PREVALENCE OF LESS SEVERE HYPERCORTISOLISM IN FRACTURED PATIENTS ADMITTED IN AN OUTPATIENT CLINIC FOR METABOLIC BONE DISEASES

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Introduction

Less severe hypercortisolism (LSH, previously named Subclinical Hypercortisolism-SH) is characterized by a discrete cortisol excess in the absence of classical signs and symptoms of Cushing syndrome (i.e. moon facies, striae rubrae, skin atrophy or buffalo hump) [(1)]. The growing attention for this condition and the easier access to diagnostic tools have shown that the LSH prevalence is higher than previously hypothesized and it is currently estimated between 0.2 and 2.0% of the general population [(2)].

The typical complications of overt hypercortisolism, including diabetes, high blood pressure and bone damage may be present also in LSH. Besides, the prevalence of vertebral fractures in patients with LSH is higher than in matched controls (between 46.3 and 82.4%), and the incidence of new asymptomatic vertebral fractures in LSH patients has been reported between 24 and 48%. Of utmost importance, adrenalectomy seems to dramatically reduce the risk of incident fractures in patients with adrenal adenoma [(3)].

Even though bone damage has not been shown in all studies on LSH patients [(4)], cases of LSH, in which the unique complication was the presence of osteoporosis and/or fragility fractures have been reported [(5-6)]. Being LSH an asymptomatic condition, possibly leading to fracture as the only consequence, it is conceivable that, although its diagnosis is often missed because of the lack of the classic signs of cortisol excess, LSH may be more prevalent in patients with bone fragility.

Indeed, several studies [7-11] evaluating the LSH presence in osteoporotic patients ~~and~~ reported variable frequencies, ranging from 0.6 to 17.6%.

Nowadays, we still do not know which is the real prevalence of LSH in patients with fragility fractures, as this information, when available, has been derived from post-hoc analysis. As a consequence, the issue of who has to be screened for LSH among osteoporotic patients is still widely debated. Some authors proposed that LSH in osteoporotic patients should be searched for when: (a) BMD is lower than age- matched controls (Z score value < -2 SD), (b) BMD declines more rapidly than expected, (c) patients fail to respond to appropriate therapy, (d) fragility fractures occur in eugonadal subjects [3, 12-13].

It is worth noting that diagnosing LSH in a patient referred for bone fragility is of utmost importance not only to tailor anti-fracture therapy, but also because LSH has been demonstrated to be associated with increased mortality for infection and cardiovascular events [14-16].

Notwithstanding the importance of the LSH recognition, studies specifically designed to assess the LSH prevalence in patients with fragility fracture are still lacking, but the widespread screening for LSH of all patients with bone fragility would result in a low specificity with a huge number of false positive results. On the other hand, scarce data are available on the possible role of the LSH related comorbidities (i.e. diabetes, hypertension and obesity) in increasing the pre-test probability of having LSH in patients with bone fragility. Additional risk factors allowing to more accurately predict LSH in patients with bone fragility could aid the identification of patients at higher risk of LSH, thus improving the efficacy of LSH screening.

The aim of the current study was to evaluate the prevalence of LSH in fractured patients and to identify possible factors able to increase the probability of diagnosing LSH in patients with bone fragility.

Patients

We evaluated all patients with fragility fracture (clinical and/or morphometric) consecutively admitted to our outpatient center for metabolic bone diseases from July 2015 to October 2018. All patients presenting with causes of secondary osteoporosis apart from diabetes mellitus (i.e. primary hyperparathyroidism and other forms of hypercalcemia, hypophosphoremia, osteomalacia, Paget's disease, chronic liver or renal failure, hyperthyroidism, overt Cushing syndrome, malabsorption, eating disorders, alcoholism, mood disorders, hematologic diseases precocious menopause, overt male hypogonadism) were excluded. Patients assuming drugs known to affect bone metabolism were also excluded.

The flow-chart of the study is illustrated in figure 1.

In all enrolled subjects data regarding gonadal status, history of diabetes, high blood pressure and dyslipidemia, measured height, weight and blood pressure, as well as fasting glycaemia, total cholesterol, triglycerides were collected. In all subjects the 1 mg overnight dexametasone suppression test (DST) was carried out and repeated in those with cortisol level >1.8 μg/dL. In case of confirmed cortisol levels after DST >1.8 μg/dL, a 2day low-dose DST (dexamethasone 0.5 mg every 6 hours for 2 days and determination of serum cortisol after 48 hours from the first dose) was performed and in those with cortisol level > 1.8 μg/dL the LSH diagnosis was established. In these patients adrenocorticotroph hormone (ACTH), late night serum cortisol and urinary free cortisol levels were assessed. In all LSH patients, abdomen computed tomography (CT) or pituitary magnetic resonance imaging (MRI) were performed to identify the site of cortisol hypersecretion on the basis of ACTH levels. In patients with ACTH level between 5 and 15 pg/mL a corticotroph hormone stimulating test was performed in order to determine the origin (i.e adrenal or pituitary) of hypercortisolism.

Methods

In all patients fasting glycaemia, total cholesterol, triglycerides were measured by standard colorimetric techniques. Plasma morning ACTH levels (mean of three determinations at 20-minute intervals) were measured by immunoradiometric assay (Brahms Diagnostica GmbH, Berlin, Germany), serum cortisol and urinary cortisol levels (after dichloromethane extraction) by immunofluorometric assay (TDXFLX, Abbott Diagnostika, Wiesbaden, Germany). The intra and inter-assay coefficients of variation were ≤ 10% for ACTH and cortisol.

In all patients, blood pressure was measured during outpatient visit. Diagnosis of hypertension, dyslipidemia, hyperglycemia and obesity were established according to the current guidelines [18]. In all patents bone mineral density (BMD) was measured by dual-energy x-ray absorptiometry (DXA) (Hologic, Waltham, MA) at the spine (DXA L1-L4, *in vivo* precision 1.0%) and femoral neck (*in vivo* precision 2.3%) and conventional spinal radiographs in lateral (T4–L4) and anteroposterior projection (L1–L4) were obtained with a standardized technique. Vertebral fractures were diagnosed by visual inspection using the semiquantitative method described by Genant et al. [17] History of non-vertebral fractures was obtained from the patient medical records.

All participants gave their witnessed consent and the study was approved by local ethic committee and in accordance with Helsinki Declaration II.

Statistical analysis

Statistical analysis was performed by SPSS version 19 (SPSS, Inc., Chicago, IL, USA). The results are expressed as mean ± SD or median and range, as appropriate. Comparison of continuous variables between the different groups was performed using Student's *t* test or Mann-Whitney U test as appropriate. Categorical variables were compared by χ^2 test or Fisher exact test, as appropriate. Univariate regression analysis was performed to evaluate the association of age,

hypertension, hyperglycemia, dyslipidemia, obesity with LSH. A p value of <0.05 was considered significant.

Results

We enrolled 101 fractured patients (75 women, 26 men, age 65±10.3 years, BMI 27.2±5.9 Kg/m^2). Fifty-five (54.5%) out of 101 were hypertensive, 57 (56.4%) dyslipidemic, 17 (16.8%) hyperglycemic, 28 (27.7%) obese patients. Five (3 females and 2 males) out of 101 (5.0%) fractured patients were diagnosed as LSH (figure 1). Among them, 2 cases were of pituitary and 3 of adrenal origin. All males and 2 out of 3 females LSH patients were hypogonadal. All LSH subjects (5/5) were hypertensive; 4/5 (80%) LSH subjects were dyslipidemic, 1/5 (20%) was hyperglycemic, 2/5 (40%) were obese ones (table 1). LSH was associated to blood hypertension [5/5 LSH vs 50/96 (without LSH (Fisher exact test, p= 0.06) hypertensive patients], although did not reach the statistical significance, but not with dyslipidemia, hyperglycemia, obesity. BMD measured at lumbar spine (LS) and femoral neck (FN), expressed as Z-score (i.e. compared to a population of the same age and gender) was not different between fractured patients with or without LSH (LS -1.5 ± 1.1 vs -1.5 ± 1.3, p= ns; FN -1.2 ± 0.6 vs -0.9 ± 0.9, p= ns). Univariate regression analysis showed the association of LSH with hypertension (Beta 0.209, p=0.036) but not with age, dyslipidemia, obesity and hyperglycemia. The characteristics of all subjects are summarized in table 1 and the ones of LSH subjects in table 2.

Discussion

The reported prevalence of LSH in subjects with apparently primary osteoporosis varies from 0.6 to 3.8% [7-11], being even higher in fractured subjects (1.9 to 17.6%). The reason for such differences is not clear but could at least partially depend on the different sizes and features of the

examined samples, as like as on the different diagnostic criteria utilized to diagnose LSH [3]. This study has been performed on a population of 101 carefully investigated fractured patients admitted to our outpatient center for metabolic bone diseases. In these subjects the main causes of secondary osteoporosis had been previously excluded and they showed no signs of overt hypercortisolism, so that they would have been classified as affected by primary osteoporosis.

In the investigated population, the prevalence of LSH was 5.0%, that is intermediate among those of different studies [7-11]. Considering that a recent study [19] suggests a cut-off of serum cortisol after DST lower than 1.8 mcg/dL for diagnosing hypercortisolism, such a prevalence could be even underestimated. On the other hand, the observed prevalence could be rather high because of the selection bias inevitably associated with the recruitment of patients in a 2nd level center for the study of bone metabolism, which in turn could lead to unintentional inclusion of a higher number of affected subjects in respect to the prevalence in the general population.

As in previous series of subjects with LSH, hypersecretion of cortisol was more commonly of adrenal origin [7, 20]. No difference was observed in the BMD values of subjects with LSH compared to those without LSH, in line with the known propensity of patients with hypercortisolism to have fractures despite a normal value of BMD [21-22].

In our study we evaluated also the prevalence of hyperglycemia, hypertension, dyslipidemia and obesity.

The prevalence of diabetes mellitus (DM) in our fractured patients (16.8%) did not differ from that of Italian people aged over 65 years [23]. Being diabetes associated to an increased fracture risk [24], a higher prevalence would have been expected. Actually, our study protocol, including only history and measurement of fasting blood glucose, could account for the relatively low prevalence of DM (20%) that we found in our LSH patients, as compared to literature data [25].

The prevalence of hypertension among our fractured patients was 54.5%, versus 65-70% of the Italian general population aged ≥65 years [26]. Although the prevalence of hypertension among LSH patients has been reported to be between 56.7 and 72% [20] we found that all our LSH patients were hypertensive. This could be due to the relatively small sample size, but it is also possible that the selection criteria could have been played a role. Indeed, since only fractured patients have been enrolled in the present study, the conceivably longstanding cortisol hypersecretion could have led to the development of both hypertension and bone fragility. In our opinion, thus, a relevant finding of our study is that in patients with bone fragility the presence of LSH was associated with hypertension, as shown also by the univariate analysis. This in turn suggests that LSH should be searched for in fractured and hypertensive patients, independent of age.

From these data, other characteristics of LSH patients do not seem to be useful in individuating fractured patients at higher risk of having LSH. Actually, the prevalence of dyslipidemia among fractured patients (56.4%) was higher than that (~35%) of the Italian general population [27], but it was not independently associated with the LSH prevalence. Similarly, 27.7% of fractured subjects were obese. This percentage is higher than that in the Italian population aged 65-74 years (15.3%) [28], though such a prevalence did not differ from that (40%) of adrenal adenoma with LSH [20]. As for dyslipidemia, obesity does not seem to be a marker for LSH in fractured patients.

Besides, the two men with LSH had mild hypogonadism, whereas two out of three women with LSH were postmenopausal. This result deserves interest, because current guidelines do not suggest to screen postmenopausal osteoporotic women for hypercortisolism.

The main strengths of this study are the careful selection of the subjects, with the exclusion of secondary osteoporosis, the stringent criteria used for the diagnosis of LSH, and the uniform clinical evaluation of the patients along with an accurate control of the biochemical and hormonal

methods, as well as the instrumental procedures. This study has also some limits. Since the study was performed in a single center, this may have caused a selection bias, with inclusion of more affected subjects in respect to their prevalence in the general population. Another possible limit, coming from the “real-world” design of our study, is the relatively low number of LSH fractured patients, and we calculated that at least 4 controls (i.e. patients without LSH) per case (i.e. patients with LSH) are needed if the probability of being affected with hypertension or hyperglycemia among LSH is 1. If this probability among controls is 0.3, we would need to study 5 cases and 20 control patients to reject the null hypothesis that the exposure rates for case and controls are equal, with a power of 0.8 and a type I error 0.01.

In conclusion, this study confirms that a non-negligible percentage of fractured subjects with apparently "primitive" bone damage actually presents an unrecognized hypercortisolism. Accordingly, regardless of age, we suggest to screen for hypercortisolism all patients with established osteoporosis and in particular hypertensive subjects.

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Table 1: clinical characteristics of the enrolled subjects

	All patients (n=101)	LSH patients (n=5)	Non-LSH patients (n=96)	p value
Age	65 ± 10.3	62.6±13.3	65± 10.3	0.60
BMI	27.2 ± 5.9	28.7 ± 5.0	27.1 ± 5.9	0.55
Hypertension	55 (54.5%)	5 (100%)	50 (49.5%)	0.06
Hyperglicemia	17 (16.8%)	1 (20%)	16 (15.8%)	1.00
Dyslipidemia	58 (57.4%)	4 (80%)	54 (53.5%)	0.39
Obesity	27 (26.7%)	2 (40%)	25 (24.8%)	0.61

p values are referred to the comparison between LSH patients and non LSH patients

Data are expressed as M ± SD or number (percentage); LSH: less severe hypercortisolism.

Table 2: characteristics of the fractured subjects with LSH

LSH patients	**Age**	**Sex**	**Hypertension**	**dyslipidemia**	**hyperglicemia**	**Obesity**	**site of cortisol hypersecretion**
1	63	F	Yes	Yes	No	Yes	Adrenal
2	62	M	Yes	No	Yes	No	Pituitary
3	63	M	Yes	Yes	No	No	Adrenal
4	72	F	Yes	Yes	No	Yes	Adrenal
5	41	F	Yes	Yes	No	No	Pituitary

LSH: less severe hypercortisolism; HT: hypertension.