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The Relevance of Insulin-Like Growth Factor-Binding Protein-3 Concentrations According to Optimal Cut-Points as a Screening Test for Diagnosis of Growth Hormone Deficiency

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Research Article

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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 binding protein-3 (IGFBP-3) are not consistent. The aim of our study was to analyze the relevance of IGFBP-3 concentration 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 IGFBP-3 concentration, laboratory reference ranges were based on age and sex. For all eligible patients, IGFBP-3 concentration was determined and an insulin tolerance test (ITT) was performed. Patients with a peak GH of ≤5.0 ng/ml were considered to be GHD. The cut-off for optimal clinical performance measures was determined from the ROC curve. Sensitivity, specificity, positive and negative predictive values were calculated for IGFBP-3 concentration.

Results: Mean age was 14.7 ±1.7 years. There were 38 males (80.9%) and 9 females (19.1%) and mean IGFPB-3 concentration was 3783.3 ±1099.7 mcg/L. Results from the ITT indicated that 21 (52.5%) had GHD. Age was not statistically significant different between GHD (14.7 ±1.9 years) and non-GHD (14.7 ±1.7 years), p=0.9. Moreover, there was non statistical significant more males (53.1%) than females (50%) in the GHD patients, P=0.9. In addition, there were not statistically significantly different (p=0.9) between GHD (3752.9 ±1295.9 mcg/L) and non-GHD (3816.8 ±867.0 mcg/L) patients. 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 IGFBP-3 concentration (r=0.103, P=0.5) (figure 1). The AUC was 43.9%. An IGFBP-3 threshold of <3665 mcg/L was

selected to emphasize sensitivity rather than specificity. We tested the diagnostic accuracy of several thresholds. With a threshold of IGFBP-3 in reference to age and sex, sensitivity was 19%, specificity was 89% and the negative predictive value for the diagnosis of GHD was 50%. With a threshold of IGFBP-3 <3665 mcg/L, sensitivity was 57%, specificity was 58% and the negative predictive value for the diagnosis of GHD was 55%. With a threshold of <3075 mcg/L, the sensitivity was 29% and the specificity was 84%. A threshold of <2175 mcg/L, gave a positive predictive value of 67% but a negative predictive value of 49%. 11 of the patients with IGFBP-3 concentration above the threshold of <3665 mcg/L (N=20) were normal and 9 had GH deficiency. These 9 GHD patients had IGFBP-3 concentration that did not differ significantly from those of their GH-sufficient counterparts (4890 ± 1080 vs 4345 ± 609 ng/dl, P=0.2). If IGFBP-3 was used as a screening test (with a concentration threshold <3665 mcg/L) and ITT as a confirmatory test, 20 (50%) out of 40 ITT would not have been performed, leading to the misdiagnosis of 9 GH-deficient adults. Thus, in our study population, such a procedure would misdiagnose 9 (43%) out of 21 GHD patients and yield a sensitivity of 57%.

Conclusion: Our study demonstrated the poor negative predictive value of IGFBP-3 concentration for the diagnosis of GHD, making it not possible to minimize the use of the "reference test" method ITT. This observation remains to be validated by population-based studies.

Keywords: Growth hormone deficiency and insulin-like growth factor-binding protein-3

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 (SS), a common problem in child population of developing countries [1,2]. Common endocrine disorder leading to SS include growth hormone deficiency (GHD) [3]. The prevalence of GHD in children with SS ranges from 2.8% to 69% with the national prevalence of 11% [4-9].

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. IGFBP3 is the most abundant IGFBP in blood and has the highest affinity for IGF1, therefore it accounts for 75–80% of the total carrying capacity. 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 [10]. If GHD is suspected, IGF-1 and IGFBP-3 levels must be measured and a study of growth hormone (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, hypothyroidism) [11]. Dynamic tests are currently recommended for the diagnosis of GHD: the insulin tolerance test (ITT) is considered as the reference test [12-15].

Reports regarding the sensitivity and specificity of IGFBP-3 are not consistent [16-27]. In general, however, IGFBP-3 is reported to have good specificity but relatively poor sensitivity for GHD. Another advantage of IGFBP-3 is that they show superior reproducibility in comparison to stimulated GH concentrations [28].

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 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 optimal cut-points of IGFBP-3 concentration as a screening test for diagnosis of GHD.

Methods

We retrospectively studied 40 patients whom were evaluated for SS at the Endocrinology Department of King Fahad Armed Forces Hospital, Jeddah, Saudi Arabia between January 2015 to December 2018. For IGFBP-3 concentration, laboratory reference ranges were based on age and sex. For all eligible patients, IGFBP-3 concentration 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 hypoglycaemic 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). 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 international 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. IGFBP-3 concentration was determined using an immunoradiometric method (Unilabs company, Germany) [29].

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 IGFBP-3 concentration to discriminate between normal and GH-deficient patients was evaluated by receiver operating characteristic (ROC) curve analysis. The cut-off for optimal clinical performance measures was determined from the ROC curve. Sensitivity, specificity, positive and negative predictive values were calculated for IGFBP-3 concentration. A greater area under the curve (AUC) indicates better predictive capability. An AUC=0.5 indicates that the test performs no better than chance, and an AUC=1.0 indicates perfect discrimination. An ideal test is one that reaches the upper left corner of the graph (100% true positives and no false positives). To determine the optimal IGFBP-3 concentration cutoff points, we computed and searched for the shortest distance between any point on the curve and the top left corner on the y-axis. Distance was estimated at each one-half unit of IGFBP-3 concentration according to the equation: Distance in ROC curve=$(1-\text{sensitivity})^2+(1-\text{specificity})$ [30-32]. Diagnostic performance of IGFBP-3 concentration in predicting GHD was assessed by calculating AUC, 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. Mean age was 14.7 ±1.7 years (Table 1). There were 38 males (80.9%) and 9 females (19.1%) and mean IGFPB-3 concentration was 3783.3 ±1099.7 mcg/L. The observed male to female ratio was 4.2:1. Results from the ITT indicated that 21 (52.5%) had GHD (Table 2). Age was not statistically significant different between GHD (14.7 ±1.9 years) and non-GHD (14.7 ±1.7 years), p=0.9. Moreover, there was non statistical significant more males (53.1%) than females (50%) in the GHD patients, P=0.9. In addition, there were not statistically significantly different (p=0.9) between GHD (3752.9 ±1295.9mcg/L) and non-GHD (3816.8 ±867.0 mcg/L) patients. 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 IGFBP-3 concentration (r=0.103, P=0.5) (Figure 1). IGFBP-3 concentration according to GH deficiency status are demonstrated in Figure 2.

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

Table 1: Demographics [mean±standard deviation or number (%)](IGFBP-3, Insulin-like Growth Factor-binding Protein-3).

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)	
IGFPB-3 (mcg/L)		3752.9 ±1295.9	3816.8 ±867.0	0.9
GH (Peak) (ng/ml)		2.2 ±1.2	10.1 ±5.7	<0.0001

Table 2: Comparison between patients with growth hormone deficiency (GHD) and non- GHD (nGHD) [mean±standard deviation or number (%)] (GH, growth hormone; IGF-1, insulin-like growth factor ; IGFBP-3, Insulin-like Growth Factor-binding Protein-3).

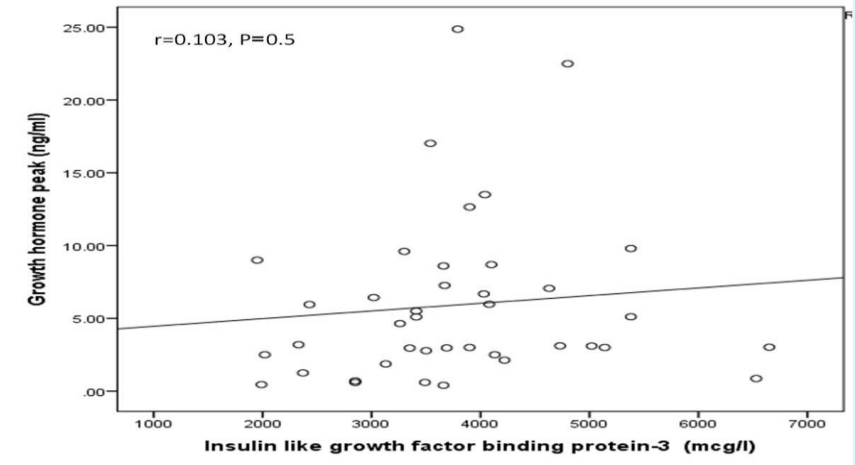

Figure 1: Correlation of insulin like growth factor binding protein-3 concentration and growth hormone peak during insulin tolerance test in the study population.

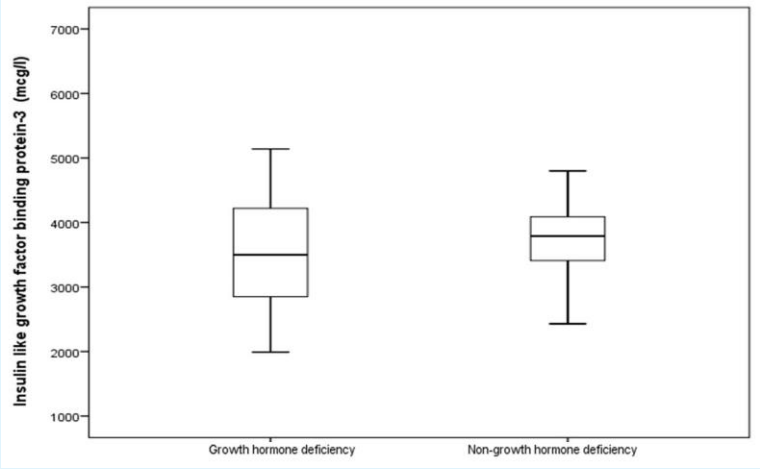

Figure 2: Insulin like growth factor binding protein-3concentration in patients with and without growth hormone deficiency: crosses represent individual data. Boxes represent 25 and 75th percentiles, split by median, with error bars representing 5th and 95th percentiles.

We plotted a ROC curve of IGFBP-3 concentration according to the diagnosis of GHD using ITT (Figure 3). The AUC was 43.9%. An IGFBP-3 threshold of <3665 mcg/L was selected to emphasize sensitivity rather than specificity. We tested the diagnostic accuracy of several thresholds (Table 3). With a threshold of IGFBP-3 in reference to age and sex, sensitivity was 19%, specificity was 89% and the negative predictive value for the diagnosis of GHD was 50%. With a threshold of IGFBP-3 <3665 mcg/L, sensitivity was 57%, specificity was 58% and the negative predictive value for the diagnosis of GHD was 55%. With a threshold of <3075 mcg/L, the sensitivity was 29% and the specificity was 84%. A threshold of <2175 mcg/L, gave a positive predictive value of 67% but a negative predictive value of 49%.

Statistic	IGFPB-3 (mcg/L)			
	Reference to Age and Sex	<3665	<3075	<2175
True positives	4	12	6	2
True negatives	17	11	16	18
False positives	2	8	3	1
False negatives	17	9	15	19
Sensitivity	19 (5 - 42)	57 (34 - 78)	29 (11 - 52)	10 (1 - 30)
Specificity	89 (67 - 99)	58 (34 - 80)	84 (60 - 97)	95 (74 - 100)
Positive Predictive Value	67 (29 – 91)	60 (44 – 74)	67 (37 - 87)	67 (16 - 95)
Negative Predictive Value	50 (44 - 56)	55 (40 - 70)	52 (43 - 60)	49 (44 - 53)
Accuracy	53 (36 - 68)	58 (41 - 73)	55 (38 - 71)	50 (34 - 66)

Table 3: Diagnostic performance of Insulin-like Growth Factor-binding Protein-3 (IGFBP-3).

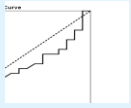

Figure 3: Receiver Operating Characteristics curve (ROC) of insulin like growth factor binding protein-3 concentration, according to the diagnosis of growth hormone deficiency established using insulin tolerance test.

11 of the patients with IGFBP-3 concentration above the threshold of <3665 mcg/L (*N* = 20) were normal and 9 had GH deficiency. These 9 GHD patients had IGFBP-3 concentration that did not differ significantly from those of their GH-sufficient counterparts (4890 ± 1080 vs 4345 ± 609 ng/dl, P=0.*2*). If IGFBP-3 was used as a screening test (with a concentration threshold <3665 mcg/L) and ITT as a confirmatory test, 20 (50%) out of 40 ITT would not have been performed, leading to the misdiagnosis of 9 GH-deficient adults. Thus, in our study population, such a procedure would misdiagnose 9 (43%) out of 21 GHD patients and yield a sensitivity of 57%.

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 diagnosis of GHD in

children with SS is very important because GHD responds better to GH treatment, compared to other causes of short stature [18]. Furthermore, appropriate replacement therapy enables the GHD child before epiphyseal fusion to achieve a normal adult height. In this 3-year retrospective study, we found that IGFBP-3 concentration was not significantly correlated with peak GH concentration during ITT. We confirmed that 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. Moreover, IGFBP-3 concentration with a thresholds <3665 mcg/L, 3075 mcg/L and 2175 mcg/L have poor positive and negative predictive values for the diagnosis of GHD. Thus, the measurement of IGFBP-3 concentration, followed by a confirmatory dynamic test ITT for patients with an IGFBP-3 concentration below the reference range for age and sex or a threshold <3665 mcg/L or lower, proved to be an invalid approach. We also observed a non-statistical significant negative correlation between age and IGFBP-3 concentration (r= -0.161, P=0.3), as seen in many reports [13,33,34].

Serum concentration of IGFBP-3 reflects the endogenous GH secretion in healthy children and exhibit little diurnal variation, which makes it potential candidates for screening of GHD [17]. However, both sensitivity and specificity of serum IGFBP-3 concentration varied greatly in previous studies [16-27]. Measurement of IGFBP-3 was confirmed useful in the diagnosis of GHD children later and suggested to be an excellent method to discriminate between GHD children and short-statured children with normal GH level [20]. IGFBP-3 is particularly useful in young children, in whom serum IGF-1 levels are in the same range in GHD and non-GHD [17]. But it has been disputed by others for the low sensitivity in spite of high specificity [16-27,35,36]. In our study, the sensitivity and specificity of IGFBP-3 concentration <2175 mcg/L are 10 and 95 %, respectively. The diagnostic characteristics are summarized in the ROC curve. The AUC wase determined to assess the discriminating ability. Rosman in our study, AUC was 0.439 (95% CI; 0.256, 0.621), indicating that IGFBP-3 had poor accuracy in GHD diagnosis.

Since GH testing is time-consuming, invasive, costly, and even hazardous, simple methods are necessary to identify those short children in whom GH testing is most appropriate. Our study indicated that IGFBP-3 concentration <2175 mcg/L had high specificity (95%) but low sensitivity (10%). The high specificity but low sensitivity suggested that in deciding whether or not a short child should be subjected to GH testing, the positive result should undergo provocative tests. Although IGFBP-3 concentration could not replace the provocative tests in the diagnosis of GHD, it could not be concluded from our finding to be used as an auxiliary method and a complementary tool to avoid repeated provocative tests. The clinical relevance of our diagnostic strategy is of critical importance. This approach could not distinguish individuals with GHD from individuals without. This affects therapeutic options, as GHD adults can be treated with recombinant GH, which may improve their height and quality of life. We are concerned by the imperfect diagnostic performance of the threshold IGFBP-3 concentration <3665 mcg/L; it misdiagnosed 9/21 patients, meaning that these 9 patients would have been denied for recombinant GH treatment. Furthermore, these patients could be the least likely to benefit from recombinant human GH treatment as suggested by their normal IGFBP-3 concentration although this is disputed by others [37,38]. Interestingly, the diagnostic procedure using a very low threshold for IGFBP-3 concentration <2175 mcg/L is not associated with a 100% positive predictive value (67%). With this threshold, 19 out of 21 patients would have been misclassified as GHD in our study population. We believe that our diagnostic procedure (i.e. IGFBP-3 concentration <3665 mcg/L) is safer than that with the low threshold (<2175 mcg/L) 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 cascade test approach had effective GHD. Conversely, with the low threshold procedure, 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 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 [17,39-41]. Coupled with ITT in a diagnostic strategy such as what is proposed here, this variability will lead to inappropriate GH therapy. It is very important to know exactly the frequency of various causes of SS from a given population in order to differentiate normal variants of growth from individual cases of short stature who 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 IGFBP-3 concentration is lower in patients with GHD than in the general population, our study

demonstrated the poor negative predictive value of IGFBP-3 concentration for the diagnosis of GHD, making it not 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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Conflict of Interests

The authors declare no conflict of interests.

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