Screening for ACTH-dependent hypercortisolism in patients affected with pituitary incidentaloma

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Abstract

Context: Pituitary incidentalomas (PIs) are commonly encountered in clinical practice. The management of these asymptomatic pituitary lesions is still controversial. Systematic screening for subclinical or mild ACTH-dependent hypercortisolism (AH) is not presently recommended, due to the limited data available thus far on the epidemiological and clinical relevance of this condition in patients with PIs. As subclinical hypercortisolism (SH) was considered to be associated with chronic complications of overt cortisol excess, such as hypertension, diabetes, and osteoporosis, this disorder should be diagnosed at the early stage.

Objective: The objective of this study was to evaluate the prevalence of hypercortisolism in a population of subjects with PIs.

Design, subjects, and methods: A total of 68 consecutive patients (48 females and 20 males, aged 18–82 years) without clinically overt hypercortisolism, who were referred for evaluation of PIs between January 2010 and March 2013, were prospectively investigated for AH. Pituitary hypercortisolism was diagnosed in the presence of cortisol >50 nmol/l after 1 mg dexamethasone suppression test, non-suppressed ACTH, and the additional finding of one of the following: urinary free cortisol (UFC) >193 nmol/24 h, and midnight serum and salivary cortisol levels >207 and 2.8 nmol/l respectively.

Results: Among patients with PIs, we found a 7.3% rate of pituitary hypercortisolism diagnosed with biochemical criteria and a 4.4% rate of histologically confirmed AH.

Conclusions: Subclinical or mild hypercortisolism may be more common than generally perceived in patients with PIs.

Introduction

The routine use of sensitive neuroradiological imaging procedures has significantly increased the incidental finding of asymptomatic pituitary lesions, the so-called ‘pituitary incidentalomas’ (PIs) in the last 30 years. They are defined as sellar lesions incidentally identified by imaging studies not performed because of signs and/or symptoms specifically related to the pituitary gland (1). The surgical series published thus far showed that the majority of PIs are pituitary adenomas (2, 3). Although these tumors are usually hormonally inactive, all patients with incidentalomas should be screened for pituitary hypo- or hyper-function (4).

In particular, a guideline for the management of PIs published in 2011 from The Endocrine Society suggests to screen all patients for hyperprolactinemia and growth hormone (GH) excess, and recommends the screening for glucocorticoid excess only in patients in whom hypercortisolism is clinically suspected (4). However, no final
agreement has been declared by the members of The Endocrine Society on this latter recommendation, due to the lack of systematic screening studies on subclinical hypercortisolism (SH) in patients with PIs.

Recent studies have demonstrated a high prevalence (5–30%) of SH (defined as a condition of biochemical cortisol excess without the classical signs or symptoms of overt hypercortisolism) in patients with adrenal incidentalomas (5, 6, 7). Interestingly, these patients showed long-term complications of cortisol excess, such as hypertension, insulin resistance, type 2 diabetes, central obesity, dyslipidemia, and an increased risk of osteoporosis and vertebral fractures, that partially reverted by adrenalectomy (8, 9, 10, 11). As no systematic screening of SH has been performed so far in patients with PIs, it is not possible to exclude the presence of a subset of subjects with mild/subclinical Cushing’s disease, possibly associated with the same comorbidities observed in overt glucocorticoid excess, in the PI population.

The aim of this study was to define the prevalence of subclinical or mild adrenocorticotropic (ACTH)-dependent hypercortisolism (AH) in a series of patients affected with PIs and to focus on the usefulness of a systematic screening for subclinical glucocorticoid excess.

**Subjects and methods**

**Subjects**

We prospectively evaluated data from 68 patients (aged 18–82, 20 males) with PIs, non-selectively and consecutively enrolled from January 2010 to March 2013 in two referral Italian Endocrinology Units: ‘Fondazione IRCCS Ca’ Granda – Ospedale Maggiore Policlinico’ and ‘San Giuseppe Hospital’ in Milan.

For the initial testing of glucocorticoid excess, serum cortisol levels were measured at 0900 h after a 1-mg overnight dexamethasone suppression test (1 mg DST; cutoff value 50.0 nmol/l, according to previous studies (12)) in all subjects.

Selection criteria were as follows:

i) Age ≥ 18 years.

ii) Detection of a pituitary lesion on magnetic resonance imaging (MRI) in the course of diagnostic testing for other clinical conditions not related to the suspicion of pituitary disease.

iii) Absence of clinical signs of glucocorticoid excess (including moon facies, striae rubrae, hypertrichosis, skin atrophy, central obesity, and posterior cervical fat pad).

All patients with absent suppression of cortisol levels under the threshold of 50 nmol/l on 1 mg DST in at least two measurements (DST+) were subjected to further screening. As widely accepted guidelines on this topic were not available, pituitary hypercortisolism was diagnosed in patients who presented abnormal DST, AH, together with at least one of the following two alterations:

i) urinary free cortisol (UFC) levels > 193 nmol/24 h (normal values 28–193 nmol/24 h), which is the cutoff of both our own and international normal reference values (6, 13) in at least two measurements;

ii) high midnight serum or salivary cortisol levels (awake value, cutoff 207 and 2.8 nmol/l respectively).

Pseudo-Cushing’s states (due to depression, anxiety disorders, poorly controlled diabetes mellitus, and alcoholism) were excluded in DST+ patients by performing 2-day low-dose-DST (LDDST), using a threshold for serum and urinary cortisol normal suppression of 50 and 27 nmol/l respectively. All patients on drugs influencing cortisol levels and dexamethasone metabolism were excluded.

We then performed corticotropin-releasing hormone (CRH) test, desmopressin (DDAVP) test, and a high-dose (8 mg) dexamethasone suppression test (HDDST) in DST+ patients in order to differentiate Cushing’s disease from ectopic ACTH secretion. An ACTH increase > 35% after CRH/DDAVP administration and a cortisol suppression below 50% after HDDST intake were attributed to the presence of Cushing’s disease (14). Inferior petrosal sinus sampling was recommended in the presence of discordant tests.

The presence of chronic complications of hypercortisolism was evaluated. Patients with systolic blood pressure (BP) ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg were defined hypertensive. Type 2 diabetes mellitus and metabolic syndrome were diagnosed using the WHO and ATPIII criteria respectively (15, 16). Written informed consent was obtained from all patients.

**Methods**

Plasma morning ACTH levels (mean of three determinations at 20-min intervals) were measured by a chemiluminescent immunometric assay (IMMULITE 2000, Siemens Medical Solutions Diagnostic, Los Angeles, CA, USA) and serum cortisol levels by an electrochemiluminescence immunoassay (ECLIA; Cobas, Roche Diagnostic GmbH). The intra- and inter-assay coefficient of variation values were ≤ 10% for ACTH and < 2.8% for serum cortisol.

UFC levels, after dichloromethane extraction, were determined immunofluorimetrically using TDX-FLX.
Abbott GmbH, Diagnostika Kits. Measurement of salivary cortisol levels was obtained by chromatography–tandem mass spectrometry LC–MS/MS using a triple quadruple mass spectrometer (TSQ Quantum Access, Thermo Scientific, Milano, Italy) equipped with a heated electrospray ionization source (H-ESI), operating in the positive ion mode (Fig. 1).

**Results**

**Assessment of hypercortisolism**

Out of 68 patients, seven were detected with non-suppressible cortisol levels after overnight 1 mg DST, therefore undergoing further evaluation. Of these seven patients, one showed an adequate suppression of cortisol levels after LDDST, while another had suppressed ACTH levels consistent with ACTH-independent hypercortisolism: she underwent an abdominal computerized tomography (CT) scan that showed an adrenal adenoma of 6 mm. Surgery was proposed but the patient preferred to continue clinical and biochemical follow-up. By performing further evaluation tests, we diagnosed AH in the remaining five patients (7.3%) (two females and three males). In fact, cortisol excess was further documented by elevated UFC excretion (patients N.1, N.3, and N.4) and/or loss of serum or salivary cortisol levels by circadian variations, which was found in all tested patients (Table 1).

**Second-line endocrine testing**

We obtained multiple ACTH measurements for all patients, always compatible with AH. We performed HDDST in two patients, which revealed a suppression of serum cortisol consistent with the diagnosis of Cushing’s disease. Furthermore, we performed CRH stimulation in two patients and DDAVP stimulation in three patients with pathological responses of ACTH and cortisol levels (Table 1).

In accordance with the current guideline, only patient N.3 underwent bilateral inferior petrosal sinus sampling because of discordant results of CRH test and HDDST: the ratios of inferior petrosal sinus to peripheral ACTH levels of 28 at basal and 36 after CRH stimulation were found consistent with the pituitary origin of hypercortisolism.

Overall then, the prevalence of hypercortisolism in our cohort was ~5% (histologically confirmed, 4.4% and biochemically diagnosed, 7.3%). This percentage is different and significantly higher than that expected in control normal groups, as shown in two published case-control series from our institution reporting the presence of ascertainment SH in <2% (3/189 in Chiodini et al. (17) and 0/83 in Chiodini et al. (14)).

**Clinical manifestations**

In AH+ patients, PI was discovered at a mean age of 55.2 years (range: 37–82 years), during investigations for: non-specific visual deficits, ischemic stroke, severe obesity, and headache (two cases, Table 2). MRI of the pituitary gland revealed a microadenoma (tumor diameter, ≤10 mm) in three patients and a macroadenoma (diameters, 13 and 44 mm respectively) in two (patients N.1 and N.5). Visual field defects (bitemporal hemianopsia and arched scotomas) were found in these two patients.
At presentation, hormonal evaluation detected hypo-gonadotropic hypogonadism in two subjects (patients N.4 and N.5), GH deficiency in one patient (patient N.4) and mild hyperprolactinemia, probably due to stalk compression, in another patient (patient N.1). On further examination, all subjects were asymptomatic and the absence of any of the typical hypercortisolism stigmata was confirmed.

Biochemical evaluation (fasting glucose and lipid levels, and electrolytes) was normal in all patients, with the exception of mixed dyslipidemia in two cases (patients N.1 and N.3).

Two patients were suffering from hypertension, in good control under pharmacological treatment, which was interpreted as essential hypertension due to known cardiovascular risk factors, i.e., obesity and dyslipidemia in patient N.3 and advanced age and family history in patient N.5. On subsequent screening tests, of the three non-hypertensive patients at first examination, one (patient N.4) showed an altered BP profile at 24-h ambulatory BP monitoring, with absent physiological night-time lowering of BP (‘non-dipper’ profile).

Dual-energy X-ray absorptiometry showed low bone mineral density at lumbar site in one out of three patients, while psychological sphere examination revealed major depression in one patient (patient N.1) and anxious depressive behavioral changes in another patient (patient N.4).

We obtained a follow-up mean duration of 30 months: in fact, two subjects were not operated and are still continuing their follow-up, while the operation was delayed due to different reasons in the three patients who underwent adenomectomy (patient’s own decision or indication to optimize other comorbidities before surgery).

Interestingly, during pre-surgery follow-up, the clinical picture of AH+ patients showed no progression and they continued to be completely asymptomatic for hypercortisolism until surgery (patients N.1, N.3, and N.4) or until their last clinical evaluation (patients N.2 and N.5). These clinical characteristics of AH+ patients are summarized in Table 2.

### Surgical outcome

Three patients (N.1, N.3, and N.4) underwent transphenoidal adenomectomy. On the day of surgery, they were treated with a standard regimen of hydrocortisone 100+100 mg, which was then replaced with cortisone acetate 37.5 mg on the 1st day after surgery and reduced to 25 mg/day from the 2nd day onwards. Cortisol measurements were always obtained before administration of glucocorticoid. Remission of hypercortisolism, defined by laboratory evidence of adrenal insufficiency (serum cortisol ≤55.18 nmol/l; in particular: 32, 40, and 20 nmol/l in patient N.1, N.3, and N.4 respectively), was observed 48 h after adenomectomy (18). Moreover, in all three cases, a pathological confirmation of the removal of an ACTH-staining adenoma was obtained and the expression of labeling index Ki67 was determined (Table 1).
The three patients showed sustained central hypo-adrenalism 2 and 6 months after surgery and only one of them showed recovery of the HPA axis after 1 year. These findings are similar to those observed in ‘classical’ Cushing’s disease after complete removal of the ACTH-secreting adenoma, although it is not possible to exclude the presence of adrenal insufficiency secondary to surgical treatment. In one subject with macroincidentaloma (patient N.1), hypoadrenalism was associated with panhypopituitarism, diagnosed according to the current guidelines. At present, these hormonal defects are still confirmed, and all patients are in good health in replacement therapy, when needed.

One subject refused surgical treatment and the last patient was not operable due to advanced age and comorbidities (N.2 and N.5 respectively).

**Discussion**

This study first investigated the presence of mild or subclinical Cushing’s disease in a consecutive and non-selected cohort of patients with PI, and estimated the prevalence of this condition to be ~5% (histologically confirmed AH, 4.4% and biochemically diagnosed AH, 7.3%). The percentage of hypercortisolism is different and significantly higher than that expected in control normal groups, as shown in few published case-control series from our institution reporting the presence of ascertained SH in 0–2% of normal population (14, 17). The only bias of this study might be related to the fact that our institute is a referral center for pituitary disorders and a pre-selection of more severe cases cannot be completely excluded.

The importance of these data comes from the consideration that the epidemiological relevance of subclinical AH has been poorly studied and likely to be underestimated until now. In particular, a systematic screening test of SH has been carried out, to date, exclusively in adrenal incidentalomas, where this condition was detected in 5–30% of patients. Moreover, SH has been associated with similar complications to chronic hypercortisolism, such as hypertension, diabetes, and osteoporosis (8, 9, 10, 11).

As for PIs, the literature only reports autopic series showing a variable prevalence of ACTH-positive pituitary adenomas in patients with no ante-mortem history of pituitary disease (18, 19). In particular, a post-mortem study conducted with 334 pituitary adenomas indicates a high prevalence of ACTH-positive immunohistochemistry (14.1%) (19), that is similar to prevalence of ACTH-positive adenomas (15%) reported in surgical series (20, 21).

With regard to the screening strategy, some authors have proposed higher cutoff values for 1 mg DST in order to reduce false-positive results (22, 23). Despite this, taking into account that both overt and SH are associated with long-term morbidity (24), we applied a screening strategy with maximal sensitivity, using the cutoff point of 50 nmol/l for 1 mg DST, as proposed by a recent consensus statement on overt Cushing’s syndrome (12).

### Table 2 Clinical characteristics of patients with ACTH-dependent hypercortisolism.

<table>
<thead>
<tr>
<th></th>
<th>Patient N.1</th>
<th>Patient N.2</th>
<th>Patient N.3</th>
<th>Patient N.4</th>
<th>Patient N.5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td><strong>Age at diagnosis</strong></td>
<td>65</td>
<td>38</td>
<td>54</td>
<td>37</td>
<td>82</td>
</tr>
<tr>
<td><strong>Reason for MRI</strong></td>
<td>Non-specific visual defects</td>
<td>Fronto-occipital headache</td>
<td>Microadenoma</td>
<td>Fronto-occipital tension headache</td>
<td>Ischemic stroke</td>
</tr>
<tr>
<td><strong>Pituitary tumor size</strong></td>
<td>Macroadenoma</td>
<td>Microadenoma</td>
<td>Normal</td>
<td>Microadenoma</td>
<td>Macroadenoma</td>
</tr>
<tr>
<td><strong>BP (mmHg)</strong></td>
<td>Normotensive</td>
<td>Hypertensive</td>
<td>Normotensive</td>
<td>Hypertensive</td>
<td>Hypertensive</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>23.2</td>
<td>25.2</td>
<td>31</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td><strong>Visual field</strong></td>
<td>Arched scotomas</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Bitemporal hemianopsia</td>
</tr>
<tr>
<td><strong>Lipid and glucose profile</strong></td>
<td>Mixed dyslipidemia</td>
<td>Normal</td>
<td>Mixed dyslipidemia</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Hormonal evaluation</strong></td>
<td>Hyperprolactinemia (stalk compression)</td>
<td>Normal</td>
<td>Normal</td>
<td>Hypogonadotropic hypogonadism, GH deficiency</td>
<td>Hypogonadotropic hypogonadism</td>
</tr>
<tr>
<td><strong>Z-score at lumbar site</strong></td>
<td>NE</td>
<td>0.9</td>
<td>−0.1</td>
<td>−2.4</td>
<td>NE</td>
</tr>
<tr>
<td><strong>Surgery</strong></td>
<td>Yes</td>
<td>Surgery refused</td>
<td>Yes</td>
<td>Yes</td>
<td>Not operable</td>
</tr>
<tr>
<td><strong>Follow-up (months)</strong></td>
<td>36</td>
<td>48</td>
<td>30</td>
<td>12</td>
<td>24</td>
</tr>
</tbody>
</table>

F, female; M, male; BP, blood pressure; NE, not evaluated.

*As candidate for bariatric surgery, patient N.3 had a Doppler ultrasound showing carotid stenosis; a subsequent brain computerized tomography (CT) showed a pituitary incidentaloma, that was confirmed by magnetic resonance (MR).

*Hypertension on therapy with two antihypertensive drugs.

The three patients showed sustained central hypo-adrenalism 2 and 6 months after surgery and only one of them showed recovery of the HPA axis after 1 year. These findings are similar to those observed in ‘classical’ Cushing’s disease after complete removal of the ACTH-secreting adenoma, although it is not possible to exclude the presence of adrenal insufficiency secondary to surgical treatment. In one subject with macroincidentaloma (patient N.1), hypoadrenalism was associated with panhypopituitarism, diagnosed according to the current guidelines. At present, these hormonal defects are still confirmed, and all patients are in good health in replacement therapy, when needed.

One subject refused surgical treatment and the last patient was not operable due to advanced age and comorbidities (N.2 and N.5 respectively).
In this study, we obtained clinical and biochemical data from five AH+ patients. The percentage of AH+ patients based on the finding of at least three abnormal parameters of cortisol secretion (abnormal DST, non-suppressed ACTH, elevated UFC levels, and/or loss of circadian rhythm of cortisol secretion) was 7.3%. Nevertheless, we are aware of some limitations about this result. It must be considered that surgical confirmation of the pituitary origin of hypercortisolism was lacking in patients N.2 and N.5 and further data corroborating the diagnosis of pituitary AH (e.g. dynamic tests) are lacking for patient N.5. Nevertheless, in light of the patient meeting all requested biochemical criteria for SH, we decided to describe the patient, contextually highlighting the percentage of histologically confirmed pituitary AH (4.4%).

A novelty of this study is the description of a silent clinical picture at presentation that, unlike what usually happens in patients with AH, did not progress over a mean follow-up duration of ~30 months. Although AH+ patients were possibly screened during an early preclinical phase, it is unlikely in those patients harboring a macroadenoma, suggesting a long duration of disease. A distinct subtype of pituitary adenoma characterized by asymptomatic clinical presentation is the so-called 'silent corticotrope adenoma’ (SCA). These tumors, which account for 1–6% of surgically removed pituitary adenomas (25, 26), show positive ACTH staining and do not exhibit clinical or biochemical features of Cushing’s disease. In fact, although these patients are promptly referred to the neurosurgeon before completing a full endocrine workup due to visual defects, studies with preoperative hormonal assessment report normal basal ACTH and cortisol levels with normal suppression after LDDST (27, 28). Few reports describe a mild elevation in ACTH levels due to secretion of high-molecular-weight ACTH without biological activity (29). Moreover, SCAs are reported to have an aggressive behavior by most (28, 30), but not all (31, 32), authors, associated with high levels of proliferative markers (mean Ki67 levels of 2–4%) (26, 28). AH+ patients described in this study appear as a clinical entity that differs from SCA, showing altered biochemical indices of cortisol secretion associated with apparently non-aggressive tumors characterized by low Ki67 levels.

Finally, as for hypercortisolism complications, although subclinical forms by definition are not associated with signs and/or symptoms specific to overt cortisol excess, such as purple striae, easy bruising, proximal muscle weakness, and plethora, some evidence suggests that even mild excesses may lead to long-term consequences of cortisol overproduction (i.e., diabetes, hypertension, obesity, and osteoporosis), as reported in patients with adrenal incidentalomas. These conditions, however, are not specific to cortisol excess, being highly frequent in the general population. The concomitant presence in the same individual of all these possible consequences of cortisol excess may suggest the presence of mild Cushing’s disease in the population described in this study, but our report is still preliminary and includes very few patients to speculate on the presence of the typical complications of chronic exposure to mild hypercortisolism in PIs.

In conclusion, the rate of mild hypercortisolism in patients with PI is not negligible (in our case series: histologically confirmed, 4.4% and biochemically diagnosed, 7.3%) and seems to be much more frequent than commonly perceived. As a consequence and in accordance with data obtained on adrenal incidentalomas (8, 9, 10, 11), we hypothesize that this condition may be associated with important clinical sequelae (hypertension, osteoporosis, insulin resistance, and diabetes). However, we are aware that limitations due to the small number of patients with AH suggest caution in providing clear-cut recommendations and that great care is required to avoid hypercortisolism overdiagnosing with consequent possibly harmful screening and therapeutic approaches.

Although further prospective follow-up studies are necessary to define the behavior of this novel challenging entity, we recommend screening for hypercortisolism in all patients with PIs at the time of diagnosis, regardless of the size of the lesion.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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