Research Article

The Relevance of Insulin-Like Growth Factor-1 and Insulin-Like Growth Factor-Binding Protein-3 Concentrations as a Screening Test for Diagnosis of Growth Hormone Deficiency

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Abstract

Background and objective: *Growth hormone deficiency (GHD) is one of the most important endocrine and treatable causes of short stature. Reports regarding the sensitivity and specificity of insulin-like growth factor-1 (IGF-1) and IGF binding protein-1 (IGFBP-3) are not consistent. The aim of our study was to analyze the relevance of IGF-1 and IGFBP-3 concentrations as a screening test for diagnosis of GHD.*

Design: *We retrospectively studied 40 patients whom were evaluated for short stature at the Endocrinology Department of King Fahad Armed Forces Hospital, Jeddah, Saudi Arabia between January 2015 to December 2018. For IGF-1 and IGFBP-3 concentrations, laboratory reference ranges were based on age and sex. For all eligible patients, IGF-1 and IGFBP-3 concentrations were determined and an insulin tolerance test (ITT) was performed. Patients with a peak GH of ≤5.0 ng/ml were considered to be GHD.*

Results: *We retrospectively included 40 patients evaluated for SS for analysis. Mean age was 14.7 ±1.7 years. There were 38 males (80.9%) and 9 females (19.1%) and mean IGF-1 concentration was 146.4 ±69.4 ng/dl. The observed male to female ratio was 4.2:1. Results from the ITT indicated that 27 (57.4%) had GHD. Age was not statistically significant different between GHD (14.7 ±1.8 years) and non-GHD (14.8 ±1.6 years), p=0.9. Moreover, there was non statistical significant more males (59%) than females (50%) in the GHD patients, P=0.7. In addition, there were not statistically significantly different between GHD and non-GHD patients in mean IGF-1 concentration (156.0 ±71.1 ng/dl vs. 140.8 ±68.1 ng/dl, p=0.5) and IGFBP-3 concentration (3752.9 ±1295.9mcg/L vs. 3816.8 ±867.0mcg/L, p=0.9). The mean peak for GH concentration was significantly lower in patients with GHD than without GHD (2.2 ±1.3 ng/ml vs. 9.9 ±5.6 ng/ml, p<0.0001). Peak GH concentration was not significantly positively correlated with IGF-1 concentration (r=0.181, P=0.3) and IGFBP-3 concentration (r=0.103, P=0.5). With a threshold of IGF-1 concentration, sensitivity was 48% (95% confidence interval (95%CI); 26%, 70%), specificity was 37% (95%CI; 16%, 62%) and the negative predictive value for the diagnosis of GHD was 39% (95% CI; 24%, 57%). With a threshold of IGF-1+IGFBP-3 concentration, the sensitivity was 19% (95% CI; 5%, 42%) and the specificity was 89% (95%CI; 67%, 99%). A positive predictive value of 67% (95% CI; 29%, 91%) but a negative predictive value of 50% (95%CI; 44%, 56%). 17 of the patients with IGF-1+IGFBP-3 concentration above the threshold (N = 34) were normal and 17 had GH deficiency. These 17 GHD patients had IGF-1+IGFBP-3 concentration below the reference range for age and sex that did not differ significantly from those of their GH-sufficient counterparts (66.7% vs 50% , P=0.7) respectively. If IGF-1+IGFBP-3 concentration was used as a screening test (with a concentration threshold below the reference range for age and sex) and ITT as a confirmatory test, 34 (85%) out of 40 ITT would not have been performed, leading to the misdiagnosis of 17 GH-deficient adults. Thus, in our study population, such a procedure would misdiagnose 17 out of 21 GHD patients (81%) and yield a sensitivity of 19%.*

Conclusion: *Our study demonstrated the good negative predictive value of IGF-1+IGFBP-3 concentration for the diagnosis of GHD, making it possible to minimize the use of the "reference test" method ITT. This observation remains to be validated by population-based studies.*

Introduction

Growth is a continuous biologic process subject to genetic, environmental, nutritional and hormonal influences. Altered growth potential may result from disturbance of any of these factors. Short stature, a common problem in child population of developing countries, is defined by height or length below 3rd percentile or less than 2 standard deviation for that specific age and sex [1, 2].

Common endocrine disorder leading to short stature includes growth hormone deficiency (GHD) [3]. Childhood onset GHD has been estimated to occur in 1 per 30 000 people per year [4]. In adult onset GHD, an annual incidence of 1.2 per 100 000 adults has been estimated [5]. The prevalence of GHD in children with short stature ranges from 2.8% to 69% with the national prevalence of 11% [6-11].

There are two growth factors; insulin-like growth factor-1 (IGF-1) and IGF-2 and up to six transporter proteins. Though IGF binding protein-1 (IGFBP-1) and IGFBP-3 are the two most studied. IGF-1 is the metabolic effectors of growth hormone (GH). It is produced by the liver and is mainly controlled by GH [12]. IGF-1 concentration is not recommended to establish the diagnosis of GHD, mainly due to the overlap of IGF-1 concentrations between normal and GH-deficient subjects [13]. If GHD is suspected, IGF-1 and IGFBP-3 levels must be measured and a study of GH secretion should be carried out. Values of IGF-1 or IGFBP-3 which are more than 2 SD below the normal range suggest a serious disorder of the GH axis, if other causes have been ruled out (malnutrition, liver diseases, and hypothyroidism) [14]. Dynamic tests are currently recommended for the diagnosis of GHD: the insulin tolerance test (ITT) is considered as the reference test [15-18].

An obvious difficulty in determining the diagnostic performance of any test for GHD is the lack of a gold standard to determine true GHD; thus, reports regarding the sensitivity and specificity of IGF-I and IGFBP-3 are not consistent. In general, however, both IGF-I and IGFBP-3 are reported to have good specificity but relatively poor sensitivity for GHD [19, 20]. Although these measures are not useful in isolation, they can be helpful when combined with other diagnostic measures with higher sensitivity [20, 21]. Another advantage of IGF-I and IGFBP-3 is that they show superior reproducibility in comparison to stimulated GH levels [22].

To our knowledge, there have been no nationwide studies using uniform diagnostic criteria. Thus, we tried to improve the simplicity and safety of the diagnosis of GHD. The use of diagnostic strategy with IGF-1 and IGFBP-3 as the first screening step and the ITT as the second confirmatory step has not been studied well in a population admitted on routine endocrinological practice for short stature. The aim of our study was to analyze the relevance of IGF-1 and IGFBP-3 concentrations as a screening test for diagnosis of GHD.

Methods

We retrospectively studied 40 patients whom were evaluated for short stature (SS) at the Endocrinology Department of King Fahad Armed Forces Hospital, Jeddah, Saudi Arabia between January 2015 to December 2018. For IGF-1 and IGFBP-3 concentrations, laboratory reference ranges were based on age and sex. For all eligible patients, IGF-1 and IGFBP-3 concentrations were determined and an ITT was performed. The ITT consisted in the IV injection of 0.1 units of insulin/kg body weight. Blood samples were collected 0 (baseline), 30, 60, 90, and 120 min for GH. Blood glucose concentration was also determined to ensure that the patient was hypoglycemic if blood glucose concentration < 2.2 mmol/l. Patients with a peak GH of ≤5.0 ng/ml were considered to be GHD and patients with a peak GH of ≥5.1 ng/ml were considered not GHD (nGHD). Peak GH secretion during provocative testing is used to assess the capacity of the pituitary to release GH [17]. Blood was centrifuged, and serum was frozen with dry ice until analysis by an independent laboratory. Blood glucose was determined using a glucose oxidase method. GH concentration was determined using a radioimmunometric test, with IS 80/505 as interna- tional standard. This kit is specific for 20 KD and 22 KD human GH. The detection limit is 0.2 ng/ml. At 1.70 ng/ml, intra and inter assay coefficients of variation are 3.9% and 2.3%, respectively. IGF-1 and IGFBP-3 concentrations were determined using an immunoradiometric method (Unilabs Company, Germany) [23].

Statistical Analysis

Data are presented as means ± standard deviation or numbers (%). Quantitative variables were compared between two groups by using the Student's test. Differences in categorical variables were analysed using the chi-square test. The relationship between continuous variables was assessed using coefficients of correlation. The ability of IGF-1 and IGFBP-3 concentrations to discriminate between normal and GH-deficient patients was evaluated. Sensitivity, specificity and positive and negative predictive values were calculated for IGF-1 and IGFBP-3 concentrations. Diagnostic performance of IGF-1 in predicting GHD was assessed by calculating sensitivity, specificity, positive and negative predictive values. P value <0.05 indicates significance. The statistical analysis was conducted with SPSS version 23.0 for Windows.

Results

We retrospectively included 40 patients evaluated for SS for analysis. Mean age was 14.7 ±1.7 years (Table 1). There were 38 males (80.9%) and 9 females (19.1%) and mean IGF-1 concentration was 146.4 ±69.4 ng/dl. The observed male to female ratio was 4.2:1. Results from the ITT indicated that 27 (57.4%) had GHD (Table 2). Age was not statistically significant different between GHD (14.7 ±1.8 years) and non-GHD (14.8 ±1.6 years), p=0.9. Moreover, there was non statistical significant more males (59%) than females (50%) in the GHD patients, P=0.7. In addition, there were not statistically significantly different between GHD and non-GHD patients in mean IGF-1 concentration (156.0 ±71.1 ng/dl vs. 140.8 ±68.1 ng/dl, p=0.5) and IGFBP-3 concentration (3752.9 ±1295.9mcg/L vs. 3816.8 ±867.0mcg/L, p=0.9). The mean peak for GH concentration was significantly lower in patients with GHD than without GHD (2.2 ±1.3 ng/ml vs. 9.9 ±5.6 ng/ml, p<0.0001). Peak GH concentration was not significantly positively correlated with IGF-1 concentration (r=0.181, P=0.3) and IGFBP-3 concentration (r=0.103, P=0.5) (Figure 1 & 2). IGF-1 and IGFBP-3 concentrations according to GH deficiency status are demonstrated in (figure 3 and 4).

Table 1: Demographics [mean±standard deviation or number (%)]

Parameters		Total
Numbers		40
Age (years)		14.7 ±1.7
Gender	Male	32 (80)
	Female	8 (20)
IGF-1 (ng/dl)		148.8 ±69.2
IGFPB-3 (mcg/L)		3783.3 ±1099.7

Table 2: Comparison between patients with growth hormone deficiency (GHD) and non- GHD (nGHD) [mean±standard deviation or number (%)]

Parameters		GHD	nGHD	P value
Numbers		21 (52.5)	19 (47.5)	
Age (years)		14.7 ±1.9	14.7 ±1.7	0.9
Gender	Male	17 (53.1)	15 (46.9)	0.9
	Female	4 (50.0)	4 (50.0)	
IGF-1(ng/dl)		156.0 ±71.1	140.8 ±68.1	0.5
IGFPB-3(mcg/L)		3752.9 ±1295.9	3816.6 ±867.0	0.9
GH (Peak) (ng/ml)		2.2 ±1.2	10.1 ±5.7	<0.0001

GH, growth hormone; IGF-1, insulin-like growth factor ; IGFBP-3, Insulin-like Growth Factor-binding Protein-3
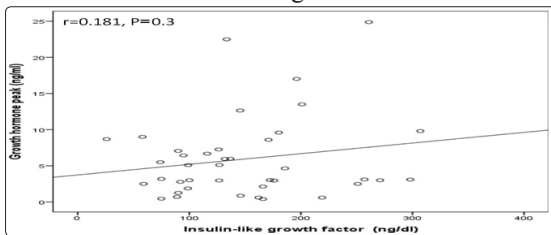

Figure 1: Correlation of insulin like growth facator-1 concentration and growth hormone peak during insulin tolerance test in the study population
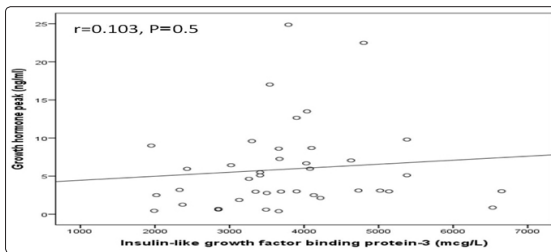

Figure 2: Correlation of insulin like growth factor binding protein-3 concentration and growth hormone peak during insulin tolerance test in the study population
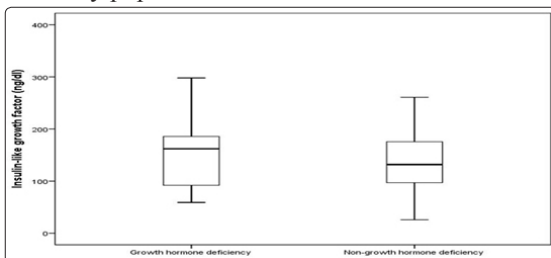

Figure 3: Insulin like growth facator-1 concentration in patients with and without growth hormone deficiency: crosses represent individual data. Boxes represent 25 and 75th percentiles, split by median, with error bars representing 5th and 95th percentiles.

Figure 4: Insulin like growth factor binding protein-3concentration in patients with and without growth hormone deficiency: crosses represent individual data. Boxes represent 25 and 75th percentiles, split by median, with error bars representing 5th and 95th percentiles.
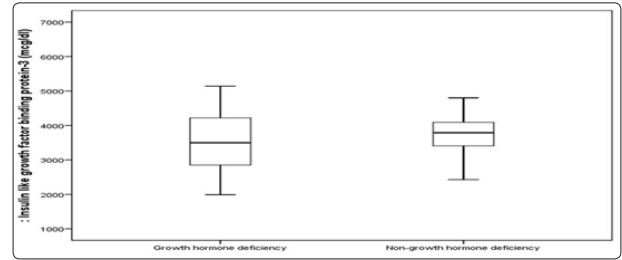
We tested the diagnostic accuracy of two thresholds using mean IGF-1 or IGF-1+IGFBP-3 concentrations with reference to age and sex according to the diagnosis of GHD as established using ITT (Table 3). An IGF-1+IGFBP-3 concentration threshold was selected to emphasize sensitivity rather than specificity. With a threshold of IGF-1 concentration, sensitivity was 48% (95% confidence interval (95% CI); 26%, 70%), specificity was 37% (95% CI; 16%, 62%) and the negative predictive value for the diagnosis of GHD was 39% (95% CI; 24%, 57%). With a threshold of IGF-1+IGFBP-3 concentration, the sensitivity was 19% (95% CI; 5%, 42%) and the specificity was 89% (95% CI; 67%, 99%). A positive predictive value of 67% (95% CI; 29%, 91%) but a negative predictive value of 50% (95% CI; 44%, 56%).

Table 3: Diagnostic performance of IGF-1 and IGF-1 + IGFBP-3 in detecting growth hormone deficiency

Statistic	Parameters	
	IGF-1	IGF-1 + IGFBP-3
True positives	10	4
True negatives	7	17
False positives	12	2
False negatives	11	17
Sensitivity	48 (26 - 70)	19 (5 - 42)
Specificity	37 (16 - 62)	89 (67 - 99)
Positive Predictive Value	45 (32 - 59)	67 (29 - 91)
Negative Predictive Value	39 (24 - 57)	50 (44 - 56)
Accuracy	43 (27 - 59)	53 (36 - 68)

17 of the patients with IGF-1+IGFBP-3 concentration above the threshold (N = 34) were normal and 17 had GH deficiency. These 17 GHD patients had IGF-1+IGFBP-3 concentration below the reference range for age and sex that did not differ significantly from those of their GH-sufficient counterparts (66.7% vs 50% , P=0.7) respectively. If IGF-1+IGFBP-3 concentration was used as a screening test (with a concentration threshold below the reference range for age and sex) and ITT as a confirmatory test, 34 (85%) out of 40 ITT would not have been performed, leading to the misdiagnosis of 17 GH-deficient adults. Thus, in our study population, such a procedure would misdiagnose 17 out of 21 GHD patients (81%) and yield a sensitivity of 19%.

Discussion

Growth is an important objective parameter of health of a child. SS although not a disease per se, may be a manifestation of several diseases. The etiology of SS ranges from normal variants like familiar SS to pathological conditions like endocrine and systemic disorders. Timely assessment is very important, because medical intervention if needed will only be effective before epiphysis fusion. In this 3-year retrospective study, we found that IGF-1 or IGFBP-3 concentration was not significantly correlated with peak GH concentration during ITT. We confirmed that IGF-1+IGFBP-3 concentration with a threshold below the reference range for age and sex has a poor positive predictive value for the diagnosis of GHD. However, an IGF-1+IGFBP-3 concentration was associated with a negative predictive value. Thus, the measurement of IGF-1+IGFBP-3 concentration, followed by a confirmatory dynamic test ITT for patients with an IGF-1+IGFBP-3 concentration below the reference range for age and sex, proved to be a valid approach. We also observed a non-statistical significant negative correlation between age and IGF-1 (r= -0.090, P=0.6) and IGFBP-3 (r= -0.161, P=0.3) concentrations, as seen in many reports [16, 24, 25].

The diagnostic procedure we proposed here was developed to limit the use of ITT which can result in adverse reactions typical of symptomatic hypoglycemia and seizure. We chose a very feasible method with large access: IGF-1+IGFBP-3 determination. It has been shown, in large groups of patients with adult GHD, that IGF-1 concentration (adjusted for age and sex) is low in a very high proportion of GHD cases [24-28]. This is not consistent with our findings: 17 out of 21 subjects with GHD had an IGF-1+IGFBP-3 concentration higher than the threshold we selected.

The clinical relevance of our diagnostic strategy is of clinical importance. This approach could not distinguish individuals with GHD from individuals without. This affects therapeutic options, as GHD patients can be treated with recombinant GH, which may improve height and quality of life [29, 30]. We are concerned by the imperfect diagnostic performance of the cascade test; it misdiagnosed 17/21 patients, meaning that these 17 patients would have been denied for recombinant GH treatment. However, these patients could be the least likely to benefit from recombinant human GH treatment as suggested by their normal IGF-1+IGFBP-3 concentration although this is disputed by others [31, 33].

Interestingly, the diagnostic procedure using a threshold for IGF-1+IGFBP-3 is associated with a 19% positive predictive value [16]. With this threshold, 2 out of 6 patients would have been misclassified as GHD in our study population. We believe that our diagnostic procedure (i.e. IGF-1+IGFBP-3) is safer than that with the IGF-1 threshold because even if some patients would not have access to GH, despite being potential candidates for this treatment, all candidates for GH treatment identified by the test approach had effective GHD. Conversely, with the IGF-1 threshold, some patients with normal GH function would receive GH therapy, which is not indicated currently.

Some limitations must be acknowledged. This is a single centre study, with a small number of patients. We had to rely on IGF-1+IGFBP-3 concentration and not on IGF-1. IGF-1 has been reported to be of greater diagnostic value by some, but not all authors [19, 34-36]. A second limitation is that IGF-1 concentration could vary greatly as shown in normal volunteers [37]. Coupled with ITT in a diagnostic strategy such as what is proposed here, this variability will not lead to inappropriate GH therapy, but simply to a possible delay of active treatment.

It is very important to know exactly the frequency of various causes of short stature from a given population in order to differentiate normal variants of growth from individual cases of short stature that need early diagnosis and treatment. Statistics addressing frequencies of various causes of growth failure in Saudi Arabia are not plentiful. This study may help to set an appropriate detection of treatable causes would be helpful in a better long-term prognosis.

In conclusion, many reports have already reported that IGF-1 concentration is lower in patients with GHD than in the general population, our study demonstrated the good negative predictive value of IGF-1+IGFBP-3 concentration for the diagnosis of GHD, making it possible to minimize the use of the "reference test" method ITT. This observation remains to be validated by population-based studies.

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