Primary aldosteronism as a cause of secondary osteoporosis

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Abstract

Objective: Patients with primary aldosteronism (PA) have a high prevalence of osteoporosis (OP) and fractures (Fx). We evaluated the presence of PA in patients admitted to our metabolic bone disease outpatient clinic.
Design: Study conducted on an in- and outpatient basis in a referral Italian endocrinology unit.
Methods: A total of 2632 patients were evaluated. 2310 were excluded because they were taking drugs known to affect bone or mineralocorticoids metabolism or were diagnosed to have a secondary cause of osteoporosis. The remaining 322 subjects (304 females, 18 males) took part in the study. Bone mineral density (BMD) and thoracic and lumbar spine vertebral morphometry were performed by dual X-ray absorptiometry. All patients were screened for PA with aldosterone-to-renin ratio. In those who had positive results, confirmatory tests were performed.
Results: Among 322 subjects, 213 were osteoporotics and 109 were not. PA was diagnosed in eleven out of 213 osteoporotic patients (5.2%) and one out of 109 non-osteoporotic subjects (0.9%, $P=0.066$). PA was observed in the 26.1% of patients with the concomitant presence of osteoporosis, hypertension and hypercalciuria. Compared with patients without PA, patients with PA had mean values of urinary calcium excretion, 4.8 ± 2.5 mmol/day vs 7.6 ± 3.2 mmol/day, $P<0.001$ and serum PTH levels, 5.4 pmol/L vs 7.3 pmol/L, $P<0.01$, significantly higher.
Conclusions: PA should be considered among the causes of secondary OP.

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Introduction

Primary aldosteronism (PA) is a disorder of the adrenal gland caused by the increased secretion of aldosterone that is relatively autonomous of normal regulatory mechanism.

Aldosterone excess is associated with hypercalciuria, hypocalcemia and subsequent secondary hyperparathyroidism both in rats and in humans (1, 2). In rats, these alterations of bone metabolism were associated with a decreased bone mass and strength (1) and were rescued by the mineralocorticoid receptor antagonist (MRA) therapy (3).

In humans, there is evidence that PA patients have an increased prevalence of low bone mass and vertebral fractures (VFs) and that treatment with adrenalectomy or MRA therapy improves the clinical picture (4, 5, 6, 7, 8, 9). Moreover, the possible relation between PA and bone was suggested by data coming from genomewide association studies showing an association between indexes of bone strength and some genes involved in aldosterone pathways (10). Finally, the presence of mineralocorticoid receptors had been demonstrated in osteoblast, osteoclast and osteocyte cells (11, 12).

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Recent data showed that a condition of an otherwise asymptomatic cortisol excess (i.e. subclinical hypercortisolism) is more prevalent than expected in patients with osteoporosis (13, 14). Given the negative role of the aldosterone excess on bone tissue, we hypothesized that in patients with osteoporosis there was a high prevalence of PA.

Therefore, the aim of the present study was to evaluate the association between osteoporosis and PA and as secondary end-point, the prevalence of PA in patients with osteoporosis consecutively recruited among the subjects referred to our outpatient clinic for metabolic bone disease.

Subjects and methods

Study design

The study was carried out at the 'IRCCS, Casa Sollievo della Sofferenza', San Giovanni Rotondo, Foggia, Italy, from November 2012 to May 2015. We evaluated 2632 consecutive patients referred to our outpatient clinic for metabolic bone disease.

Exclusion criteria were use of drugs (bisphosphonates, denosumab, teriparatide, raloxifene, bazedoxifene, corticosteroids) or diseases (hypogonadism, hypercortisolism, primary hyperparathyroidism and thyrotoxicosis, bowel diseases, chronic kidney and hepatic disease, alcoholism, eating disorders, rheumatologic, hematological diseases or oncologic diseases) (15), apart from hypercalciuria, known to affect bone or mineralocorticoids metabolism. Moreover, hypertensive patients have been recruited after their antihypertensive therapy had been stopped and/or shifted to not interfering antihypertensive medications according to the Endocrine Society Guidelines (16).

According to these exclusion criteria, 2310 were excluded while 322 subjects were included in the study (Fig. 1). All subjects included in the study underwent to a dual X-ray absorptiometry for measuring bone mineral density (BMD) and a thoracic and lumbar spine radiograph for assessing the presence of asymptomatic VFs. In postmenopausal women and men aged 50 years and older, osteoporosis and established osteoporosis were diagnosed in the presence of T-score <−2.5 and fragility fractures, respectively, according to WHO criteria (17). In premenopausal women or men less than 50 years of age, according to the International Society for Clinical Densitometry recommendations, *Z*-scores were used, with *Z*-scores values of −2.0 or lower considered as low BMD for age. Both patients with densitometric diagnosis of osteoporosis or low BMD for age and patients with VFs regardless of BMD values were included in the osteoporotic group.

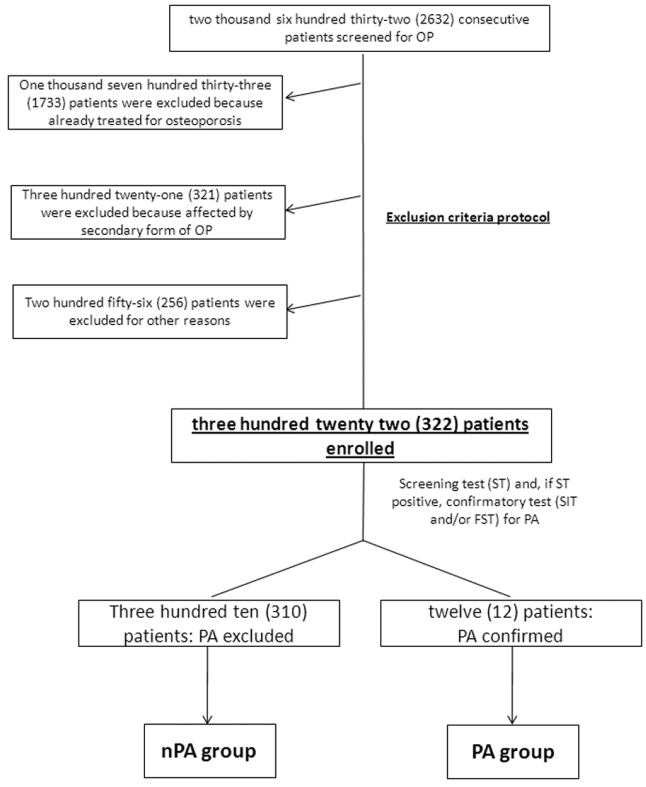

Figure 1

Study design. ST was positive if aldosterone-to-renin ratio >300 and plasmatic aldosterone concentration >416 pmol/L. SIT was considered positive if plasmatic aldosterone concentration >277 pmol/L, negative if plasmatic aldosterone concentration <139 pmol/L, indeterminate if 139 pmol/L > plasmatic aldosterone concentration < 277 pmol/L. If SIT was indeterminate, FST was performed. FST was considered positive if plasmatic aldosterone concentration >166 pmol/L. FST, fludrocortisone suppression test; nPA, not primary aldosteronism; OP, osteoporosis; PA, primary aldosteronism; ST, screening test; SIT, saline infusion test.

All patients were screened for PA with aldosterone-to-renin ratio and in those whose results were positive, confirmatory tests were performed according to Endocrine Society Guidelines. The presence of a possible glucocorticoid remediable aldosteronism (GRA) was ruled out by the search for the chimeric gene with the protocol previously described (18).

In patients with PA, abdomen CT or MRI was asked to be performed in order to identify adrenal lesions, possibly causing PA.

All subjects gave their witnessed informed consent before entering. The study was approved by Ospedale Casa Sollievo della Sofferenza, IRCCS and was carried out in accordance with the Declaration of Helsinki II.

Methods

In all patients, serum calcium, albumin, phosphorous, creatinine, 24-h urinary calcium and creatinine excretion were measured by standard colorimetric techniques. Total calcium was corrected for serum albumin (Ca alb-adj) according to the formula: Ca alb-adj (mg/dL) = total calcium + ((4.0 − albumin expressed as g/dL) × 0.8) (19). Serum intact parathyroid hormone (PTH) levels were measured by an immunochemiluminometric assay (ICMA) (LIAISON N-TACT PTH 2 Assay; Diasorin, Stillwater, MN, USA), with intra- and interassay coefficients of variation (CVs) of 5.1% and 8.2%, respectively. Serum 25-hydroxyvitamin D (25OHD) concentration was measured by radioimmunoassay (RIA) (Diasorin), whose intra and interassay CVs were 7.2% and <12%, respectively. In all patients, plasma aldosterone concentration (PAC) was measured by RIA (Diasorin), whose intra- and interassay CVs were 3.8% and 7%, respectively. Plasma renin activity (PRA) levels were measured by RIA (Diasorin), whose intra- and interassay CVs were 7.5% and 8.1%, respectively. Blood samples for the screening test, aldosterone-to-renin ratio (ARR), were collected at mid-morning, after the patient had been up (sitting, standing or walking) for at least two hours and seated for five to 15 min (16). The saline infusion test (confirmatory test in patients screened positive) was performed while the patients were in the recumbent position for at least one hour before and during the infusion of two L of 0.9% saline i.v. over four hours, starting between 08:00 h and 09:30 h. Blood samples for PRA, aldosterone, cortisol and plasma potassium were drawn at time zero and after four hours. If the saline infusion test did not exclude or confirm the diagnosis of PA, the fludrocortisone suppression test was performed. For the fludrocortisone suppression test, patients received 0.1 mg oral fludrocortisone every six hours for four days, together with slow-release KCl supplements and slow-release NaCl supplements plus sufficient dietary salt to maintain a urinary sodium excretion rate of at least 3 mmol/kg of body weight. On day five, plasma aldosterone and PRA were measured at 10:00 h with the patient in the seated posture, and plasma cortisol was measured at 07:00 h and 10:00 h (16).

BMD was measured by dual-energy X-ray absorptiometry (GE Lunar Prodigy, GE Healthcare Lunar) at lumbar spine (LS; *in vivo* precision 1.0%), total hip and femoral neck (TN and FN; *in vivo* precision 2.3% and 1.8%, respectively). Individual BMD values are expressed as s.d. units (*Z*-values) in relation to our reference age- and gender-matched population (20). Fractured vertebrae were excluded from BMD measurement. Conventional spinal radiographs in lateral (T4–L4) and anteroposterior projection were obtained in all subjects. Two trained radiologists who were blinded to BMD and hormonal data, independently reviewed the radiographs and evaluated the presence of fractures according to the semi-quantitative Genant method (21); the interobserver reliability between the two radiologists was good ($k = 0.82$).

There were no patients that received MRA when they were doing absorptiometry and urine electrolyte measurement.

Statistical analysis

Data are expressed as mean ± s.d. if not differently specified. Normality of distribution was evaluated by Kolmogorov–Smirnov test. Comparisons between continuous or categorical variables were performed by unpaired *t* test or Mann–Whitney *U* test and by chi-square or Fisher exact test respectively, as appropriate. Bivariate correlations between continuous variables were performed by Pearson correlation or Spearman's rho correlation, as appropriate. In all patients, logistic regression analysis was used to assess the association between the presence of osteoporosis or fractures as dependent variables and age, body mass index (BMI), presence of PA, essential hypertension (EH) and spinal BMD (only for fractured patients, expressed as *Z* score) as independent variables; we chose such variables because they affect bone mass and/or contribute to the development of VFs, apart from PA and EH.

The analyses were performed with SPSS 19. A *P* value <0.05 was considered significant.

Results

The clinical characteristics of the whole population included are summarized in Table 1.

Of the 322 subjects who took part in the study, 115 were hypertensive patients and 207 were not,

Table 1 Clinical characteristics of all subjects ($n=322$) enrolled in the study. Data are expressed as mean ± S.D. or median (range), as appropriate. The prevalence and percentage of hypertension, hypercalciuria, vertebral fractures and osteoporosis are reported.

Characteristics	Values
Age (years)	61.1 ± 9.5
BMI (kg/m^2)	25.5 (30.6)
PAC (pmol/L)	128 (559)
SBP (mmHg)	120 (100)
DBP (mmHg)	76 (55)
K^+ (mEq/L)	4.31 ± 0.38
Serum calcium albumin adjusted (mmol/L)	2.24 ± 1.01
Phosphorous (mmol/L)	1.10 ± 0.15
Creatinine (μmol/L)	62.9 ± 12.4
PTH (pmol/L)	5.4 (15.2)
25OH_VitD (nmol/L)	44.9 (187.5)
Urinary calcium (mmol/day)	4.9 ± 2.6
uCa/kg (μmol 24 h/kg)	78 ± 40
BMD_LS (*Z* score)	−0.94 ± 1.25
BMD_FN (*Z* score)	−0.61 ± 0.89
BMD_TN (*Z* score)	−0.50 ± 1.00
Hypertension, *n* (%)	115 (35.7)
Osteoporosis, *n* (%)	213 (66.1)
Vertebral fractures, *n* (%)	72 (22.4)
Hypercalciuria, *n* (%)	77 (23.9)
Primary aldosteronism, *n* (%)	12 (3.7)

DBP, diastolic blood pressure; PAC, plasma aldosterone concentration; SBP, systolic blood pressure; uCa/kg, urinary calcium in a day/kg (weight).

according to the guidelines for the management of arterial hypertension of the European Society of Cardiology (22).

PA was diagnosed in 11 out of 213 osteoporotic patients (5.2%) and 1 out of 109 non osteoporotic subjects (0.9%, $P=0.066$). The overall prevalence of PA was 3.7% (12/322 subjects), but it increases to 5.2, 6.9, 9.1 and 9.6%, when considering only osteoporotic patients (eleven to 213), fractured patients (five to 72), hypercalciuric patients (seven to 77) and hypertensive patients (eleven to 115) respectively (Fig. 2).

The prevalence of PA among patients that were both: osteoporotics and hypertensive was 13.9% (ten to 72); fractured and hypertensive was 14.8% (four to 27); osteoporotics and hypercalciuric was 9.5% (six to 63); fractured and hypercalciuric was 11.1% (two to 18); osteoporotics, hypertensive and hypercalciuric was 26.1% (six to 23); fractured, hypertensive and hypercalciuric was 33.3% (two to six) (Fig. 2).

The clinical features of patients with PA and without PA (nPA group) are reported in Table 2. Age and BMI were comparable between the two groups. There was one premenopausal woman in the PA group and twenty-one women in the nPA group; all men were eugonadal.

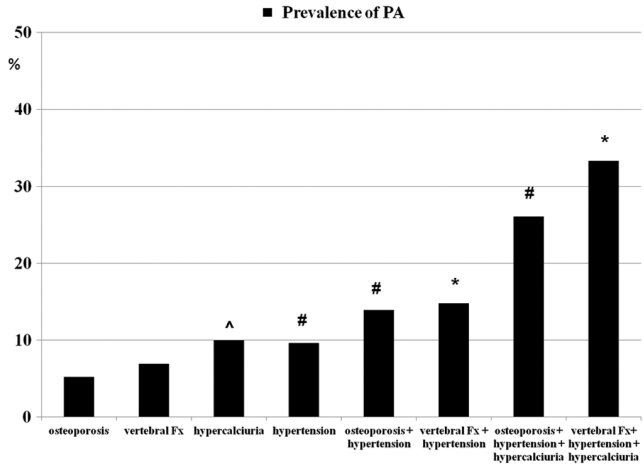

Figure 2
Prevalence of primary aldosteronism. OP, osteoporosis; Fx, vertebral fracture. *$P<0.05$; ^$P<0.01$; #$P<0.001$ in respect to the subjects without features reported in the column.

In PA subjects, as expected, PAC was significantly higher, while potassium levels were significantly lower than that in the other subjects. In the PA group, there was a significant higher systolic blood pressure (SBP) and

Table 2 Clinical characteristics of subjects with and without PA enrolled in the study. Data are expressed as mean ± S.D. or median (range), as appropriate. The prevalence and percentage of hypertension, hypercalciuria, vertebral fractures and osteoporosis are reported.

	nPA ($n=310$)	PA ($n=12$)
Age (years)	61.1 ± 9.3	60.4 ± 13.5
BMI (kg/m^2)	25.6 (30.6)	23.9 (21)
PAC (pmol/L)	126 (539)	243 (420)[a]
SBP (mmHg)	120 (95)	140 (90)[a]
DBP (mmHg)	75 (55)	83 (35)[b]
K^+ (mEq/L)	4.33 ± 0.38	3.97 ± 0.34[a]
Serum calcium albumin adjusted (mmol/L)	2.24 ± 0.10	2.23 ± 0.13
Phosphorous (mmol/L)	1.11 ± 0.15	1.01 ± 0.10[c]
Creatinine (μmol/L)	62.8 ± 12.4	61.9 ± 11.5
PTH (pmol/L)	5.4 (14.2)	7.3 (14.7)[b]
25OH_VitD (nmol/L)	45 (188)	29 (123)
Urinary calcium (mmol/day)	4.8 ± 2.5	7.6 ± 3.2[a]
uCa/kg (μmol 24 h/kg)	75 ± 40	113 ± 35[a]
BMD_LS (*Z* score)	−0.95 ± 1.25	−0.70 ± 1.25
BMD_FN (*Z* score)	−0.61 ± 0.89	−0.68 ± 0.78
BMD_TN (*Z* score)	−0.51 ± 1.00	−0.26 ± 1.03
Hypertension, *n* (%)	104 (33.5)	11 (91.7)[a]
Osteoporosis, *n* (%)	202 (65.2)	11 (91.7)
Vertebral fractures, *n* (%)	67 (21.6)	5 (41.7)
Hypercalciuria, *n* (%)	70 (22.6)	7 (58.3)[b]

[a]$P<0.001$ vs nPA; [b]$P<0.01$ vs nPA; [c]$P<0.05$ vs nPA.
DBP, diastolic blood pressure; nPA, not primary aldosteronism; PA, primary aldosteronism; PAC, plasma aldosterone concentration; SBP, systolic blood pressure; uCa/kg, urinary calcium in a day/kg (body weight).

diastolic blood pressure (DBP) as well as there was a higher prevalence of hypertensive patients (Table 2).

In the twelve patients, with PA there was a significantly higher urinary calcium excretion as well as a higher prevalence of hypercalciuric patients (i.e. uCa >100 µmol/kg body weight/day) (23) than in the other subjects. Moreover, serum PTH levels were significantly higher, while serum phosphorous levels were lower in PA than those in nPA group (Table 2).

In the whole sample, there was a weak but significant direct association between serum aldosterone and daily urinary calcium excretion (r=0.123, P=0.028) or urinary calcium/weight ratio (µmol Ca/kg body weight) (r=0.124, P=0.027). Furthermore, there was a significant direct association between serum aldosterone and PTH (r=0.160, P=0.005).

Logistic regression analysis showed that (i) osteoporosis was significantly associated with age (OR 1.06, CI 1.03–1.09, P=0.001), BMI (OR 1.11, CI 1.05–1.17, P=0.001) and PA (OR 10.42, CI 1.21–90.91, P=0.033), but not with presence of EH (OR 1.23, CI 0.72–2.10, P=0.46); (ii) fractures were significantly associated with age (OR 1.06, CI 1.03–1.10, P=0.001) and spinal BMD (OR 1.71, CI 1.30–2.25, P=0.001), but not with the presence of PA (OR 3.22, CI 0.90–12.20, P=0.071) and of EH (OR 1.02, CI 0.55–1.89, P=0.95).

In ten out of the 12 PA patients, abdomen CT or MRI was performed, while two patients refused to perform imaging studies. In three patients, a unilateral nodular adrenal lesion was detected while in five patients bilateral or monolateral adrenal hypertrophy was found. Imaging studies were negative in the remaining two patients. None of the patients agreed to undergo surgery; therefore, adrenal veins catheterization was not performed (16).

Discussion

The present study suggests the role of aldosteronism as a secondary cause of osteoporosis. Indeed, we found a 5.2% prevalence of aldosteronism among patients with osteoporosis and 6.9% in those with fractures, after exclusion of secondary causes of osteoporosis (24) apart from hypercalciuria, according to our protocol (15). Interestingly, our data showed that about a quarter of patients with osteoporosis, hypertension and hypercalciuria were affected with PA, thus providing for the first time the clinical features of a typical patient with primary hyperaldosteronism and coexistent osteoporosis.

Aldosteronism has been associated with cardiovascular and renal injury (inflammation, remodeling and fibrosis) (25) and more recently with bone damage (4, 5, 6, 7, 8, 9). In an animal model, as well as in humans, aldosterone excess was associated with an increased urinary and fecal loss of Ca++ and Mg++, in turn inducing hypocalcemia, hypomagnesemia and secondary hyperparathyroidism (1, 2, 3, 4, 5, 6), that was rescued by adrenalectomy or treatment with MRA (4, 5). These alterations seem to lead to low bone mass and fragility fractures, whereas surgery or MRA improved bone mass (4, 5). Moreover, spironolactone reduced fracture risk in men with congestive heart failure (26). For these reasons, we decided to search for an excess of mineralocorticoids in consecutively recruited patients with osteoporosis.

The direct associations we found between serum aldosterone and urinary calcium excretion and between serum aldosterone and PTH confirm the effect of aldosteronism on renal calcium handling, resulting in hypercalciuria and consequent hyperparathyroidism. Although it is well known that hypercalciuria and secondary hyperparathyroidism can cause osteoporosis (27), we believe that they do not fully explain the high prevalence of PA patients we observed among osteoporotic or fractured subjects (5.2% and 6.9% respectively). It is interesting to note that the recent genome wide association study, aimed at identifying new candidate genes for bone strength, showed a strong association between phenotypes of bone strength and genes belonging to the mineralocorticoid pathway (10). In this regard, it should be mentioned that the expression of mineralocorticoid receptors on bone cells (11, 12) could actually suggest a still unknown direct effect of mineralocorticoids on the skeletal tissue. Indeed, in an animal model, Fumoto *et al*. showed that pharmacological inhibition of mineralocorticoid function with eplerenone resulted in increased bone mass, with stimulation of bone formation and suppression of resorption (12). The treatment with eplerenone as well as the specific deletion of mineralocorticoid receptor in osteocytes improved the cortical bone thinning in the prednisolone-treated mouse (12).

The presence of PA in patients with osteoporosis and/or fragility fracture, in the absence of a picture of severe hypertension as commonly believed in PA patients, is not completely unexpected, since a high prevalence of aldosteronism among normotensive patients has been reported (28, 29).

In our report, we excluded hypertensive patients whether we could not change antihypertensive therapy with verapamil and doxazosin; thus, we could have not identified some hypertensive patients with PA. Therefore,

in these patients, we suspect that the prevalence of aldosteronism could be even higher. Consequently, the prevalence of PA patients in osteoporosis should be higher than we actually observed.

Our study has several limitations. Firstly, its cross-sectional design shows association and not causality. Intervention studies should be planned to evaluate the effect of surgical or pharmacological treatment on bone mass and incidence of fractures in patients with PA. This study due to the assumption of antihypertensive drugs and/or drugs for the treatment of osteoporosis, can result in a selection bias since more than two thousand subjects were excluded from the analysis and this could have affected the true prevalence of PA among patients with osteoporosis. The studied sample size is relatively small due to exclusion criteria above described. Nevertheless, our findings are fairly coherent, and in keeping with data from animal models and patients treated with spironolactone.

Notwithstanding these limitations, the study has an important clinical implication. Indeed, it suggests PA is a cause of osteoporosis and should be searched for in patients with an unexplained form of osteoporosis, particularly in hypertensive subjects with hypercalciuria.

Declaration of interest

All 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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