

## EVALUATION OF INSULIN-LIKE GROWTH FACTOR-1 AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 IN DIAGNOSIS OF GROWTH HORMONE DEFICIENCY IN SHORT-STATURE CHILDREN

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**Background:** Growth Hormone Deficiency (GHD) is conventionally diagnosed and confirmed by diminished peak Growth Hormone (GH) levels to provocative testing. Serum Insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) are under the influence of GH and reflect the spontaneous 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 Exercise and L-Dopa stimulation tests in the diagnosis of growth hormone deficiency in short stature children using ITT as gold standard. **Methods:** This validation study was conducted at Department of Chemical Pathology and Endocrinology, AFIP, Rawalpindi, from November 2005 to October 2006. Fifty-two short stature children were included in the study. Basal samples for GH levels and simultaneous IGF-1 and IGFBP-3 measurements were obtained and afterwards all children were subjected to sequential exercise and L-Dopa stimulation tests. Insulin Tolerance Test (ITT) was performed one week later with all the necessary precautionary measures. On the basis of ITT results, children were divided into two groups, i.e., 31 growth hormone deficient and 21 Normal Variant Short Stature (NVSS). **Results:** The diagnostic value of exercise stimulation test remained highest with sensitivity 90.3%, specificity 76.0%, Positive Predictive Value (PPV) 84.84%, Negative Predictive Value (NPV) 84.2% and accuracy 84.6%. The conventional L-Dopa stimulation had sensitivity 96.7%, specificity 38.0%, PPV 69.7%, NPV 88.8 % and accuracy 73.0%. The serum IGF-1 and IGFBP-3 levels were positively correlated with post ITT peak GH levels ( $r=0.527$ ,  $r=0.464$  respectively, both  $p<0.001$ ). The diagnostic value of IGF-1 had sensitivity 83.87%, specificity 76.2%, PPV 83.87%, NPV 76.2% and accuracy 80.76%. The diagnostic value of IGFBP-3 had sensitivity 54.83%, specificity 90.47%, PPV 89.47%, NPV 57.57% and accuracy 69.23%. With combined use of IGF-1 and IGFBP-3 diagnostic value had sensitivity 69.35%, specificity 83.33% PPV 86%, NPV 64.81% and accuracy 75%. **Conclusion** Growth Hormones provocative tests still remain the most useful investigations for the diagnosis of GHD. Measurements of IGF-1 and IGFBP-3 have shown comparable diagnostic performance with growth hormone stimulation tests and are valuable for patients' convenience and ease of performance and can be useful in the initial workup of short stature.

**Keywords:** Growth Hormone Deficiency, Insulin-like Growth Factor-1, IGF-1, Insulin-like Growth Factor Binding Protein-3, Normal Variant Short Stature

### INTRODUCTION

Short stature is a common cause of concern among children and their parents. The majority of short stature presentations in children are normal variations reflecting the expected distribution of height according to the age and sex of the individuals which may or may not be familial.<sup>1</sup> The common causes of short stature include familial short stature, malnutrition, chronic illnesses, hypothyroidism, constitutional delay, psychosocial dwarfism, growth hormone deficiency (GHD), and other endocrine and genetic disorders.<sup>2</sup> GHD is suspected in subjects with short stature and reduced growth velocity in whom other causes of poor growth have been excluded. The incidence of GHD is estimated to be in 1:4000 to 1:3500.<sup>2</sup> GHD is responsible for 14% cases of the short stature in hospital setting.<sup>3</sup> The

diagnosis of GHD cannot be made on the basal values of serum growth hormone (GH) because of the pulsatile nature of GH secretion, being low in day time and increasing during sleep, stress, exercise and after meals.<sup>2-4</sup> The diagnosis of GHD rests upon demonstration of an inadequate rise in serum GH levels after exercise or pharmacological stimulation or upon some other measures of GH secretion.<sup>2-4</sup> Since 10% or more of healthy children do not have an adequate rise in GH levels with only one stimulation test, at least two stimulation tests are required for assessing GH reserves before the diagnosis of GHD is assigned.<sup>2,4</sup> The main disadvantages of these provocative tests are: (a) lack of generally agreed values of response of GH levels after these tests, (b) time consuming, unpleasant and rather distressing nature of the tests, and (c) other technical/logistic problems related to these tests.<sup>3-5</sup> Generally a

rise above 10 ng/ml in serum GH level in a single test rules out GHD.<sup>24</sup>

Insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) measurements are relatively newer methods for evaluating GHD or GH adequacy. IGF-1, produced by liver and bound to IGFBP-3 and acid labile subunit (ALS) in circulation, is the main mediator of actions of GH.<sup>6-8</sup> IGF-1 and IGFBP-3 are dependent on GH status and correlate significantly with the integrated GH secretion.<sup>6-8</sup> Individual measurements of these factors may provide information about GH secretion integrated over previous several days.<sup>6-8</sup> 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.<sup>7,8</sup> IGF-1 has better sensitivity in GHD, whereas IGFBP-3 has greater specificity.<sup>9,10</sup> Moreover, there are certain conditions in which either of these two markers have been reported to be of low diagnostic yield. Therefore, IGF-1 and IGFBP-3 complement each other.<sup>9,10</sup>

## MATERIAL AND METHODS

The study was conducted in the department of Chemical Pathology and Endocrinology; Armed Forces Institute of Pathology (AFIP), Rawalpindi, from November 2005 to October 2006. It was a validation study and was approved by the ethical committee of the institute. Fifty two (n=52) short stature children were included in the study. Sampling was done by non probability convenience. Children less than 15 years of age irrespective of sex and ethnic groups reporting to AFIP for evaluation of short stature were included in the study whereas patients with known endocrinopathies (e.g., hypothyroidism), patients on treatment for GHD, malnutrition (weight <10<sup>th</sup> percentile) and with other chronic ailments like congenital heart diseases, respiratory diseases and renal diseases were excluded from the study. Full informed consent was obtained from the parents of the all the children who were selected for the study based on the above-mentioned inclusion and exclusion criteria. Parents were briefed to accompany their children at 8:00 AM with overnight fasting for exercise stress test and L-Dopa stimulation test. After an overnight fast, subjects were made to relax for half an hour before the actual test procedure and 4 ml of blood was drawn for basal GH level and simultaneous IGF-1 and IGFBP-3 estimation. The children were then asked to exercise on a treadmill for 15–20 min under the supervision of the attending doctor. Children were continuously motivated by the attending doctor and parents for putting in maximum effort to maintain the heart rate between 160–180 beats/min, which was regularly monitored by the attending doctor. Blood samples for serum GH response were drawn 5–10 min after the completion of exercise. Oral L-Dopa tablets

were given along with routine breakfast at 9:00 AM as per the standard regimen: 125 mg if weight is less than 15 Kg; 250 mg if weight is between 15–30 Kg; 500 mg if weight is more than 30 Kg. Blood samples were drawn 90 min after oral L-Dopa for serum GH level response. Insulin Tolerance Test (ITT) was performed after one week with all the necessary precautionary measures. The strict ITT protocol was followed, i.e., 1) child must fast overnight, 2) blood glucose must be >3.0 mmol/l at 0 minutes, i.e., just before insulin administration, and 3) oxygen, glucose, and hydrocortisone must be available. An indwelling intravenous cannula was passed and was kept open with a 100 IU Heparin solution for subsequent sampling. After an overnight fast, subjects were made to relax for half an hour before the actual test procedure. Blood was then collected for fasting glucose and basal GH levels. Plain insulin at the dose of 0.1 unit/Kg body weight was administered intravenously. Child was then observed for development of hypoglycaemic symptoms like palpitations, headache, feeling of hunger, dizziness, somnolence, and perioral and frontal head sweating. At the time of induction of hypoglycaemic symptoms blood was collected for glucose levels. A blood glucose level <2.2 mmol/l (checked on the spot with glucometer) was used as the biochemical confirmation of hypoglycaemia. After the blood collection during induction phase, hypoglycaemia was reverted by infusion of 25% dextrose at the dose of 0.8 ml/kg body weight. Whole procedure was supervised by the attending doctor. Sample for IGF-1 and IGFBP-3 were stored in aliquots at -25 °C till further analysis in batches. Samples stored at or below -25 °C are stable for 12 months.<sup>11</sup>

The serum GH, IGF-1 and IGFBP-3 were assayed by chemiluminescence method on Immulite 1000, an automated immunoassay analyser by Diagnostic Products Corporation (DPC), Los Angeles, USA. Analytical sensitivity of the GH assay was 0.01 ng/mL (0.03 mIU/L). The inter-assay coefficient variation (CV) was 5.7% at the mean concentration of 3 ng/ml and 6.1% at the mean concentration of 18 ng/ml. The intra-assay (within-assay) CV was 5.3% at the mean concentration of 1.7 ng/ml and 6.5% at the mean concentration of 31 ng/ml.<sup>12</sup> For converting the GH results from ng/ml to mIU/L and/or vice versa, the conversion factor of 2.6 was used.<sup>12</sup> Serum IGF-I concentrations were measured after separation of IGF-1 from IGFBPs by acid-ethanol extraction. Analytical sensitivity of the IGF-1 assay was 20 ng/ml. At the mean IGF-1 concentration of 49 ng/ml, the intra-assay CV was 3.1% and total CV was 6.1%. At the mean IGF-1 concentration of 955 ng/ml, the intra-assay CV was 3.5% and total CV was 5.8%.<sup>13</sup> Analytical sensitivity of the IGFBP-3 assay was 0.02 µg/ml. At the mean IGFBP-3 concentration of 0.55µg/ml, the

intra-assay CV was 3.6% and total CV was 9.1%. At the mean IGFBP-3 concentration of 7.80 µg/ml, the intra-assay CV was 4.2% and total CV was 8.5%.<sup>14</sup>

All the data including demographical, clinical details and biochemical parameters were stored and compiled for SPSS Version 15.0. Descriptive statistics were carried out to summarise the data. Mean and standard deviation were calculated and recorded for numerical data including age, post-stimulation GH levels after exercise, L-Dopa and after ITT (Table-1). IGF-1 and IGFBP-3 levels of individual children were compared with reference values (at central 95% confidence interval) provided in the kit literature according to the age groups. Frequency and percentage were calculated for qualitative data including Growth hormone deficient, and normal variant short stature (NVSS) children. Pearson's correlation test was used to correlate the serum IGF-1 and IGFBP-3 levels with the peak GH levels achieved with the ITT (Table-2). A *p*-value of <0.05 was considered as significant. Considering ITT as gold standard the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of exercise stimulation test, L-Dopa stimulation test, IGF-1 and IGFBP-3 were calculated by using 2x2 contingency table.

**RESULTS**

Out of the total 52 subjects males were 32 (62%) and females were 20 (38%). In this study mean age of the children who presented for short stature evaluation was 11.0±2.40 years. Serum GH ≥20 mIU/L were taken as cut-off levels for a normal response. For the IGF-1 and IGFBP-3 levels, the lower limit of the reference range at 2.5 centile (calculated at central 95% confidence interval) provided by the kit manufacturer was taken as

the cut-off level for GH sufficiency or deficiency. The diagnosis of GHD was made on maximal growth hormone response on ITT of < 20 mIU/L. The children who had the maximal growth hormone response of >20 mIU/L were diagnosed as NVSS. On the basis of ITT results, children were divided into two groups, i.e., 31 growth hormone deficient (GHD) and 21 normal variant short stature (NVSS). The serum IGF-1 and IGFBP-3 levels were positively correlated (Table-2) with post-ITT peak GH levels (*r*=0.527, *r*=0.464 respectively, both *p*<0.001). The Sensitivity, Specificity, PPV and NPV of exercise stimulation test, L-Dopa stimulation test, and serum IGF-1 and serum IGFBP-3 individually and in combination were calculated taking ITT as gold standard. The results of diagnostic performance of individual tests are given in Table-3.

**DISCUSSION**

This study evaluated the utility of IGF-1 and IGFBP-3 levels in diagnosing GHD in comparison with the GH stimulation tests, i.e., exercise stimulation test and L-Dopa stimulation test in short stature children. The studied group was divided in two categories, i.e., GHD and NVSS.

The results showed that exercise stimulation test was the single most sensitive (90.3%) and specific (76%) test, with PPV 84.84%, NPV 84.2% and an overall accuracy of 84.6% for the diagnosis of GHD. This is in accordance with the studies carried out by Nicol<sup>15</sup> and Liberman<sup>16</sup>. However the usefulness of exercise stimulation test is limited by the fact that continuous motivation of the child is required and any unwilling or less motivated child may result in a false positive case. Moreover very short children or children of less than 5 years of age can hardly perform it.

**Table-1: GH levels achieved after individual GH stimulation test**

	GHD* (Mean±SD)		NVSS**	
	Mean	SD	Mean	SD
Exercise stimulation test	8.30 mIU/l	±6.20	26.80 mIU/l	±8.0
L-Dopa stimulation test	3.85 mIU/l	±4.30	16.50 mIU/l	±8.0
Insulin Tolerance test	8.95 mIU/l	±6.0	27.1 mIU/l	±6.0

\*GHD= Growth hormone deficient children, \*\*NVSS= Normal variant short stature children

**Table-2: Correlation of IGF-1 & IGFBP-3 with post-ITT GH levels**

		IGF-1 (ng/ml)	IGFBP-3 (µg/ml)	Post ITT GH (mIU/L)
IGF-1 (ng/ml)	Pearson Correlation	1	0.477	0.527
	<i>p</i> -value		0.000	0.000
IGFBP-3 (µg/ml)	Pearson Correlation		1	0.464
	<i>p</i> -value	0.000		0.001
Post ITT GH (mIU/L)	Pearson Correlation	0.527	0.464	1
	<i>p</i> -value	0.000	0.001	

**Table-3: Diagnostic performance of individual tests**

Measure	Sensitivity	Specificity	PPV	NPV	Accuracy
Exercise simulation test	90.3%	76.0%	84.84%	84.2%	84.6%
L-Dopa stimulation test	96.7%	38.0%	69.76%	88.8%	73%
Serum IGF-1	83.87%	76.19%	83.87%	76.19%	80.76%
Serum IGFBP-3	54.83%	90.47%	89.47%	57.57%	69.23%
Combined (IGF-1 & IGFBP-3)	69.35%	83.33%	86.0%	64.81%	75%

On the other hand L-Dopa stimulation test had greater sensitivity (96%) but very low specificity (38%). One of the reason for its greater sensitivity was the more number of GHD (n=31/52) than NVSS (n=21/52) subjects in the study population. In this study, L-Dopa could only detect 8 individuals out of 21 NVSS subjects yielding low specificity. The reason for inability of the L-Dopa stimulation test to raise the GH levels to the threshold of 20 mIU/L was probably the sampling timings. In this study the protocol that was followed was to collect blood samples at 90 min interval after ingestion of L-Dopa, while various studies recommend 30, 60, 90, and 120 min sequential sampling as there is greater inter-individual variability in the GH response after L-Dopa administration.<sup>17,18</sup> Secondly, there is considerable variation in the potency of various stimulation tests. As shown in this study (Table-1) that the peak GH levels after L-Dopa stimulation test in NVSS were 16.50±8.0 mIU/L, that were much below the cut-off level. Biller *et al*<sup>19</sup> have recommended that the test specific cut-off levels be followed while interpreting the results of GH provocative tests. Their study revealed that the mean peak response in GHD individuals was 0.13±0.22 µg/L, while in control subjects the mean peak GH response was 3.3±4.9 µg/L.

We demonstrated a significant positive correlation between peak GH levels after ITT and IGF-1 and IGFBP-3 levels ( $r=0.527$  and  $r=0.464$  respectively, both  $p<0.001$ ), which is in accordance with the different studies carried out by Blum WF *et al*<sup>20,21</sup> and Biller *et al*<sup>19</sup> and others. In this study IGF-1 was the second best test after exercise stimulation test. Serum IGF-1 had a sensitivity of 83.87% and specificity of 76.19%, which is in agreement with the studies carried out by Hasegawa *et al*<sup>22</sup>, Granada *et al*<sup>23</sup> and Juul *et al*.<sup>24</sup> Cianfarani *et al*<sup>25</sup> in their study have documented 86% sensitivity and 99% specificity of IGF-1. In the same study they documented 91% specificity of IGF-1 measurement in children younger than 11 years and only 53% in the older ones, probably reflecting the lack of appropriate reference standards for bone age and pubertal stage. However, other intrinsic factors may affect the accuracy of IGF-1 assessment in the diagnosis of GHD. The biological variability in IGF-1 measurements is up to 32% in the same subject when tested on different days.<sup>26</sup> Furthermore, genetic determinants account for 40% of the variability in serum IGF-1.<sup>27</sup> With a PPV 83.87%, NPV 76.19% and an overall accuracy of 80.76%, IGF-1 is a very useful screening test for GHD.

Serum IGFBP-3 measurements in the present study had sensitivity 54.83% and specificity 90.47%. The findings of this study are consistent with the studies carried out by Granada *et al*<sup>23</sup> and Cianfarani *et al*<sup>28,29</sup> Only few studies have reported IGFBP-3

sensitivity equal or greater than 90%<sup>30-32</sup> whereas many previous studies have shown IGFBP-3 sensitivity ranging between 22–79%.<sup>33-36</sup> One of the mechanisms proposed to explain the low sensitivity of IGFBP-3 measurement in the diagnosis of GHD is that IGFBP-3 concentrations reflect the total circulating IGF concentrations, i.e., IGF-1+IGF-2, but IGF-2 is less GH dependent than IGF-1.<sup>37,38</sup> The other reason is that there are two immunoreactive forms of IGFBP-3, a 42-kDa and a 29-kDa form, the former representing the intact form, the latter the major fragment of IGFBP-3.<sup>25,39</sup> Cianfarani *et al* in their study have reported that IGFBP-3 proteolysis is increased in GHD, at least up to young adulthood, and might affect the results of IGFBP-3 measurements.<sup>25,28</sup> On the other side, higher specificity of IGFBP-3 for the diagnosis of GHD has been generally reported by various studies to be high.<sup>23-25,40</sup>

The high sensitivity of IGF-1 and higher specificity of IGFBP-3 as evidenced in this study and other studies makes the combined use of IGF-1 and IGFBP-3 very much rational and improves the diagnostic value. In this study, the combined diagnostic value had sensitivity 69.3%, specificity 83.33%, and accuracy up to 75%. This is in agreement with the findings of Jaruratanasirikul.<sup>10</sup>

The findings of this study support the evidence that IGF-1 and IGFBP-3 reflect the GH status of spontaneous endogenous secretion and can be useful as screening investigations for initial work up of GH evaluation in short stature children. Therefore, short stature children whose IGF-1 and IGFBP-3 levels fall clearly within the normal range for chronological age, support the exclusion of GHD. On the contrary, short children whose IGF-1 and IGFBP-3 are very low for chronological age are very much likely to be GHD.

Because of the comparable performance of IGF-1 and IGFBP-3 as compared to GH stimulation tests, the normal levels of IGF-1 and IGFBP-3 can exclude GHD. Low concentrations of IGF-1 and IGFBP-3 suggest GHD and the borderline low IGF-1 or IGFBP-3 concentrations may indicate insufficient GH secretion. In these latter two categories GH provocative tests are indicated for definite diagnosis.

However, interpretation of IGF-1 and IGFBP-3 concentration has a number of significant limitations. IGF-1 concentrations are markedly age-dependent. The levels are low in children younger than 5 years of age making it difficult to discriminate between normal and GHD children in this age group. In addition, the various factors can affect the IGF-1 and IGFBP-3 concentration such as under-nutrition, chronic illness, hypothyroidism, renal failure, hepatic failure, and diabetes mellitus.<sup>41-43</sup> Therefore, all these conditions need to be evaluated before measuring IGF-1 and IGFBP-3 concentrations.

## CONCLUSION

Growth hormone stimulation tests performed relatively better but the estimation of serum IGF-1 and IGFBP-3 have given comparable diagnostic performance in the evaluation of GHD when compared with exercise and L-Dopa stimulation tests. Measuring serum IGF-1 and IGFBP-3 are valuable for patients' convenience and ease of performance and can be used in the initial work up of GHD in short stature children.

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