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ORIGINAL RESEARCH

Validation of Glucagon Stimulation Test in Establishing GH and ACTH Deficiency in Hypopituitarism

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ABSTRACT

Background: Diagnosis of GH deficiency (GHD) and secondary adrenal insufficiency entails evaluation by multiple dynamic stimulation tests in most cases. Insulin tolerance test (ITT), the gold-standard test for evaluation of GHD and hypothalamo-pituitary-adrenal (HPA) axis, carries risks and hence avoided in many institutes. Glucagon stimulation test (GST) is a safe alternative to test for GHD and HPA axis.

Materials and Methods: We studied the diagnostic performance of GST compared to ITT in adult patients with hypopituitarism and HPA axis suppression utility of AACE/ACE proposed lower GH cut-point of 1 µg/L for adult GHD. GST and ITT were performed on consecutive days as per standard protocol. Main outcome measures were the GH and Cortisol response to GST and ITT. Hypopituitarism was due to Sheehan's syndrome in 13 patients, pituitary tumors in 4 subjects and empty sella syndrome in 1 patient. Two patients with HPA axis suppression due to exogenous glucocorticoids were also evaluated with both GST and ITT.

Results: Sixteen patients had ≥3 pituitary hormone deficiencies. Peak cortisol levels obtained during GST were significantly lower than the values obtained during ITT (5.1±4.8 vs. 6.2±5.7 µg/dl; P = 0.004). Peak GH responses were not significantly different between GST and ITT (0.4±0.7 vs 1.4±3.4 µg/L; P = 0.445). Using ITT as gold standard, GH cut of 3 µg/L in GST had 100% sensitivity, 100% NPV, 89% PPV, and 90% accuracy in diagnosing GHD. Adopting AACE/ACE proposed lower GH cut-point of 1 µg/L did not add further to the diagnostic accuracy of GST in GHD overall or in overweight/obese subjects.

Conclusion: We conclude that GST can be reliably used in the evaluation of GHD as well as HPA axis in hypopituitarism

Keywords: Hypopituitarism, GST, ITT.

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INTRODUCTION

Reliable and safe tests to establish the diagnosis of hypopituitarism is vital for timely initiation of treatment. Many causes of hypopituitarism are irreversible and the treatment once initiated is likely to be life-long. Testing the patients, who are already on replacement hormones, for hypopituitarism is even more complex. The gold standard for checking the hypothalamo-pituitary adrenal (HPA) axis and GH-IGF-1 axis is insulin tolerance test (ITT). Many centres avoid or limit doing ITT due to logistic and safety reasons. ITT should be carried under direct medical supervision in a closely supervised setting in a day procedure unit or hospital bed. It is risky to subject patients with epilepsy and underlying ischemic heart

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disease to induced hypoglycaemia. Glucagon stimulation test (GST) is a safer alternative to ITT to assess GH-IGF-1 axis and HPA axis. Its reliability and reproducibility was established by earlier studies.[1-7] In Kashmir valley of northern India, access to specialised medical centres is limited to Srinagar. The Himalayan terrain makes transport difficult. Many women have home deliveries in remote villages. Obstetric complications like post-partum haemorrhage often get delayed treatment in referral hospitals due to distance and difficult terrain. Sheehan's syndrome is a common cause of hypopituitarism in Kashmir. A safe test which could reliably identify hypopituitarism but can be done in any hospital would help institute required treatment in a timely manner. We sought to compare the reliability of GST in assessing HPA axis and GH-IGF-1 axis in hypopituitarism.

MATERIALS & METHODS

GST and ITT were performed on consecutive days as per standard protocol. Sampling was performed during the patient's routine clinical care for endocrinological evaluation of pituitary function after pituitary surgery. For ITT, patients received 0.15 IU/kg of regular insulin (Actrapid Novo Nordisk, Mainz, Germany) intravenously to achieve blood glucose levels below 40 mg/dl and until symptoms of hypoglycemia had developed. Blood samples for GH, cortisol, and glucose were collected at 10, 0, 15, 30, 45, 60, 90, and 120 min. In addition, all patients underwent testing with glucagon (Glucagen Diagnostic Kit, Novo Nordisk) on a separate occasion by injecting 1 mg of glucagon (1.5 mg in patients having >\90 kg body weight) subcutaneously, and blood samples for GH, cortisol, and glucose were collected at K10, 0, 90, 120, 150, 180, 210, and 240 min. The maximum interval between the two dynamic tests was 7 days. Further assessment of anterior pituitary function was done by baseline hormonal testing as well as by provocative tests as required. Serum GH levels were determined by a Chemiluminescent immunometric assay (Immulite 2000 assay, Siemens AG, Erlangen, Germany). All samples from each individual patientwereanalyzed together. The assay was calibrated against the WHO 1st international standard (80/505) for human GH. Intra- and interassay coefficients of variation (CV) values for a low point of the standard curve were 5.4 and 7.9% respectively. For ITT, a peak GH response below 3 mg/l established the diagnosis of severe GHD according to the data given in the literature. Serum cortisol levels were determined by competitive immunoassay using commercial kits (Advia Centaur, Bayer). The analytical sensitivity of the assay was 5.5 nmol/l. Intra-assay variations as CV for various cortisol values were 3.7% (107.1 nmol/l), 3.1% (155.3 nmol/l), 2.9% (391.0 nmol/l), 3.8% (759.6 nmol/l), and 3.0% (1025.0 nmol/l) respectively. Inter-assay variations for the cortisol concentrations mentioned above were 5.5, 3.8, 3.1, 1.9, and 4.0% respectively. A peak cortisol !500 nmol/l was used to define AI. TSH deficiency was defined by low serum free thyroxine level without appropriate elevation in serum TSH. In males, secondary hypogonadism was defined by low serum testosterone with inappropriately low gonadotropin level, in premenopausal females by amenorrhea in the presence of low serum estradiol level without a rise in gonadotropin level, and in postmenopausal females by inappropriately low serum gonadotropin concentrations. Vasopressin deficiency was defined by increased serum sodium and plasma osmolality and low urinary osmolality. All other parameters were determined by routine methods.

RESULTS

Hypopituitarism was due to Sheehan's syndrome in 13 patients, pituitary tumors in 4 subjects and empty sella syndrome in 1 patient. Two patients with HPA axis suppression due to exogenous glucocorticoids were also evaluated with both GST and ITT. Sixteen patients had ≥3 pituitary hormone deficiencies. Median age was 38.5 years (range 23 to 70 years), BMI

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mean±SD was 25.1±3.7 (range 17.8 to 33.6). Eight patients (40%) had BMI of ≥25 kg/m2. [Table 1] compares the GH and Cortisol levels by ITT and GST respectively.
Peak cortisol levels obtained during GST were significantly lower than the values obtained during ITT (5.1±4.8 vs. 6.2±5.7 µg/dl; P = 0.004). [Figure 1] Peak GH responses were not significantly different between GST and ITT (0.4±0.7 vs 1.4±3.4 µg/L; P = 0.445). [Figure 2] Correlation between GH and Cortisol levels is shown in [Table 2&Figure 3,4]
Using ITT as gold standard, GH cut of 3 µg/L in GST had 100% sensitivity, 100% NPV, 89% PPV, and 90% accuracy in diagnosing GHD. Adopting AACE/ACE proposed lower GH cut-point of 1 µg/L did not add further to the diagnostic accuracy of GST in GHD overall or in overweight/obese subjects.

Table 1:Comparison of Growth hormone and cortisol levels as assessed by ITT and GST

	Growth Hormone		Cortisol	
	ITT	**GST**	**ITT**	**GST**
No.	20	21	20	21
Minimum	0.012	0.016	0	0
Maximum	15.976	14.098	17.600	15.630
Mean ± SD	1.873 ± 4.522	0.996 ± 3.074	6.402 ± 5.856	5.079 ± 5.041
p value	0.002*		0.002*	

Table 2: Correlation between ITT and GST for Growth hormone and cortisol levels

	No.	**Correlation Coefficient**	**p value***
Growth Hormone	20	0.861	<0.001
Cortisol	20	0.978	<0.001

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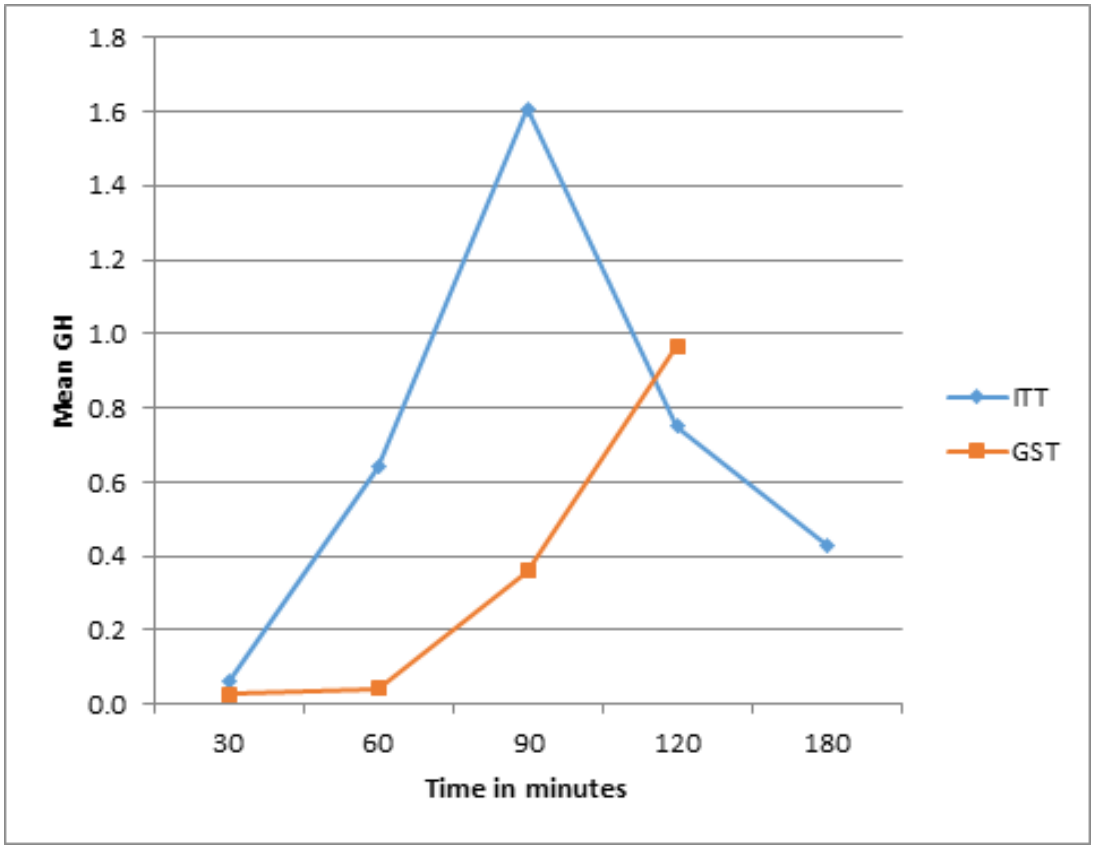

Figure 1: Mean Growth hormone levels for ITT and GST at different intervals of time

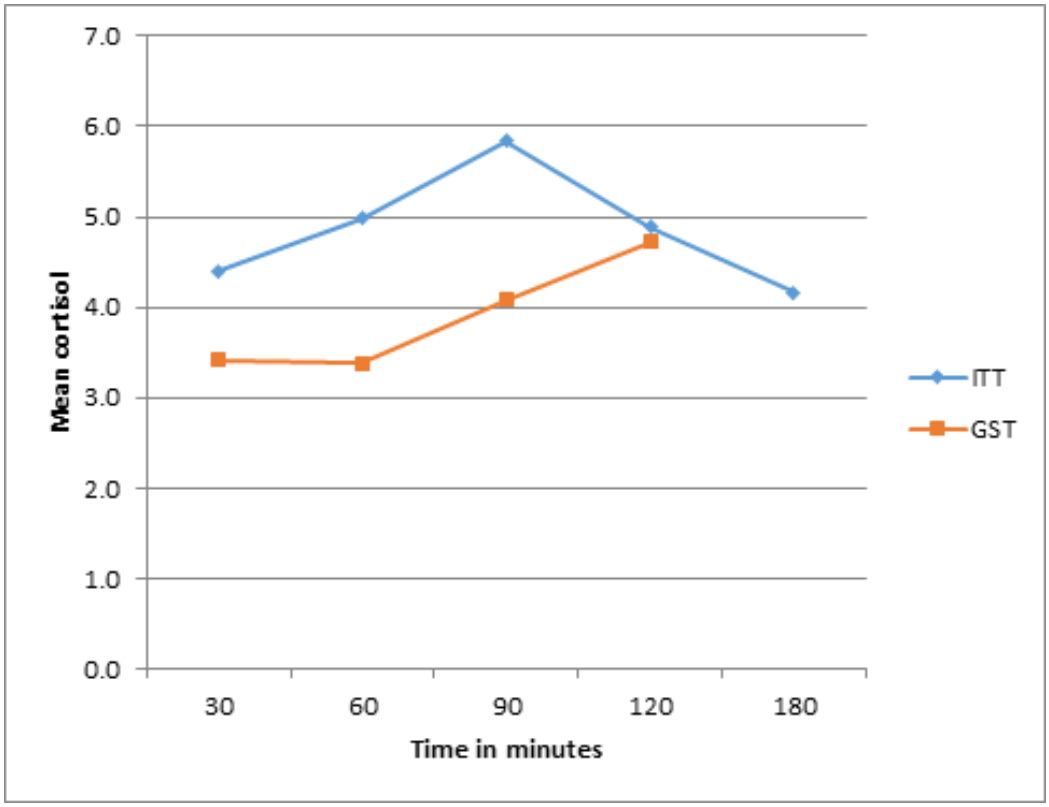

Figure 2: Mean Cortisol levels for ITT and GST at different intervals of time

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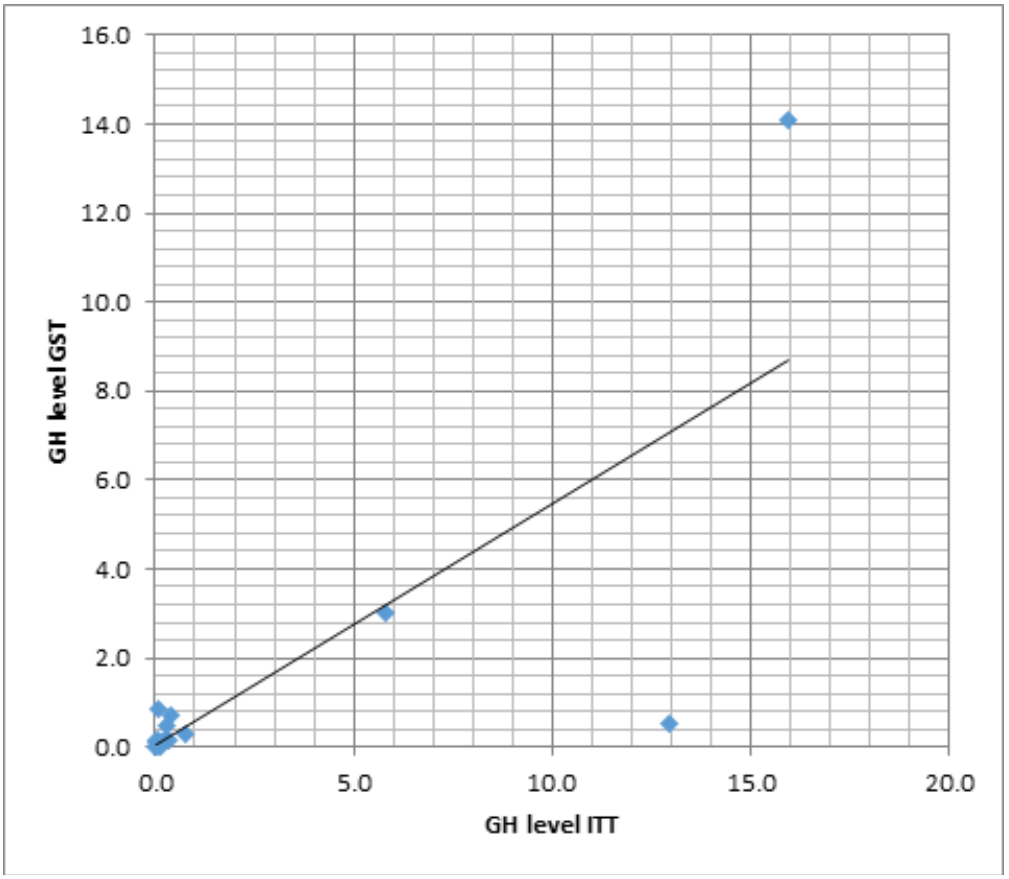

Figure 3: Scatter diagram showing correlation between peak GH levels for ITT and GST

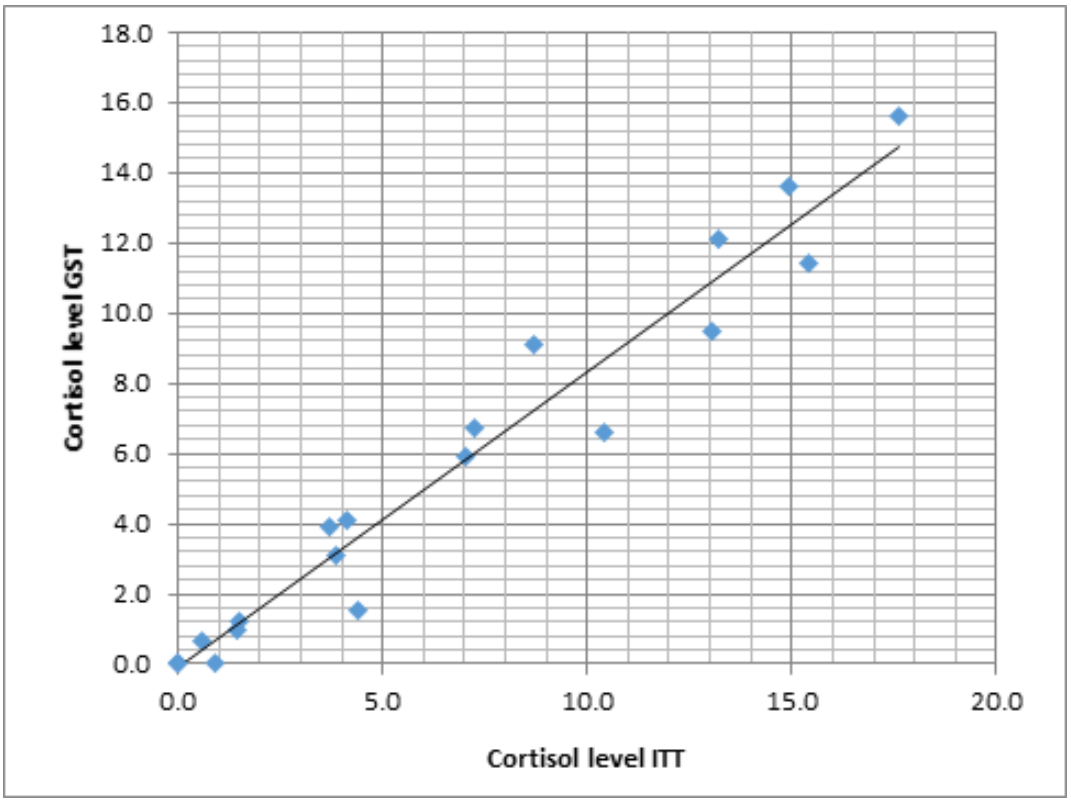

Figure 4: Scatter diagram showing correlation between peak Cortisol levels for ITT and GST

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Statistical analysis

Results (mean + S.E.M.) are expressed as absolute values for GH and cortisol. Spearman's rank correlation analysis was used to determine relationships between the variables. For further statistical analysis, two-way ANOVA and the Mann–Whitney and the Wilcoxon tests were performed wherever appropriate.

DISCUSSION

In the present prospective study, we demonstrated that the GST is a potential alternative test for the assessment of GH reserve in patients with pituitary disease, but a moderately precise test for ACTH reserve. We determined new cut-off values for the currently used assays and their sensitivity and specificity in the diagnosis of adrenal and GH deficiencies. Accurate assessment of the hypothalamic–pituitary axis is essential in patients with pituitary disease. Alternative tests other than the ITT have been sought for evaluating the hypothalamo pituitary (HP) axis, such as arginine,[1] GHRH +arginine,[2–4] GH-releasing peptide-6 and combined GH-releasing peptide-6 plus GHRH,[5,6] and CRH tests.[7] In a recent review by Yuen et al. the GST is recommended as the best alternative test when the ITT is not desirable or when the GHRH–ARG test is unavailable, based on the GST's reliability and availability, its accurate and reliable discrimination between normal and true GH deficiencies, safety, and lack of influence by BMI and gender.[8] Of note, the GST has only few contraindications such as underlying pheochromocytoma and insulinoma.[9,10] For GH, we found a strong positive correlation between peak responses during ITT and GST, which was comparable to the results of Spathis et al.[11] Previous studies reported that the GST is a potent and reliable stimulus of GH and cortisol secretion in healthy adults and patients.[12-15] The glucagon-induced cortisol release has been shown to be ACTH dependent.[11,16] The detailed physiological mechanisms by which glucagon induces both GH and cortisol release are unclear. Some of the hypothesized mechanisms include the glycemic fluctuations during the test where blood glucose levels increase initially before decreasing later in the test,[17] the generation of a peptidyl fragment associated with the GH- and ACTH-releasing activity,[14] and the induction of norepinephrine secretion in stimulating GH and ACTH release via a-receptors.[15]Soliman et al. concluded previously that glucagon was at least as good as the ITT in stimulating GH release.[18] Moreover, Aimaretti et al. found that although the ITT was a more effective stimulus than L-arginine, L-dopa, and clonidine, glucagon was the strongest stimulus after ITT.[19] By ROC curve analysis, we found that a GH cut-off of 2.5 ng/ml is optimal to prove GH sufficiency when applying a current GH assay. Owing to this, the GST was useful for the correct interpretation of GH status in 71% of GH-sufficient patients, and 29% (6/21) of these patients were misdiagnosed. Hence, the GST tends to overestimate the prevalence of GHD. In comparison, Conceicao et al. determined a cut-off of 3 ng/ml for the GST when categorizing the patients by ITT using a slightly higher threshold of 5 ng/ml.[20] Similar to our study, sensitivity for GHD was high with 97% showing somehow lower specificity of 88%. Moreover, Gomez et al. suggested the same cut-off of 3 ng/ml for the GST with high sensitivity and specificity of each 100%.[21]It has to be stressed that their control group did not include patients with pituitary disease, but healthy subjects. Furthermore, three different GH assays were used in that study including two different polyclonal RIA in 37% of the patients, which may give higher GH concentrations compared to the currently used immunoassays. Both test- and assay-specific cut-offs are of special importance to avoid misinterpretation, so caution is required before adopting or extrapolating cut-off values from other laboratories.[22]

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For cortisol, the correlation between ITT and GST peak values was somehow lower than that for GH. Cortisol peaks were significantly lower in GST than in ITT. Thereby, ROC analysis with emphasis on the high sensitivity for AI revealed a cut-off of 599 nmol/l with rather low specificity. In addition to this upper threshold, which conversely has high specificity for AS, we determined a lower threshold with high specificity for AI. Thereby, half of the patients were correctly diagnosed, leaving the other half with peak levels in the gray zone for subsequent investigation by alternative tests such as the ITT. So far, there are limited data in the literature regarding the validity of the GST in assessing the pituitary–adrenal axis. By comparing CRH testing and GST in children, Boettner et al. suggested a cut-of of 450 nmol/l with a sensitivity of 89% and specificity of 87%.[23] We found the maximum GH and cortisol release with the GST between 120 and 180 min, which is consistent to the studies by Andler et al.[24] Goodwin et al.[25] and Littley et al.[6] Hence, we propose that the shortened GST can be used without losing its diagnostic utility, which could simplify the test in clinical practice reducing costs and resources. In general, the GST was tolerated well. We observed occasional nausea with vomiting, sweating, and headache in 10%. Therefore, our data are consistent with the studies by Gomez et al.[11] and Leong et al.[15] showing low prevalence of side effects and good tolerability, while Aimaretti et al. described 15% of patients with vomiting during the GST. Owing to the advantages that the GST is simple to perform, inexpensive, readily accessible, and has few side effects, we propose that the GST can be used as the first-line stimulation test in patients with relative contraindications for the ITT. If both the upper and lower cut-offs are used, the GST allows a correct diagnosis of adrenal function in almost half of the patients, reserving patients in the gray zone for additional testing by ITT. A correct diagnosis of GHD is obtained in almost 90% of the patients. We conclude that the GST is a simple and safe method for stimulating GH and ACTH secretion in patients with hypothalamic–pituitary disease following pituitary surgery. It is a potential alternative test for the assessment of GH reserve, but is a poor test for ACTH reserve, as demonstrated by comparison with the ITT. Test-specific cut-offs should be applied to avoid misinterpretation.

CONCLUSION

GST is safe and effective alternative to ITT for evaluation of HPA axis.

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