

CLINICAL STUDY

Significant GH deficiency after long-term cure by surgery in adult patients with Cushing's disease

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Abstract

Objective: Impaired GH secretion usually accompanies Cushing's syndrome and a variable proportion of patients reportedly fail to recover normal GH secretion after successful treatment. This wide variability is most probably due to differences in the treatment (i.e. surgery and/or radiotherapy), timing of patient re-evaluation after surgery and dynamic tests employed to challenge GH secretion, and hinders a precise assessment of risk of GH deficiency after cure. The aim of the present study is to evaluate GH secretory status after long-term cure of Cushing's disease achieved by surgery alone.

Design and methods: We studied 34 patients (27 females and 7 males, age range 21–68 years) formerly affected by Cushing's disease. Patients were studied 2–20 years (median 3.3 years) following remission of hypercortisolism; all patients underwent transsphenoidal surgery with the removal of an ACTH-secreting adenoma; repeat pituitary surgery for relapse was performed in two patients while bilateral adrenalectomy was necessary in two patients. In all subjects, the GH response to GHRH + arginine stimulation was evaluated. At the time of testing, 13 patients were still on steroid replacement therapy.

Results: In long-term surgical remission, 22 patients (65.0%) presented subnormal GH secretion; partial GH deficiency (GH peak <16.5 µg/l) was found in 11 patients and severe GH deficiency (GH peak <9 µg/l) in another 11. Male gender and length of hypercortisolism were risk factors for postsurgical GH deficiency.

Conclusions: This study demonstrates the presence of GH deficiency in a high percentage of patients with Cushing's disease after long-term remission of hypercortisolism obtained by surgery alone. Male gender and length of hypercortisolism are the most significant predictors of postsurgical GH deficiency. This finding is significant as it highlights that even the most favourable therapeutical course, i.e. remission achieved by surgery alone, is accompanied by impaired GH secretion. Assessment of GH secretion is therefore recommended for all patients cured from Cushing's disease, even if not submitted to radiotherapy. Studies on the clinical impact of GH deficiency and the use of GH replacement therapy seem warranted in patients cured from Cushing's disease.

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Introduction

Excessive glucocorticoids unquestionably inhibit growth hormone (GH) secretion. Indeed, GH secretion is impaired in both children (1) and adult patients (2, 3) with exogenous or endogenous hypercortisolism. In contrast, to what extent recovery of GH secretion follows the normalisation of cortisol concentrations is less well established (4, 5). This uncertainty is due to the fact that patients were often tested shortly after remission from hypercortisolism (6, 7) and remission was achieved by several treatment modalities, including radiation therapy which is known to progressively impair anterior pituitary function over time (8). Further, patients cured from Cushing's disease

frequently do not recover normal weight and this may confound the interpretation of GH status. A high prevalence of GH deficiency has also been reported after pituitary surgery/radiotherapy in paediatric Cushing's disease together with a favourable outcome obtained by early GH replacement (6, 7). We currently lack the information on GH secretory status in patients with Cushing's disease treated only by surgery, which in fact is the most frequent therapeutic course. In order to clarify this issue, we studied 34 patients with Cushing's disease after long-term remission obtained with surgery only. We used the GHRH + arginine test as this challenge is unaffected by glucocorticoids and can be evaluated according to the body mass index (BMI) (9).

Patients and methods

Patients

Thirty four patients (7 males, 27 females, age 41.2 ± 2.1 years, range 21–68 years) previously affected by Cushing's disease were studied. The diagnosis had been based on the usual diagnostic criteria (10) and confirmed by pathology of the surgical specimen. Two patients presented a macroadenoma and the remainder a microadenoma. Of the 34 patients, 13 were of normal weight ($BMI < 25 \text{ kg/m}^2$), 18 overweight ($BMI 25\text{--}30 \text{ kg/m}^2$) and 3 obese ($BMI > 30 \text{ kg/m}^2$).

Transsphenoidal surgery was performed as the first and only therapeutic approach in 31 patients. Two patients were resubmitted to pituitary surgery, followed by bilateral adrenalectomy in one, due to recurrence of hypercortisolism. One last patient was cured by bilateral adrenalectomy after failure of the first transsphenoidal surgery. The study was approved by both Institutions' Ethical Committees and written informed consent was obtained from all patients. Assessment of GH status was performed at least 2 years (59.3 ± 8.57 months, range 24–261 months) after remission of hypercortisolism by cross-sectional design. At the time of GH testing, all patients presented normal parameters of adrenal function (urinary free cortisol (UFC), serum cortisol levels, normal cortisol suppression by low dose dexamethasone test). Thirteen patients, including the two adrenalectomised patients, were still on steroid replacement therapy (12.5–37.5 mg cortisone acetate or 30 mg hydrocortisone daily). All other patients were off the replacement therapy, since at least 6 months and none were taking drugs known to interfere with adrenal or pituitary secretion. Seven patients were on thyroid replacement therapy for primary hypothyroidism; post-surgical diabetes insipidus requiring desmopressin therapy was present in one patient. Gonadal function was normal in all patients with two men on androgen replacement therapy.

Assessment of GH secretion

GH was measured after stimulation with growth hormone-releasing hormone (GHRH) + arginine; an indwelling catheter was placed into a forearm vein between 0700 and 0800 h and continuously flushed with saline. Two baseline samples were collected at least 15 min apart prior to the injection of $1 \mu\text{g/kg}$ body weight GHRH (Geref, Industria Farmaceutica Serono, Rome) as an i.v. bolus and infusion of 0.5 g/kg body weight (maximal dose 30 g) arginine (S.p.A. Laboratorio Farmacologico, Turin) over 30 min through a separate i.v. access. Blood samples were then collected 15, 30, 45, 60 and 90 min after the beginning of the infusion. Blood samples were centrifuged at 1000 g for 10 min at 4°C , serum collected by aspiration and stored at -20°C until assayed for GH. Insulin-like

growth factor-I (IGF-I) was measured in the samples collected at baseline. On the day of testing, patients on steroid replacement therapy were administered the drug at the end of the test.

Assays

Plasma adrenocorticotrophin (ACTH) and serum cortisol were measured by immunometric assay (Liason, Nichols Institute, San Juan Capistrano, CA, USA). UFC was measured by radioimmunoassay after urine extraction with dichloromethane (Byk-Sangtec Diagnostica, Dietzenbach, Germany). Intra- and interassay coefficients of variations were 3.2 and 8.2% for ACTH, 3.0 and 4.7% for serum cortisol respectively and 3.5 and 6.2% for UFC. Normal ranges are 10–50 pg/ml for ACTH, 5–25 $\mu\text{g/dl}$ for serum cortisol and 10–80 $\mu\text{g}/24 \text{ h}$ for UFC (11). IGF-I serum concentrations were determined by chemiluminescent immunoassay (CLIA, Nichols Institute, San Juan Capistrano, USA). Sensitivity of the assay is $6 \mu\text{g/l}$. IGF-I s.d. score (SDS) was calculated according to the formula: $(\log\text{IGF-I} + 0.00625 \times \text{age} - 2.555)/0.104$ (12). IGF-I scores comprised between -2 and $+2$ SDS (i.e. $3^\circ\text{--}97^\circ$ centile) are normal. GH serum concentrations were measured by CLIA (Nichols Institute); sensitivity of the assay is $0.01 \mu\text{g/l}$; baseline values up to $4 \mu\text{g/l}$ are considered normal.

Peak of GH levels during GHRH + arginine were used to define the response as normal ($\text{GH} > 16.5 \mu\text{g/l}$), partial GH deficiency ($\text{GH} < 16.5 \mu\text{g/l}$) or severe GH deficiency ($\text{GH} < 9 \mu\text{g/l}$) (13). Weight-adjusted thresholds for GH deficiency were also taken into consideration; GH peak $< 11.5 \mu\text{g/l}$ in normal weight, $< 8 \mu\text{g/l}$ in overweight and $< 4.2 \mu\text{g/l}$ in obese subjects (9, 14).

Statistical analysis

The results obtained were analysed using Student's *t*-test or Mann–Whitney's test for unpaired data, as appropriate. Bonferroni's correction was used for multiple comparisons. Qualitative differences were established by χ^2 or Fisher's exact test, according to the sample size. Linear regression analysis was used to evaluate association between the variables. Kaplan–Meier survival was calculated using GH deficiency as endpoint and difference among binary variables assessed by log-rank test (Statview, Abacus Concepts, Berkeley, CA, USA). Cox proportional hazard analysis was used to establish predictors of GH deficiency. Statistical significance was accepted for $P < 0.05$. Data are given as mean \pm s.e.m.

Results

According to established criteria for the GHRH + arginine test (13), 22 out of 34 (65.0%) patients

cured from Cushing's disease by pituitary surgery alone had impaired GH secretion at prolonged follow-up (Fig. 1). Eleven patients (32.5%) presented partial GH deficiency and an equal number (32.5%) severe GH deficiency. The mean of GH peak was $34.5 \pm 3.96 \mu\text{g/l}$ for normal responses, $11.9 \pm 0.59 \mu\text{g/l}$ for partial GH deficiency and $4.74 \pm 0.83 \mu\text{g/l}$ for severely GH deficient patients ($P < 0.001$ for normal versus GH deficient, $P = 0.05$ for partial versus severe GH deficiency). Of note, the two patients operated twice at the pituitary were both severely GH deficient; the patient with diabetes insipidus was partially GH deficient and the two adrenalectomised patients were partially and severely GH deficient respectively. GH deficiency, both partial and severe, was present in 52.4% of patients off steroid replacement therapy and in 84.6% of patients still treated with cortisone acetate/hydrocortisone ($P = 0.07$); severe GH deficiency was equally prevalent among patients on/off steroids (38.5 vs 28.6%, NS).

GH peak responses to GHRH+arginine stimulation were negatively correlated with BMI ($r = -0.385$, $P < 0.02$) and body weight ($r = -0.474$, $P < 0.005$) even though the body weight of our patients was mostly normal. In fact, evaluation of the GH response using the recently proposed weight-corrected cut-off values for GH deficiency (9) revealed that 3 out of 13 normal weight patients and 7 out of 18 overweight patients were GH deficient; conversely, all 3 obese patients presented peak GH values $> 4.2 \mu\text{g/l}$. Overall, these results are comparable with the prevalence of severe GH deficiency as assessed by standard criteria (13) and indicate that GH deficiency occurred independently of weight in our patients.

As expected, the GH response to GHRH+arginine was smaller in male than in female patients (6.9 ± 2.77 vs $20.3 \pm 3.03 \mu\text{g/l}$ respectively, $P < 0.007$) leading to a greater preponderance of severe GH deficiency in males (71.5 vs 22.2%, $P = 0.024$). Of note, BMI was

comparable among sexes (25.0 ± 2.34 vs $25.9 \pm 5.2 \text{ kg/m}^2$, NS). Partial GH deficiency developed in equal proportion among males and females. No association between GH peak and hypothalamic-pituitary-adrenal (HPA) axis parameters (e.g. UFC, serum cortisol, cortisol suppression by low dose dexamethasone, ACTH values in the active phase and at the time of testing) was detected. Further, no significant differences were found for the same variables between partially and severely GH deficient patients. No association was found between GH deficiency and prolactin levels. As only two patients presented a macroadenoma, no analysis on the size of the tumour could be performed.

In keeping with the gender difference reported above, male patients presented an increased rate of severe GH deficiency development at Kaplan–Meier analysis (males versus females $P < 0.05$, Fig. 2). Of note, no difference in anthropometric and HPA parameters was observed between male and female patients. Proportional hazard analysis revealed that the only predictor of GH deficiency was the length of hypercortisolism (positive predictor, coefficient = 0.218, $P < 0.005$). No relationship was found between GH status and age, BMI, HPA axis parameters in the active phase and at the time of testing at either parametric or conditional logistic regression analysis.

IGF-I scores below -2 s.d. was displayed by 17 patients (50%); among them, 3 patients presented normal responses to GHRH+arginine, 8 were partially GH-deficient and 6 presented complete GH deficiency. IGF-I SDS were lower in patients with complete or partial GH deficiency compared with patients with normal GH reserve (-2.9 ± 0.68 and -2.7 ± 0.52 vs -1.6 ± 0.31 SDS respectively, both $P < 0.05$). No difference was observed between IGF-I scores in patients with partial or complete GH deficiency. (Fig. 3). Peak GH levels were significantly correlated with IGF-I SDS ($r = 0.37$, $P < 0.05$); no association was

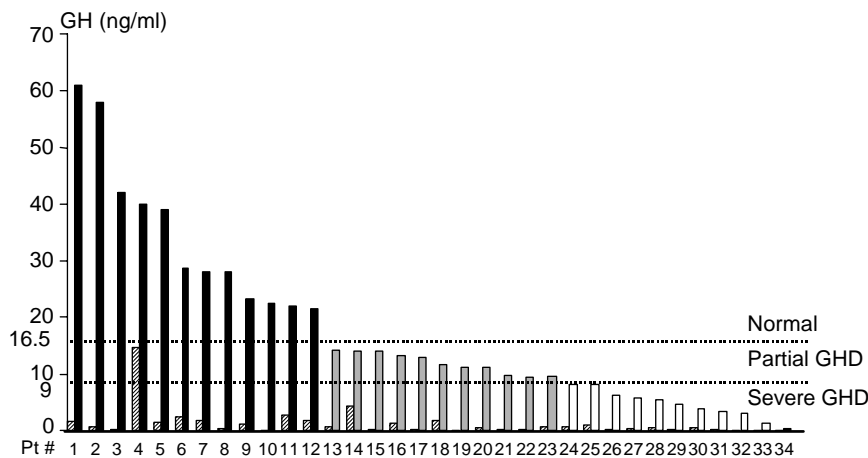


Figure 1 GH response to GHRH+arginine in individual patients cured from Cushing's disease by surgery alone. Baseline (shaded bar) and peak (black/grey/white bars) GH levels. Dotted lines indicate thresholds for GH deficiency.

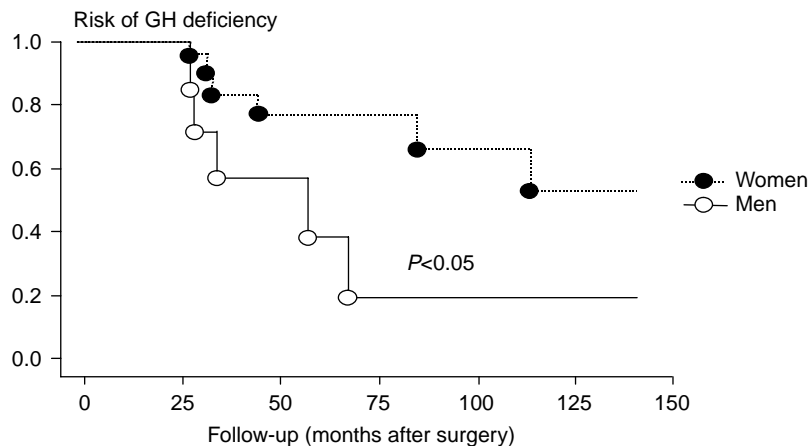


Figure 2 Kaplan–Meier survival plot for risk of severe GH deficiency in patients cured from Cushing's disease by surgery alone subdivided according to sex. Analysis was terminated at 150 months as <20% of the initial population was still available to follow-up.

observed between IGF-I scores and age, gender, BMI, length of follow-up, replacement therapy or prolactin secretion.

Discussion

Our study shows that a considerable proportion of patients with Cushing's disease treated by surgery alone displays impaired GH secretion after long-term remission of hypercortisolism. This is a relevant finding as it shows that even the most favourable therapeutical outcome, i.e. remission achieved by surgery alone, is associated with impaired GH secretion. High prevalence of GH deficiency had previously been reported in patients cured from Cushing's syndrome, but the past series contained patients treated by radiation therapy in addition to transsphenoidal surgery (15–20). Radiotherapy carries a considerable risk of causing hypopituitarism (21) and the GH axis is considered the most vulnerable in this respect, indeed, GH production may fail before gonadotrophin secretion (21). In other studies, patients had been tested early after resolution of Cushing's disease (6, 7, 22, 23) but the GH axis has been shown to recover over time (5, 24), thus testing after more prolonged follow-up appears necessary. On balance, published studies do not allow a precise estimation of GH deficiency in patients cured from Cushing's disease, especially in patients in whom surgery was sufficient to obtain remission of disease which, thankfully, is the majority. Our findings now provide this information.

We used a single test, GHRH + arginine stimulation – a test which has never before been used in patients cured from Cushing's disease – to assess GH secretory reserve. In fact, a variety of tests had been employed in this context, often even more than one in the same series, adding to the heterogeneity of reported data (8, 19, 22, 25). GHRH + arginine testing appears to be a reliable and convenient alternative to insulin tolerance testing (ITT) for the diagnosis of GH deficiency in adults

(26), as it appears unaffected by exogenous steroids (27) and reliable across a large age span (28). The latter is an additional advantage as we evaluated patients ranging from 21 to over 60 years of age. In addition, the response to GHRH + arginine stimulation can be analysed according to the recently proposed weight-adjusted criteria (9, 14). Evaluation using the latter as well as standard thresholds yielded superimposable results; in fact, our series comprised mostly overweight and normal weight subjects for whom the recommended cut-offs (8.0 and 11.5 $\mu\text{g/l}$ respectively) are close to the standard 9.3 $\mu\text{g/l}$ threshold. Of note, all three our obese patients presented GH responses above 9.3 $\mu\text{g/l}$.

In our series, severe GH deficiency was detected 2 years after the cure of hypercortisolism in one-third of patients (32.5%) and an equal number presented partial GH deficiency, totalling 65%; these results were substantiated by the detection of lower IGF-I SDS in these patients. The length of hypercortisolism and male sex appeared to be the most consistent predictors of postsurgical GH deficiency. The duration of disease has been associated with the persistence of some Cushingoid features after resolution of hypercortisolism, such as

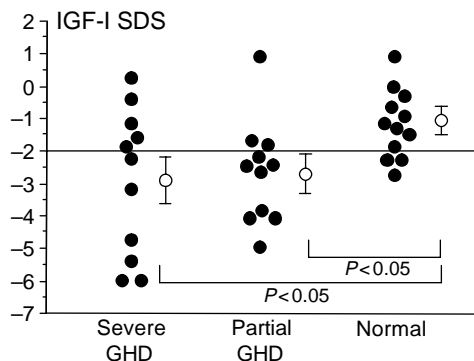


Figure 3 IGF-I s.d. in patients cured from Cushing's disease by surgery alone. Subnormal values are below -2 SDS, hard line. Empty circles indicate mean \pm s.e.m.

hypertension (29, 30), increased carotid artery thickness (31) and obesity (30), thus recovery of somatotroph function may also depend on the length of exposure to excess corticosteroids. As for the greater prevalence of GH deficiency among our male patients, it is noteworthy that lower GH responses to GHRH+arginine have been reported in healthy males compared with healthy females, with higher BMI in males contributing to this difference according to some but not all authors (32, 33). However, this gender-related difference does not seem to affect the diagnostic cut-offs (32) and the sensitivity of the test and an equal prevalence of GH deficiency was detected among male and female patients tested by both GHRH+arginine and ITT (14). Our observation appears therefore unique to Cushing's disease and is in accordance with the more severe clinical presentation, cortisol hypersecretion and worse postsurgical outcome in adult male patients with active Cushing's disease (34) although the reasons underlying these gender-specific differences are as yet obscure. Somatotroph failure was also slightly more prevalent among patients on glucocorticoid replacement therapy, although this difference fell short of statistical significance. This difference is unlikely to result from the interference of exogenous steroids on GH secretion as the GHRH+arginine test is reportedly unaffected by administration of steroids at pharmacological dosages (27, 35), i.e. far greater than those employed to correct postsurgical hypocortisolism.

Active Cushing's disease is a known condition of GH deficiency although the pathophysiology of this alteration is not completely understood. Prolonged exposure to suprphysiological levels of cortisol has been postulated to impair GH secretion through several mechanisms. Excess glucocorticoids have been hypothesised to reduce GHRH release (36) and/or increase somatostatinergic tone (37) or inhibit GH secretion by pituitary somatotrophs (38). In fact, spontaneous 24 h GH secretion (22) as well as GH responsiveness to L-dopa (22), GHRH+arginine (39) and ITT (5) are impaired in patients with active Cushing's disease. As we and others have shown, GH secretion does not always normalise after cure. Several hypotheses have been put forward to explain the persistence of this defect, including surgical manipulation of the pituitary during the search for small tumours. In fact, in large neurosurgical series, GH deficiency developed after pituitary surgery in 14–30% of operated patients and this occurrence was independent of the size of the lesion (40, 41). Impaired GH secretion after recovery from hypercortisolism may also likely result from an irreversible damage of the GHRH/GH axis during the active phase of the disease induced by sustained hypercortisolism, as suggested by comparison of postsurgical GH deficiency among different pituitary tumours (42). We have not tested the GH axis in these patients prior to surgery and thus cannot compare the GH status before and after surgery. On

balance, both surgical and presurgical factors contribute to postsurgical GH deficiency in patients with Cushing's disease.

The impact of impaired GH secretion after cure of Cushing's disease, even in patients who were not treated by radiation therapy, should not be underestimated. Indeed, GH deficiency impairs catch-up growth in short children cured from Cushing's disease and treatment with GH has proven greatly beneficial in these patients (6, 7). In adults, GH deficiency could contribute to metabolic derangements, increased cardiovascular risk and osteoporosis, which have been observed years after successful resolution of Cushing's disease (30, 31, 43). Accordingly, a study on a small group of patients has shown that adult patients with Cushing's disease who develop GH deficiency fare worse than age-matched patients with hypopituitarism due to other disorders, both prior to initiating rhGH therapy and during the course of treatment (17). In addition to patients with severe GH deficiency, we observed a considerable proportion of patients cured from Cushing's disease with partial GH deficiency, a condition which is claiming an increasing attention. Indeed, patients who fall in this 'grey area' of impaired GH secretory status have recently been shown, in spite of normal IGF-I scores, to present several metabolic abnormalities and possible progression towards full GH deficiency (44). Thus, patients cured from Cushing's disease merit careful evaluation, both with regard to the benefits of GH treatment in patients with severe GH deficiency and clinical surveillance of patients with partial GH deficiency with low IGF-I SDS.

In conclusion, our study demonstrates the presence of GH deficiency in a high percentage of patients with Cushing's disease even after long-term remission of hypercortisolism obtained only by surgical means. Male gender and length of hypercortisolism appeared to be the most significant predictors of postsurgical GH deficiency. These findings highlight the need for re-evaluation of the GH/IGF-1 axis in all patients cured of Cushing's disease, even if not submitted to radiotherapy, and establish the need for studies on the possible use of GH replacement therapy in patients cured from Cushing's disease with impaired GH secretion.

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