Brief Communication

Growth Hormone Cut-Off Post Glucagon Stimulation Test in an Indian Cohort of Overweight/Obese Hypopituitary Patients for the Diagnosis of Adult Growth Hormone Deficiency

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Abstract

Obesity has been associated with reduced growth hormone (GH) secretion, which might lead to the over diagnosis of adult GH deficiency (GHD) in overweight (OW)/obese hypopituitary patients. Currently, there are no body mass index (BMI)-specific peak GH cut-offs for the glucagon stimulation test (GST) for assessing adult GHD in India, given the BMI cut-offs vary for Asians. The study's main objective was to determine a peak GH cut-off level for the diagnosis of adult GHD in overweight (OW)/obese individuals utilizing the GST. Forty OW/obese subjects were studied in two groups of 20 each. The first group included 20 OW/obese hypopituitary adults and the second group included 20 control subjects. The intervention consisted of a 3 h GST. The main outcome measured was the peak GH level on GST. The mean age of control subjects was lower (33.15 ± 7.67 v/s. 42.10 ± 13.70 years; $P = 0.017$) in comparison with hypopituitary adults. The mean BMI (27.93 ± 1.63 v/s. 25.81 ± 1.66 kg/m2; $P < 0.001$), mean IGF1 (272.81 ± 38.57 v/s. 163.75 ± 42.42; $P < 0.001$, and mean HOMA IR (11.8 ± 9.7 v/s. 6.02 ± 3.14; $P = 0.02$) was greater in OW/obese controls. The mean GH peak was significantly higher in control subjects (5.41 ± 3.59 ng/mL v/s. 1.49 ± 1.25 ng/mL; $P < 0.001$) compared to hypopituitary subjects. ROC curve analysis demonstrated a GH cut-off of 3.3 ng/mL with a moderate sensitivity of 70% and high specificity of 95%, with an AUC of 0.838 ($P < 0.001$; 95% confidence interval [CI] of 0.710–0.965) for the diagnosis of GHD in overweight/obese hypopituitary adults. This study demonstrates that a cut-off of 3.3 ng/mL would diagnose GHD in Indian overweight/obese hypopituitary adults.

Keywords: BMI, glucagon stimulation test, growth hormone deficiency, overweight/obesity

Introduction

Adult-onset growth hormone deficiency (AO-GHD) is a distinct disorder characterised by a myriad of metabolic perturbations such as decreased lean body mass, increased fat mass, dyslipidaemia, cardiac dysfunction, decreased fibrinolysis and premature atherosclerosis, decreased muscle strength and exercise capacity, decreased bone mineral density, increased insulin resistance and impaired quality of life.[1] It commonly occurs as a consequence of hypothalamic–pituitary tumours and their treatment.[2] Recent studies have shown increased mortality in patients with hypopituitarism.[3] Establishing the diagnosis of AO-GHD is very difficult given the poor diagnostic value of Insulin-like growth factor 1 (IGF1), insulin-like growth factor binding protein 3 (IGFBP3) and 24 h GH secretion.[4,5] Consequently, GH stimulation tests are usually required for diagnosing AO-GHD. Insulin tolerance test (ITT) is the gold-standard test for the assessment of adult GHD.[2] The cumbersome nature of ITT limits its use in routine clinical practice.[6] Results from the ANSWER programme show glucagon stimulation test (GST) as the most frequently used test after 2009 and should be considered if the ITT cannot be performed or is contraindicated.[7,8] Obesity is considered a state of relative GHD[9,10] and earlier physiologic studies in obese individuals have shown that spontaneous GH

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secretion is reduced, GH clearance is enhanced and stimulated GH secretion is reduced.[11-13] Previous studies investigating the diagnostic utility of the GST in adult GHD have not considered body mass index (BMI)[14,15] or included only controls with normal BMIs.[16,17] Many recent retrospective studies questioned the diagnostic accuracy of the GST when the GH cut-point of 3 ng/mL is applied to overweight/obese adults.[18-21] There are no studies on the BMI-specific cut-offs for the GST in the Indian population. The main study objective was to determine the peak GH cut-off for the diagnosis of adult GHD in overweight (OW)/obese individuals utilising the GST.

Materials and Methods

Approval for this study was obtained from the Institutional Ethics Committee of Gandhi Medical College/Hospital (IEC/GMC/2020/02/09). Study procedures were conducted in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. Written consent was obtained from each subject before the study. This was a hospital-based cross-sectional study, conducted in the Department of Endocrinology, Gandhi Medical College/Hospital between March 2020 and March 2022 for 25 months. Twenty OW/obese hypopituitary adults and 20 control subjects attending the endocrinology outpatient department (OPD) were taken for the study as it was intended to be a pilot project.

Subjects

Hypopituitary subjects: Patients with a clear diagnosis of hypopituitarism, 1–4 non-GH pituitary deficits, and a BMI of ≥23 kg/m^2 were included**.** Those with childhood-onset GHD, a history of traumatic brain injury, BMI <23 kg/m^2, and patients with known hypothalamic or pituitary disease with no pituitary deficiencies were excluded.

Control subjects: Subjects ≥18 years with a BMI of ≥23 kg/m^2 and stable weight in the past 3 months were included. Subjects with chronic illness, diabetes mellitus, smoking and those with pituitary disorders were excluded.

Glucagon Stimulation Testing

GST was performed using the same protocol in obese subjects and hypopituitary patients. Intramuscular glucagon was administered at a fixed dose of 1 mg (GluGon-united biotech SC/IM 1 mg). In both groups, fasting GH levels were measured at time zero and then at 60, 120, 150 and 180 min for a total of five samples.

Laboratory Assays: HbA1c measurements were performed using high-performance liquid chromatography (Bio-Rad D-10 USA). Serum insulin was measured using insulin enzyme-linked immunosorbent assay (ELISA) (Calbiotech Inc., USA) kit, based on solid phase sandwich ELISA method with an intra-assay and inter-assay Coefficient of variation percentage (CV%) of 2.8–4.2 and 5.5–6.74, respectively. Serum IGF1 was measured using a DRG IGF1 600 ELISA kit (DRG Instruments GmbH, Germany), which is a solid phase enzyme immunoassay with intra-assay variability of 6.39% to 7.39% and inter-assay variability of 10.34% to 14.84%. GH was measured by hGH (human growth hormone) ELISA kit (Calbiotech Inc, USA) based on the solid phase sandwich hGH method. The sensitivity of the test kit was 0.012 ng/mL. The intra-assay and inter-assay CV% were 4.90–7.67% and 4.53–8.59%, respectively. All estimations were performed using Thermo Fisher Varioskan LUX Multimode microplate reader.

Statistical analysis

Data were entered in MS Excel and analysed in SPSS V25. Descriptive statistics were represented with percentages for qualitative data, mean with SD (standard deviation) or median with IQR (interquartile range) for quantitative data. Shapiro–Wilk test was applied to find normality. The Fisher exact test was applied for the comparison of proportions. Independent *t*-test and Mann–Whitney *U* test were applied for comparison between means and medians. receiver operating characteristic (ROC) curve was drawn. The area under the curve was calculated. Sensitivity and specificity were calculated. $P < 0.05$ was considered as statistically significant.

Results

All the baseline clinical characteristics have been summarised in Table 1. The mean age of control subjects was lower in comparison with hypopituitary subjects. The mean BMI, mean IGF1 and mean homeostatic model assessment for insulin resistance (HOMA IR) were greater in OW/obese controls. Systolic blood pressure was higher among the hypopituitary subjects. There were no significant differences among all the other parameters.

Among the hypopituitary patients, hypogonadism and hypocortisolism were common after hypothyroidism. Most of them required surgical intervention followed by radiation and medical therapy.

The majority of the hypopituitary group had non-functioning pituitary adenoma followed by Sheehan's syndrome and craniopharyngioma [Figure 1].

The mean GH peak was significantly higher in control subjects (5.41 ± 3.59 ng/mL v/s. 1.49 ± 1.25 ng/mL; $P < 0.001$) compared to hypopituitary subjects [Table 2].

In the hypopituitary adults, there was no GH peak (undetectable GH at all time periods) in 45%. GH peak occurred between 120 and 180 min in the remaining 45%. Among the control subjects, GH peak occurred in 70% of the subjects at 120–150 min [Graph 1].

ROC curve analysis demonstrated a GH cut-off of 3.3 ng/mL with a moderate sensitivity of 70% and high specificity of 95%, with an AUC of 0.838 ($P < 0.001$; 95% CI of 0.710–0.965)

Table 1: Baseline clinical and biochemical characteristics of two study groups

Variable		Hypopituitary subjects (m±SD)	Control subjects (m±SD)	*P*
Age (y)		42.10±13.70	33.15±7.67	0.017
Gender (*n* (%))	Male	14 (70%)	16 (80%)	0.72
	female	6 (30%)	4 (20%)	
BMI (kg/m^2)		25.81±1.66	27.93±1.63	<0.001
Waist circumference (cm)		88.10±6.77	89.85±5.98	0.26
Hip circumference (cm)		95.10±4.95	95.45±4.44	0.82
W: H ratio		0.92±0.06	0.94±0.05	0.22
SBP (mmHg)		126.80±5.93	121.60±4.97	0.01
DBP (mmHg)		77.80±6.52	75.70±5.85	0.29
IGF 1 (ng/mL)		163.75±42.42	272.81±38.57	<0.001
HOMA IR		6.02±3.15	11.81±9.77	0.02
Total cholesterol (mg/dL)		201.75±28.57	201.20±14.11	0.62
HDL (mg/dL)		48.60±3.72	49.10±5.74	0.75
LDL (mg/dL)		124.60±23.43	120.55±14.06	0.66
TGL (mg/dL)		144.65±27.87	156.95±14.02	0.09
Hormone deficiencies: *n* (%)				------
Hypocortisolism		16 (80%)		
Hypothyroidism		20 (100%)		
Hypogonadism		18 (90%)		
Testosterone/estradiol use		10 (50%)		
FSH/LH use		1 (5%)		
Diabetes insipidus		3 (15%)		
DDAVP use		3 (15%)		
Treatment history: *n* (%)				-------
Medical		4 (20%)		
Surgery		9 (45%)		
Radiation		7 (35%)		

Abbreviations: BMI=Body mass index, W=H ratio-waist to hip ratio, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, IGF 1=Insulin-like growth factor 1, HOMA IR=Homeostatic model assessment for insulin resistance, HDL=High density lipoprotein, LDL=Low density lipoprotein, TGL=Triglycerides, FSH=Follicle stimulating hormone, LH=Luteinising hormone, DDAVP=1-deamino-8-D-arginine-vasopressin

Table 2: GH characteristics after glucagon stimulation test in the two study groups

Variable	Hypopituitary subjects (*n*=20)	Control subjects (*n*=20)	*P*
Mean GH peak±SD ng/mL	1.49±1.25	5.41±3.59	<0.001
Median GH peak ng/mL (IQR) ng/mL	1.49 (2.25)	5.80 (5.91)	
Timing of GH peak: *n* (%)			
No peak	9 (45%)	0	
60 min	2 (10%)	1 (5%)	
120 min	3 (15%)	7 (35%)	
150 min	3 (15%)	7 (35%)	
180 min	3 (15%)	5 (25%)	

for the diagnosis of GHD in overweight/obese hypopituitary subjects [Figure 2].

Discussion

Our study demonstrated that a GH cut-off of 3.3 ng/mL would be able to diagnose OW/obese hypopituitary subjects with moderate sensitivity and good specificity. Control subjects were younger (33.15 ± 7.67 v/s. 42.10 ± 13.70 years; $P = 0.017$) in comparison to OW/obese hypopituitary subjects. As GH decreases with age, controls were not age-matched as it might reduce the cut-off further. They also had a higher BMI (27.93 ± 1.63 v/s. 25.81 ± 1.66 kg/m^2; $P < 0.001$). Previous studies on GST in adult GHD have not considered BMI[14,15] or included only controls with normal BMIs.[16,17] HOMA IR was significantly increased in control subjects (11.8 ± 9.7 v/s. 6.02 ± 3.14; $P = 0.011$) compared with hypopituitary subjects, contrary to the expectation. The possible explanation is inadequate cortisol replacement in those with hypocortisolism. A previous study showed that the insulin sensitivity measured by the euglycemic hyperinsulinaemic clamp in hypopituitary adults not on GH replacement was similar to individuals with normal pituitary function, despite presenting with higher fat mass percentage.

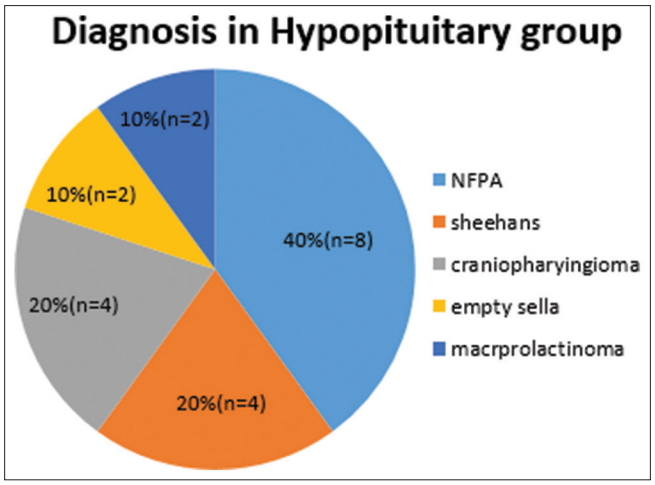


Figure 1: Pie chart showing diagnosis in hypopituitary group

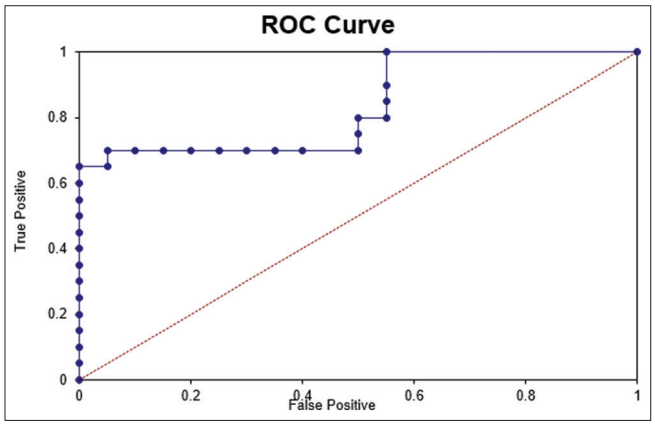
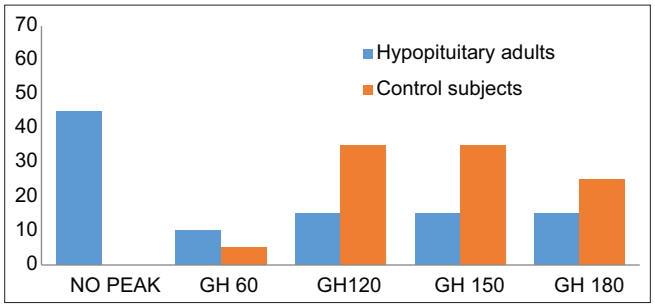


Figure 2: ROC curve of peak GH level on GST for detecting GHD. The AUC is 0.838 ($P < 0.001$)



Graph 1: Detailing the timing of GH peak among the two groups

HOMA-IR may not be a good method for assessing insulin sensitivity in hypopituitary adults.[22] GH peak occurred by 120 to 150 min in the majority of the control subjects. In the present study, a fixed dose regimen was used. Previous studies, comparing fixed-dose and weight-based dosing regimens of glucagon, reported a similar peak with the fixed-dose regimen but a later peak at 150–180 min with weight-based doing.[22] Previous studies demonstrated that reducing the peak GH cut-off to 1 ng/mL would reduce the overdiagnosis of GHD in overweight/obese hypopituitary patients.[18] However, the ROC curve in our study demonstrated that a cut-off of 3.3 ng/mL would diagnose GHD in OW/obese hypopituitary subjects with 70% sensitivity and 95% specificity. This cut-off is similar to the traditional cut-off of 3 ng/mL suggested by many endocrine societies.[23] These differences might be explained by the higher BMI (> 30 BMI kg/m^2) of the subjects in the Caucasian population. There are no studies on the BMI-specific cut-off diagnosing GH deficiency in overweight/obese Indian patients using GST. Large-scale studies with a diverse BMI range would help in knowing the true GH cut-off for diagnosing GHD in the overweight/obese Indian population.

Limitations of our study include a small sample size. The study assumes GH deficiency in all hypopituitary subjects and GH sufficiency in all controls. Serum growth hormone was measured using ELISA, which is not a commonly used assay, and the results may not be generalisable. Also, GST results were not compared with the gold standard test such as ITT.

Conclusions

Our study demonstrated that a cut-off of 3.3 ng/mL would diagnose GHD in Indian overweight/obese hypopituitary subjects. It would be prudent to continue using the traditional cut-off of 3 ng/mL in Indian overweight/obese hypopituitary patients until large-scale studies have been completed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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