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Subclinical hypercortisol-assessment of bone fragility: experience of single osteoporosis center in Sicily

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Abstract. – AIM: Hypercortisolism is known to cause osteoporosis. Some evidence suggests that osteoporotic fractures may be the presenting manifestations of otherwise-asymptomatic hypercortisolism. The aim of our research was to investigate the prevalence of subclinical hypercortisolism (SH) in postmenopausal women evaluated for bone fragility.

PATIENTS AND METHODS: One hundred consecutive postmenopausal women attending the Osteoporosis Centre in the Department of Internal Medicine of the University of Messina (Messina, Italy), for the first time, were screened and a total of 50 patients (age 58±5 years) were studied. Hypercortisolism was diagnosed by unsuppressed serum cortisol levels after 2 day low dose dexamethasone suppression test.

RESULTS: Among the 50 postmenopausal women studied, 3 had SH. This prevalence was 6%. The three patients with SH had a normal bone mineral density (BMD) at lumbar spine and were osteopenic at femoral neck, and presented one or more vertebral fractures at spinal radiography.

CONCLUSIONS: Physicians should always consider SH among the causes of bone fragility, especially in individuals with vertebral fractures and the presence of an only slightly reduced BMD.

Key Words:
Bone density, Fractures, Hypercortisolism, Menopause, Osteoporosis.

Introduction

Classical overt Cushing's Syndrome (CS), the consequence of longstanding excess of circulating glucocorticoids, is characterized by typical signs and symptoms of hypercortisolism, such as plethora, moon face, buffalo hump, central obesity, easy bruising, deep purple striae, proximal muscle weakness, menstrual irregularities, acne, hirsutism, glucose intolerance and osteoporosis[1-3]. Classic Cushing's syndrome is a rare disease with an estimated incidence of 0.7 to 2.4 per 1 million persons annually[4]. Studies of patients with diabetes, hypertension, and hirsutism showed a prevalence of Cushing's syndrome from 2% to 4%, 1%, and 0.25%, respectively[5-7]. Autonomous glucocorticoid production without specific signs and symptoms of Cushing's syndrome is termed "subclinical Cushing's syndrome" or "subclinical hypercortisolism (SH)". With an estimated prevalence of 79 cases per 100,000 persons, subclinical Cushing's syndrome is much more common than classic Cushing's syndrome[8]. This prevalence is probably underreported due to the lack of symptoms or signs in these patients. Several cross-sectional and longitudinal studies have suggested that these patients are at high risk of hypercortisolism complications, such as diabetes and osteoporosis[9-13]. Some evidence suggests that osteoporotic fractures may be the presenting manifestations of otherwise-asymptomatic hypercortisolism[14]. Endogenous CS results from chronic exposure to excess glucocorticoids produced by the adrenal cortex. It may be caused by excess ACTH production (80-85%), usually by a pituitary corticotroph adenoma [Cushing's disease (CD)], less frequently by an extra pituitary tumour (ectopic ACTH syndrome), or very rarely by a tumour secreting CRH (ectopic CRH syndrome). CS can also be ACTH-independent (15-20%) when it results from excess secretion of cortisol by unilateral adrenocortical tumors, either benign or malignant, or by bilateral adrenal hyperplasia or dysplasia[3].

Incidentally discovered adrenal masses (incidentalomas) have recently become a relatively common finding in patients evaluated by imaging techniques, such as ultrasound (US), computed tomography (CT) and magnetic resonance (MR), for unrelated disorders[15-17]. This evidence is in accordance with data from autopsy series documenting microscopic or macroscopic adrenal nodules in 2-9% of patients who had never had

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signs of adrenal dysfunction[18,19]. Incidentalomas are mostly benign and asymptomatic. They may be nonfunctioning or producing adrenocortical hormones in amounts insufficient to cause a clinically apparent disease[20]. Recent studies show a significant prevalence of SH in patients with chronic diseases such as hypertension or diabetes mellitus[21-24]. Are little data on the prevalence of subclinical Cushing in patients with osteoporosis. The aim of our research was to investigate the prevalence of SH in postmenopausal women evaluated for bone fragility.

Patients and Methods

The subjects were recruited from 100 consecutive postmenopausal women attending the Osteoporosis Centre in the Department of Internal Medicine of the University of Messina (Messina, Italy), for the first time. Enrolment took place during the period between September 2008 and June 2009. Exclusion criteria were cushingoid appearance, precocious or surgical menopause, thyrotoxicosis, chronic liver or renal failure, bowel diseases, eating disorders, hematologic diseases, depression or other mood disorders, chronic anxiety and alcoholism, the use of drugs influencing bone, cortisol, and dexamethasone metabolism or cortisol secretion. A total of 50 patients (age 58±5 years) were studied. None of the patients displayed moon facies, striae rubrae, skin atrophy or buffalo hump. Patients not included in the study were evaluated and treated according to common treatment guidelines: among these, the only patient who apparently had a phenotype suggestive of Cushing's, reported negative results in subsequent tests (see below) that allowed us to exclude Cushing's disease. All patients had spinal and femoral dual-energy x-ray absorptiometry using a Hologic 4500 machine and a conventional radiography of the spine in anteroposterior and lateral projections to search for morphometric fractures that were subsequently classified according to the semiquantitative method proposed by Genant et al[25]. After an overnight fast, all participants underwent routine tests such as count blood cells (CBC), blood glucose, electrolytes (sodium, potassium, calcium, phosphorus), renal function (BUN, creatinine), coagulation, transaminases, alkaline phosphatase, lipid profile (cholesterol, triglycerides); 25-hydroxyvitamin D3 (normal values 20 to 120 ng/ml), PTH (normal values 2-72 pg/ml), TSH (normal values 0.27 to 4.2 μU/ml), ACTH (normal values 5-50 pg/ml), BGP: Bone Gla Protein: osteocalcin (normal value 1.6 to 17.4 pg/ml) were also assayed. A 2-hour fasting morning urine sample was collected at the same time to assess urinary excretion of pyridinium cross links [pyridinoline (PYD) and deoxypyridinoline (DPD)]. PYD (normal range, 26 to 91 pmol/μmol creatinine) and DPD (normal range, 3 to 21 pmol/μmol creatinine) were measured by using high-performance liquid chromatography (Bio-Rad Laboratories, Hercules, CA, USA). At the same time, a 24h urine sample was collected to measure urinary cortisol and cortisone. Urinary cortisol (normal range from 9.2 to 45.2 g/24h) and cortisone (normal range 14.5-94.6 g/24h) were determined by using high-performance liquid chromatography; serum cortisol (normal range from 5 to 25 g/dl) was measured by solid-phase competitive chemiluminescent enzyme immunoassay (Immulite 2000 Cortisol, Diagnostic Products Corporation, Los Angeles, CA, USA), ACTH by sequential immunometric assay (Immulite 2000 ACTH, DPC, USA). All routine blood tests were carried out using automated routine procedures. Patients with increased urinary cortisol values performed the rapid dexamethasone suppression test (Nugent's test): 1 mg of dexamethasone was given to these patients at 11.00 p.m. and the following morning plasma cortisol was measured (normal response: cortisol < 1.8 μg/dl[3]. In case of no suppression of serum levels of cortisol after overnight low-dose (1 mg) dexamethasone suppression test, the patient underwent a 2 day low-dose dexamethasone suppression test (dexamethasone 0.5 mg every 6 hours for 2 days) and determination of serum cortisol at 8:00 am after 48 hours from the first dose. Diagnosis of SH was based on the presence of the following alterations of HPA (hypothalamic-pituitary-adrenal) axis: (1) increased urinary free cortisol (UFC) levels > 70 g per 24 h (193 nmol/24h); (2) unsuppressed serum cortisol levels after 1-mg overnight dexamethasone suppression test [serum cortisol after dexamethasone > 1.8 g/dl (50 nmol/liter)] and (3) unsuppressed serum cortisol levels after 2 day low dose dexamethasone suppression test [serum cortisol after dexamethasone > 1.8 g/dl (50 nmol/liter)]. The intra- and inter-assay coefficient of variation (CV) were < 10% for all the tests. Once subclinical hypercortisolism was diagnosed, the dosage of ACTH was performed (normal values 5-50 pg/ml). The study protocol was approved by the

Ethics Committee of our Hospital and was performed according to the principles of the Declaration of Helsinki; informed consent was obtained in every case.

Statistical Analysis

Statistical analyses were performed using StatSoft software (release 4.5). All values were expressed as mean ± SD. Comparisons between the groups were performed by Student's *t*-test for unpaired data when the data were normally distributed, or by non-parametric statistical analysis (Wilcoxon rank-sum test) when they were not. All p values are two-sided, and values of < 0.05 were considered to indicate statistical significance.

Results

The characteristics of all patients are shown in Table I and Table II. Among the 50 women enrolled, 5 (10%) had urinary cortisol greater than normal values (9.2 to 45 g/24h). A subsequent Nugent's test performed on these 5 patients, detected an inadequate suppression of plasmatic cortisol level (cortisol values remained above 1.8° g/dl) after administration of 1 mg of dexamethasone in 3 of them. Inadequate suppression of plasmatic cortisol level was also confirmed by classical 2-day low dose dexamethasone suppression test carried out on the same patients (range of values obtained 2.57 µg/dl-3.81 µg/dl). Basal ACTH in our patients was 15, 14, 16.5 pg/ml respectively. These results suggested an ACTH-independent SH. A computed tomography of the abdomen was done and showed a homogeneous, hypodense, and well-shaped adrenal mass consistent with adrenocortical adenoma in all three patients (Table III).

Table I. Characteristics of patients.

Characteristics all patients	(No = 50)
Age (years)	58.00 ± 5.00
BMI (kg/m^2)	26.11 ± 1.96
Menopausal age (years)	50.00 ± 2.00
BMD lumbar spine (g/cm^2)	0.78 ± 0.11
T-score lumbar spine (SD)	-2.67 ± 1.13
BMD femoral Neck (g/cm^2)	0.65 ± 0.09
T-score femoral neck (SD)	-2.12 ± 0.69
Patients with vertebral fractures (%)	6.25
Systolic blood pressure (mmHg)	120 ± 15
Diastolic blood pressure (mmHg)	80 ± 10
Glucose (mg/dL)	96 ± 6

Values are mean ± SD.

In accordance with WHO criteria for the diagnosis of osteoporosis[26] evaluated by dual-energy x-ray absorptiometry, all three patients with SH had a normal bone mineral density (BMD) at lumbar spine and were osteopenic at femoral neck (Table IV).

The fifty patients studied were divided into two groups: group with SH (n=3) and group without SH (n=47). Age did not differ between groups. Menopausal age in the group with SH was significantly greater than in the group without SH ($p < 0.05$). The SH group had a greater, but not significantly, body mass index (BMI) than the SH free group. Patients with SH had a significantly greater lumbar spine BMD in comparison to the SH free group ($p < 0.01$). Femoral neck BMDs in the SH group were greater, but not significantly, than in the SH free group.

Table II. The variables in patients with (SH) and without (No-SH) subclinical hypercortisolism (SH); results are expressed as means ± SD.

Variable	No-SH (n = 47)	SH (n = 3)
Age (years)	58.00 ± 4.00	57.00 ± 3.00
BMI (kg/m^2)	26.00 ± 2.00	28.00 ± 2.00
Menopausal Age (years)	47.00 ± 4.00	52.00 ± 1.00*
BMD lumbar spine (g/cm^2)	0.76 ± 0.10	0.92 ± 0.04
T-score BMD lumbar spine (SD)	-2.90 ± 0.95	-0.76 ± 0.15**
BMD femoral neck (g/cm^2)	0.67 ± 0.08	0.70 ± 0.04
T-score BMD femoral neck (SD)	-1.60 ± 0.60	-1.33 ± 0.30
Patients with vertebral fractures (%)	10.6	100**
Systolic blood pressure (mmHg)	125 ± 10	120 ± 10
Diastolic blood pressure (mmHg)	80 ± 10	80 ± 10
Glucose (mg/dL)	98 ± 4	92 ± 6

*$p < 0.05$ versus no SH; **$p < 0.01$ versus no SH.

Table III. Biochemical and imaging data of patients with subclinical hypercortisolism.

Patients	Cortisol level (after dex 0.5 mg every 6 h for 2 days) µg/dl	ACTH levels pmol/L	24-hour urinary free cortisol µg/24h	Imaging findings	Diagnosis
1	2.93	15	99.09	1 cm adrenal mass	Adrenal SH
2	3.81	14	111.24	1.5 cm adrenal mass	Adrenal SH
3	2.57	16.5	119.69	1 cm adrenal mass	Adrenal SH

Serum levels of calcium, phosphorus, parathyroid hormone, 25-hydroxyvitamin D3 and bone turnover markers were not significantly different between the groups.

All three patients with SH presented one or more vertebral fractures at spinal radiography. According to Genant et al classification[25], patient 1 presented one mild vertebral fracture at D11, patients 2 and 3 presented two mild vertebral fractures (D7-D11 and D8-D9 respectively) (Table IV). In the SH free group, the percentage of vertebral fractures was 10.6% (five patients with one vertebral fracture).

Patients with subclinical Cushing, in which an adrenal mass was identified, were included in follow-up. After 6 months, urinary cortisol and adrenal CT images were similar at baseline in these patients.

Discussion

As in patients with Cushing's syndrome[27], in which trabecular bone at lumbar spine is predominantly affected[28], patients with subclinical hypercortisolism (SH) show a higher prevalence of vertebral fractures only partially explained by reduced spinal BMD[29,30]. Recently, Chiodini et al[30] have documented that in patients with SH an increased Spine Deformity Index (SDI) is observed. SDI is a surrogate marker of bone microarchitecture and provides information on bone quality; it is a semiquantitative index that integrates both the number and severity of fractures reflecting the total burden of the spine. SH affects bone quality and increases SDI[31,32].

Our three patients with SH presented one or more vertebral fractures in normal lumbar BMD. Subtle cortisol excess may exert an integrated detrimental effect on trabecular bone quality. The negative influence of SH on connective tissue and muscle strength could also contribute to increased fracture risk[33,34].

The potential danger of subclinical Cushing's syndrome is not a progression to clinically manifest hypercortisolism, which is rarely seen, but rather, the possibility that an excess of cortisol, albeit modest, but maintained chronically, could favour the onset of classical cardiovascular and metabolic complications of Cushing's syndrome. In our patients, none showed higher systolic or diastolic blood pressure or higher glucose.

With regard to involvement of the bone, cortisol exerts direct and indirect effects on bone tissue, promoting resorption at first, and then inhibiting formation as reflected by reduced BGP levels. Direct effects of glucocorticoid excess include inhibited differentiation of pluripotent mesenchymal cells toward the osteoblastic lineage and enhancement of their differentiation into the adipocytic pathway. Cortisol also reduces the apoptosis of osteoclasts and promotes that of osteoblasts and osteocytes[35-40]. Cortisol excess reduces the production of growth factors and bone matrix proteins, and decreases calcium absorption[41]. In our patients with SH, there were no differences in bone formation evaluated with bone alkaline phophatase and in bone resorption evaluated with pyridinium cross links.

Table IV. BMD at lumbar spine and femoral neck and vertebral fractures in patients with subclinical hypercortisolism (SH).

Patients	Age y	Menopausal age y	L-BMD g/cm²	T-score SD	FN-BMD g/cm	T-score 2SD	VF
1	59	52	0.92	-0.70	0.70	-1.20	D11
2	57	51	0.88	-0.90	0.67	-1.66	D7-D11
3	53	50	0.94	-0.60	0.73	-1.13	D8-D9

Table V. Urinary cortisol, cortisone and their ratio in patients with subclinical hypercortisolism (SH) and without subclinical hypercortisolism (no-SH).

	Urinary cortisol (9.2-45.2 µg/24h)	Urinary cortisone (14.5-94.6 µg/24h)	Cortisol/cortisone ratio (0.19-0.76)
SH patients	110.01 ± 10.35*	49.28 ± 33.49	3.01 ± 1.94*
No SH patients	20.15 ± 9.32	40.28 ± 19.85	0.57 ± 0.35

*$p < 0.001$ vs no SH.

The effect of GC excess on bone may be mediated through increased expression of osteoblastic 11β-hydroxysteroid dehydrogenase type1 that converts cortisone into cortisol[42] or the presence of the N363S or Bcl1 polymorphism in the GC receptor that appears to be associated with an increased sensitivity to the same GC[43-44]. In patients with SH, we have documented an increased cortisol/cortisone ratio in urine and thus support a possible role of an altered balance of the enzyme 11β-hydroxysteroid dehydrogenase type 1 and type 2 in the pathogenesis of bone fragility (Table V).

11β-hydroxysteroid dehydrogenase type 2 is found in tissues that express high levels of GC receptors, such as liver and adipose tissue, and acts as an inactivating enzyme by converting cortisol to cortisone. This enzyme was also identified in rat and human osteosarcoma cells, where GC inactivation by this mechanism was demonstrated. By contrast, 11β-hydroxysteroid dehydrogenase type 1 is primarily a GC activator, converting cortisone to cortisol. This enzyme is widely expressed in target tissues of GC action, including bone. The activity of 11β-hydroxysteroid dehydrogenase type 1 and its potential to generate cortisol from cortisone in human osteoblasts are increased by proinflammatory cytokines and by GCs themselves[45].

In our population, the percentage of subclinical hypercortisolism is 6%. It was a cross-sectional evaluation of a referral population. The prevalence of SH may be lower in a community-based population, although, middle-aged or older patients have common disorders, such as obesity, diabetes, hypertension which should be studied by the physician to search for hypercortisolism as a possible cause of these diseases.

Our records show that patients with SH exhibit higher values of BMD: this is not surprising when one considers that female patients with SH have had a more recent menopause; patients with subclinical Cushing also have greater body weight, and higher femoral BMD values were observed in subjects with higher BMI, most likely due to a major mechanical loading.

Conclusions

Our data clearly show that subtle hypercortisolism is associated with a high prevalence of fractures independent of bone mineral density. In a careful radiographic examination of the spine in lateral projection, all patients with subclinical Cushing had at least one mild vertebral fracture in relation to the Genant et al classification, thus demonstrating increased bone fragility that BMD alone cannot express. In conclusion, our work has limitations. Although screening for SH in patients with osteoporosis is desirable, there is still no validated diagnostic test that allows a correct diagnosis; there are also few data on the natural history of SH and, in particular, data on the effect of SH resolution on bone mass and fracture risk are currently unavailable. Physicians should always consider SH among the causes of secondary osteoporosis especially in individuals with diabetes, hypertension and obesity with vertebral fractures, and in the presence of an only slightly reduced BMD. Therefore, careful evaluation of subjects referred to clinics for the prevention of osteoporosis, may reveal cases of SH in patients with osteopenia or BMD in the normal range, with consequent possible savings in health costs through early detection and prevention of complications.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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