
| | >10 years | 87.5 |
| **IGFBP-3** | Total population | 81.1 |
| | ≤10 years | 96.2 |
| | >10years | 75 |
| **Two test combined IGF-1 or IGFBP-3** | Total population | 84.4 |
| | ≤10 years | 84.6 |
| | >10 years | 84.4 |
| **Two test combined IGF-1 and IGFBP-3** | Total population | 83.3 |
| | ≤10 years | 96.2 |
| | >10 years | 78.2 |

The sensitivity and specificity of IGF-1 in the total population was 86.1 % and 87% respectively. Similarly IGFBP-3 had a sensitivity of 61.1% and specificity of 94.4%. In the younger age group IGFBP-3 had higher specificity (100%). Even though the sensitivity of IGF-1 was high (100%) in the younger age group its specificity was low (76.5%). In the older age group IGF-1 was the sensitive test (81.5%). Sensitivity of IGFBP-1 was very low (51.5%). Both the tests had same specificity. Combining the tests improved the specificity in all the age groups (Table 1).

In the whole population PPV of IGF-1 and IGFBP-3 were 81.6% and 88% respectively. In the younger age group PPV of IGF-1 was very low (62.2%).But IGFBP-3 had a 100% PPV and a comparable NPV (94.4%). In the older age group both PPV and NPV of IGF-1 was better than IGFBP-3 (Table 2).

The accuracy of IGF-1 was maximum in the older age group whereas IGFBP-3 had a better accuracy in the younger age group (Table 3).

Sensitivity and specificity of IGF-1 and IGFBP-3 were assessed by creating a Receiver operating Curve (figure 1). Area under the curve showed that IGF-1 as a good diagnostic test (AUC= 0.742) and IGFBP-3 as a sufficient test (AUC= 0.689). P value of both the tests were significant. It means that both the diagnostic tests actually do discriminate between those with growth hormone deficiency and those without deficiency (Table 4).

**Table 4: Area under curve.**

| Variable | Area | Std. Error | P Value | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| **S IGF 1** | 0.742 | 0.051 | 0 | 0.641 | 0.843 |
| **S IGFBP 3** | 0.689 | 0.056 | 0.003 | 0.578 | 0.799 |

## DISCUSSION

The diagnosis of growth hormone deficiency is conventionally achieved by the use of provocative growth hormone stimulation test which has been considered as the gold standard. But these tests are time consuming, costly and unpleasant for the patients. Therefore additional parameters to assess the growth hormone deficiency have been searched for years.[14]

90 children with short stature were included in the study. We studied the diagnostic parameters in the whole population and in the prepubertal (<10 years) and pubertal (>10 years) age groups separately. 40% of the study population had growth hormone deficiency (positive clonidine stimulation test). 42.2% and 27.7% patients had IGF-1 and IGFBP-3 positivity respectively.

In the prepubertal group IGFBP-3 had maximum specificity (100%) and PPV (100%), even though it was IGF-1 which had more sensitivity. Multiple factors might have played their role in this age group especially nutritional status.

In the pubertal age group IGF-1 had better performance with high sensitivity and PPV. The ROC showed that IGF-1 is a "good diagnostic test" and IGFBP-3 a "sufficient diagnostic test". Combined use of IGF-1 and IGFBP-3 improved the diagnostic values considerably.

In a similar study done by Anders Juul at Denmark 15showed almost similar findings. In their study both tests had sufficient PPV in the prepubertal age group. But in the pubertal age group (>10years) the predictive value of IGF-1 and IGFBP-3 was diminished. The diagnostic superiority of IGFBP-3 over IGF-1 in younger age group has been supported by other studies like that of Ranke MB16. In their study IGFBP-3 had a sensitivity of 97% and specificity of 95% in younger children.

**Table 5: Performance of IGF-1 in previous studies and present study-comparison.**

| Study | Sensitivity (%) | Specificity (%) |
|---|---|---|
| **Granada et al[17]** | 86.2 | 99.3 |
| **Cianfarani[19]** | 69 | 81 |
| **Mitchell et al[20]** | 62 | 47 |
| **Juul et al[15]** | 76 | 72 |
| **Hasegawa[18]** | 100 | 82 |
| **Blum et al[7]** | 92 | 54 |
| **Present study** | 86.1 | 87 |

**Table 6: Performance of IGFBP-3 in previous studies and present study-comparison.**

| Name of the study | Sensitivity | Specificity |
|---|---|---|
| **Granada et al[17]** | 70.4 | 96.7 |
| **Cianfarani[19]** | 27 | 100 |
| **Mitchell[20]** | 14.9 | 98 |
| **Juul et al[15]** | 68 | 79 |
| **Hasegawa[18]** | 92 | 69 |
| **Blum et al[7]** | 97 | 95 |
| **Present study** | 61.1 | 94.4 |

Comparison of performance of IGF-1 and IGFBP-3 in previous studies and present study are shown in Table 5 and Table 6 respectively.

Our findings regarding the diagnostic value of IGF-1 and IGFBP-3 are in accord with some of the above mentioned studies (Granada et al, Hasegawa etc.), but in contrast to those of Cinafarani and Mitchell who found an extremely poor diagnostic value for IGFBP-3.[17-20]

In the study by Mitchell et al the diagnostic value of IGFBP-3 was less in both prepubertal and pubertal age group. This discrepancy may be due to the fact that these researchers studied a relatively different sample size and used a different cut off value for clonidine stimulation test. In addition other factors like BMI and nutritional status might have affected the diagnostic values.

## CONCLUSION

On conclusion IGF-1 and IGFBP-3 either alone or together can be used for the diagnosis of growth hormone deficiency instead of the unpleasant provocative tests. The diagnosis of growth hormone deficiency is hurdled with various reasons in resource limited countries like India. This restricts the availability of growth hormone therapy to only a segment of the children in India. An early initiation of therapy could increase the chance of achieving normal adult height. So similar studies from other parts of India will help to fill the gaps in diagnosis and treatment of GHD. Simple diagnostic methods like IGF-1 and IGFBP-3 deserves further exploration as it might reduce the investigation of GHD to a single blood test.

## ACKNOWLEDGEMENTS

Authors would like to thank Research department, Jubilee Mission Medical College, Dr Sharma and Mrs Kumudham for the support during the course of the research.

*Funding: No funding sources*
*Conflict of interest: None declared*
*Ethical approval: The study was approved by the Institutional Ethics Committee*

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**Cite this article as:** Thomas HC, Kumar S, Bisto AA. The usefulness of serum IGF-1 and serum IGFBP-3 for the diagnosis of growth hormone deficiency in comparison to clonidine stimulation test: a prospective cohort study. Int J Contemp Pediatr 2021;8:327-32.