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THE OCCURRENCE OF SUBCLINICAL HYPERCORTISOLISM AND OSTEOPOROSIS IN PATIENTS WITH INCIDENTALLY DISCOVERED UNILATERAL AND BILATERAL ADRENAL TUMORS

PREVALENCA SUPKLINIČKOG HIPERKORTICIZMA I OSTEOPOROZE KOD PACIJENATA SA SLUČAJNO OTKRIVENIM UNILATERALNIM I BILATERALNIM ADRENALNIM TUMORIMA

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Summary

Background: Adrenal incidentalomas (AI) are clinically silent adrenal masses that are detected incidentally during imaging procedures performed for unrelated diseases. The aim of this study was to investigate the prevalence of subclinical hypercortisolism (SH) and associated co-morbidities in patients with unilateral AI (UAI) and bilateral AI (BAI).
Methods: We evaluated 152 patients, 105 (69.1%) with UAI and 47 (30.9%) with BAI. SH was diagnosed in the presence of serum cortisol levels after 1 mg dexamethasone suppression test (DST) or after 2-day low-dose DST (LDDST) > 50 nmol/L with at least one of the following parameters: midnight serum cortisol > 208 nmol/L, 24-h urinary free cortisol > 245 nmol/24 h, or ACTH < 10 ng/L. Bone mineral density (BMD) was measured at lumbar spine (LS) and femoral neck (FN).
Results: Age, BMI, and waist circumference were comparable, and diabetes, hypertension and dyslipidemia occurred

Kratak sadržaj

Uvod: Adrenalni incidentalomi (AI) jesu tumori otkriveni različitim vizualizacionim metodama učinjenim bez sumnje na adrenalnu patologiju. Cilj studije je bio da se ispita prevalenca supkliničkog hiperkorticizma (SH) i pridruženih bolesti kod pacijenata sa unilateralnim (UAI) i bilateralnim AI (BAI).
Metode: Ispitivanje je obuhvatilo 152 pacijenta, 105 (69,1%) sa UAI i 47 (30,9%) sa BAI. SH je definisan na osnovu serumskog kortizola >50 nmol/L nakon prekonoćnog deksametazon (1 mg) supresivnog testa (DST) ili nakon dvodnevnog niskodoznog DST (LDDST) sa 2 mg deksametazona, uz još jedan navedeni parametar (ponoćni serumski kortizol > 208 nmol/L, 24-h urinarni slobodni kortizol > 245 nmol/L ili ACTH < 10 ng/L). Kostna gustina je merena na nivou lumbalnog dela kičme i vrata femura.
Rezultati: Pacijenti sa BAI se nisu razlikovali u odnosu na pacijente sa UAI po starosti, BMI, obimu struka, učestalosti

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List of abbreviations: AI, adrenal incidentalomas; BMD, bone mineral density; BMI, body mass index; CT, computerized tomography; DHEAS, dehydroepiandrosterone-sulfate; DST, dexamethasone suppression test; DXA, dual-energy X-ray absorptiometry; FN, femoral neck; GR, glucocorticoid receptor; HOMA-IR, homeostasis model assessment – insulin resistance index; LDDST, low-dose dexamethasone suppression test; LS, lumbar spine; MRI, magnetic resonance imaging; OGTT, oral glucose tolerance test; PAC, plasma aldosterone concentration; PRA, plasma renin activity; SH, subclinical hypercortisolism; T2DM, type 2 diabetes mellitus; UAI, unilateral adrenal incidentalomas; BAI, bilateral adrenal incidentalomas; UFC, urinary free cortisol; WHR, waist-to-hip ratio.

with similar frequency in both groups. The overall prevalence of SH was 20.5% based on post-1 mg DST, and 20.0% based on post-LDDST cortisol levels, and it was more prevalent in BAI than UAI patients (31.1% *vs* 15.2%, respectively, p=0.026). LS BMD was lower in BAI than in UAI patients (0.96±0.14 *vs* 0.87±0.15, p=0.002). There were no differences in FN BMD. The prevalence of osteoporosis was higher in BAI compared to UAI patients (37.1% *vs* 15.9%, respectively, p=0.011).

Conclusions: Patients with BAI had higher prevalence of SH and osteoporosis than those with UAI. Frequency of other co-morbidities was similar. This may be due to the higher degree of autonomous cortisol secretion or different tissue-specific sensitivity to glucocorticoids.

Keywords: adrenal incidentaloma, osteoporosis, subclinical hypercortisolism

dijabetesa, hipertenzije i dislipidemije. Prevalenca SH u celoj grupi iznosila je 20,5% (prekonoćni DST), odnosno 20,0% (LDDST) i bila je značajno veća kod pacijenata sa BAI u odnosu na pacijente sa UAI (31,1% *vs* 15,2%, p=0,026). Kostna gustina na nivou kičme je bila značajno niža kod pacijenata sa BAI u odnosu na UAI (0,96±0,14 *vs* 0,87±0,15, p=0,002). Nije nađena razlika u kostnoj gustini na nivou vrata femura. Prevalenca osteoporoze je bila značajno veća kod pacijenata sa BAI u odnosu na UAI (37,1% *vs* 15,9%, p=0,011).

Zaključak: Pacijenti sa BAI imaju značajno veću prevalencu SH i osteoporoze, ali ne i bolesti pridruženih u metaboličkom sindromu, u odnosu na pacijente sa UAI. To može biti posledica veće autonomne sekrecije kortizola ili različite tkivno-specifične osetljivosti na kortikosteroide.

Ključne reči: adrenalni incidentalomi, osteoporoza, supklinički hiperkorticizam

Introduction

Adrenal incidentalomas (AI) are clinically silent adrenal masses detected incidentally during imaging procedures performed for other reasons than adrenal diseases (1). It has been shown that the mean prevalence of AI is 2.3%. The prevalence increases with age, ranging from 0.2% in young subjects to 6.9% in patients more than 70 years old (2). These tumors are mostly unilateral, but in about 15% of cases they are bilateral (3). Most of these tumors are nonfunctional, although some of those demonstrate an autonomous subtle cortisol hypersecretion in the absence of signs of overt hypercorticism. This condition is defined as subclinical hypercortisolism (SH) (4), and it occurs in 5–30% of patients with AI, depending on the applied diagnostic criteria (5, 6). SH has been associated with obesity, arterial hypertension, dyslipidemia, diabetes mellitus, and with an increased risk of osteoporosis (7–10). These disorders may also be part of the metabolic syndrome, being highly prevalent in the general population. Since 2011, six studies have investigated the prevalence of SH and related comorbidities among patients with unilateral (UAI) and bilateral (BAI) adrenal incidentalomas with controversial results, probably due to the lack of consensus on diagnostic criteria for SH (11). The pathogenesis of the adrenal tumors is unknown. Bilateral formation of nodules indicates that the pathogenesis of BAI may differ from that of UAI. Multiple molecular mechanisms may be involved in the pathogenesis of bilateral adrenal tumors with subclinical or overt Cushing's syndrome, suggesting that it may be a heterogeneous group of diseases with a common presentation (12).

The primary objective of the current study was to analyze the characteristics of patients with UAI and BAI, and the prevalence of SH based on the different cut-off values of serum cortisol levels after dexamethasone suppression tests (DST). The secondary objectives were to evaluate the prevalence of arterial hypertension, obesity, type 2 diabetes mellitus, dyslipidemia, and osteoporosis.

Materials and Methods

Subjects

In this cross-sectional study, we evaluated 152 patients, average age 58.3 years (range, 25–84 years) of whom 105 (69.1%) with UAI and 47 (30.9%) with BAI. All adrenal masses were diagnosed by ultrasonography, computerized tomography (CT) or magnetic resonance imaging (MRI). Ultrasound findings were confirmed with CT or MRI. Inclusion criteria were: 1) discovery of an adrenal mass by radiological investigation performed for unrelated reasons; and 2) absence of symptoms and signs of overt Cushing's syndrome, while the exclusion criteria were: 1) presence of congenital adrenal hyperplasia, pheochromocytoma, primary hyperaldosterinism, adrenal carcinoma, metastasis of extraadrenal tumors, and 2) presence of other diseases affecting bone metabolism.

Study protocol

The study was approved by an institutional ethics committee. All subjects included in the study gave informed written consent prior to its beginning. Anthropometric characteristics included body weight, height, waist and hip circumference. Body mass index (BMI) was calculated as [body weight (kg)/height^2 (m^2)]. Patients with a BMI > 25 kg/m^2 were defined as overweight and those with BMI ≥ 30 kg/m^2 were designated as obese (13). Subjects were defined as hypertensive if they had a systolic blood pressure of 130 mmHg or greater and/or diastolic blood pressure of 85 mmHg or more and/or had been treated with antihypertensive drugs (14).

Type 2 diabetes mellitus (T2DM) was diagnosed if the subject had a prior diagnosis or had been treated with anti-diabetic therapy. In non-diabetic patients, oral glucose tolerance test (OGTT) with 75 g glucose was used with determination of plasma glucose and insulin levels at baseline and after 30, 60, 90 and 120 min. The criteria used for defining T2DM were in

accord with the American Diabetes Association recommendations (15). Dyslipidemia was defined as serum triglyceride (TG) levels of ≥ 1.7 mmol/L, or high-density lipoprotein cholesterol (HDL-C) levels < 1.0 mmol/L in men and < 1.3 mmol/L in women (16). Insulin resistance was assessed by homeostasis model assessment (HOMA) index [(fasting glucose (mmol/L × fasting insulin (mIU/L)/22.5] (17).

Hormonal evaluation included: morning plasma cortisol at 08.00 h and midnight cortisol at 24.00 h, 24-h urinary free cortisol (UFC), and morning plasma concentration of adrenocorticotropin hormone (ACTH), plasma 17-OH-progesterone, total testosterone, androstenedione and dehydroepiandrosterone-sulfate (DHEAS). We performed an overnight dexamethasone suppression test (DST: 1 mg orally at 23.00 h and blood samples for serum cortisol were collected the following morning at 08.00 h). We also performed a 2-day low-dose dexamethasone suppression test (LDDST: 0.5 mg orally, every 6 h for 2 days with determination of serum cortisol 8 h after the last dose of dexamethasone). To exclude tumor hormonal functionality other than cortisol production, we collected 24-hour urine samples for measurement of catecholamine concentrations, assessed plasma renin activity (PRA) and plasma aldosterone concentration (PAC), and calculated the PAC to PRA ratio.

The diagnosis of SH was made on the basis of post 1 mg-DST cortisol levels > 50 nmol/L or post-LDDST cortisol levels > 50 nmol/L and at least one of the following criteria: 1) ACTH level < 10 ng/L; 2) midnight serum cortisol > 208 nmol/L; and 3) UFC > 245 nmol/24 h, corresponding to the upper normal limit of the reference range of our method (5). According to the suggestions given by ESE and ENSAT guidelines on adrenal incidentaloma, we used post-DST serum cortisol level ≤ 50 nmol/L as a diagnostic criterion for the exclusion of autonomous cortisol secretion; post-DST cortisol between 50.1 and 137.9 nmol/L for 'possible autonomous cortisol secretion'; and post-DST cortisol levels ≥ 138 nmol/L as evidence of 'autonomous cortisol secretion' (18).

Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DXA) (Hologic Discovery W, MA, USA) at lumbal spine and femoral neck. Z - and T - scores were calculated according to the manufacturer's reference curves. Normal BMD was defined as a T score more than -1 in post-menopausal women and patients over 50 years of age, and as a Z score above -2 in non-menopausal women or patients under age 50. Osteopenia was defined as a T score less than -1 in post-menopausal patients or those over age 50, and as a Z score less than -2 in non-menopausal women or patients under age 50. In patients over age 50, a T score less or equal to -2.5 was considered to indicate osteoporosis (19).

Assays

Serum total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C) and TG concentrations were measured by using commercial enzymatic methods. Plasma glucose was determined by the glucose oxidase method (Beckman Glucose Auto-Analyzer, Fullerton, CA, USA). Insulin concentration was tested by radioimmunoassay (RIA) (Institute for the Application of Nuclear Energy [INEP], Belgrade, Serbia); lower limit of sensitivity was 3.0 mU/L, whereas intra and inter-assay coefficients of variations were < 10.0%. Serum ACTH was determined by immunoradiometric assay (CIS Bio International). The reported sensitivity of the assay was 5 pg/mL, with intra- and inter-assay coefficients of variation ranging from 3.1% to 8.9%. Serum cortisol was measured by RIA (CORT-CT2; CIS Bio International, Gif-Sur-Yvette Cedex, France). The lower detection limit was 4.6 nmol/L; intra- and inter-assay coefficients of variation were 5.4% and 7.3%, respectively. UFC levels were measured by high-performance liquid chromatography (HPLC) after solid phase extraction (Waters, HLB 30 mg, Milford, MA, USA; reference range 40–245 nmol/24 h).

Statistical analysis

Results for continuous variables are expressed as mean ± SD, and for binary variables as proportions (percentages). Normality of the distribution was tested by Kolmogorov-Smirnov test. Differences in investigated continuous variables (normally distributed) were assessed by using ANOVA. Differences in categorical variables were assessed by using Chi-square test, while for continuous variables, in case the distribution was not normal, Mann-Whitney U test for two independent samples was applied. $P<0.05$ is considered statistically significant. The SPSS 17.0 statistical software package (SPSS Inc, Chicago, IL, U.S.A.) was used to perform the statistical analysis.

Results

Characteristics of patients

Demographic, clinical and metabolic data of patients with UAI and BAI are shown in *Table I*. The mean age, BMI, waist circumference and waist-to-hip ratio (WHR) were similar in both groups. Most patients had central obesity (waist circumference ≥ 94 cm for male, and ≥ 80 cm for female). Women were more frequent in the group with BAI compared to group with UAI (80.9% *vs* 64.8%, respectively, $p=0.046$). Patients with BAI experienced larger adrenal tumors than patients with UAI, but there was no significant difference between the longest diameter of the largest mass between two groups ($p=0.254$). The prevalence of T2DM and hypertension were of similar frequency in both groups. There was no significant

Table I Demographic, clinical and metabolic characteristics of 152 patients with UAI and BAI.

Characteristics	Unilateral	Bilateral	p value
Patients, n (%)	105 (69.1)	47 (30.9)	
Age, years	58.0±11.1	59.2±10.2	N.S.
Gender, n (%) Male Female	 37 (35.2) 68 (64.8)	 9 (19.1) 38 (80.9)	0.040
Tumor size, mm (range)	30±12 (10–65)	32±10 (14–58)	N.S
BMI, kg/m^2 BMI category, % 18.5–25.0 25.1–29.9 ≥ 30.0	28.3±5.5 26.7 40.0 33.3	28.2±5.1 23.4 48.9 27.6	N.S N.S
WC, cm	96.2±12.8	96.4±15.2	N.S
WC ≥ 94 cm (m), ≥ 80 cm (f), %	81.1	81.8	N.S.
WHR	0.91±0.9	0.90±0.9	N.S.
Hypertension, %	77.1	66	N.S.
Duration of hypertension, years	6.8±8.0	6.9±8.8	N.S.
SBP (mmHg)	141±24	141±22	N.S.
DBP (mmHg)	86±13	87±12	N.S.
Diabetes mellitus, %	24.0	19.1	N.S.
TC, mmol/L	5.8±1.1	6.0±1.0	N.S.
HDL-C, mmol/L HDL < 1.03 mmol/L (male), % HDL < 1.29 mmol/L (female), %	1.3±0.3 45.2	1.4±0.4 39.1	N.S. N.S.
LDL-C, mmol/L	3.7±1.0	3.8±0.8	N.S.
TG, mmol/L	1.7±0.7	1.8±0.9	N.S.
TG >1.7 mmol/L, %	42.3	46.8	N.S.
HOMA IR	3.4±2.1	3.3±1.6	N.S.

Data are expressed as mean±standard deviation ($\bar{x}$±SD), or percentages.
BMI, Body Mass Index; WC, Waist Circumference; WHR, Waist to Hip Ratio; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; TC, Total Cholesterol; HDL, High-Density Lipoprotein cholesterol; LDL-C, Low-Density Lipoprotein cholesterol; TG, triglycerides; HOMA-IR, Homeostasis Model Assessment-Insulin Resistance index; N.S. – not significant.

difference in duration of hypertension, mean values of systolic and diastolic blood pressure. The prevalence of reduced HDL-C levels, and elevated TG levels had similar frequency in both groups. There was no difference in HOMA-IR index between groups.

Hormonal evaluation

Hormonal evaluation is shown in *Table II*. No difference was noted in morning and midnight serum cortisol concentrations between patients with UAI and those with BAI. Although patients with BAI had higher levels of UFC, nevertheless there was no statistically significant difference (p=0.450). Patients with BAI had significantly lower ACTH values (17.07±10.87 *vs* 23.95±9.0, p=0.023), and significantly higher post 1 mg-DST (91.94±82.54 *vs* 59.23±55.00, p=0.005) and post-LDDST cortisol values (87.8± 11.92 *vs* 54.51±5.33, p=0.04) in comparison to UAI. The post-1 mg-DST diagnostic criterion for exclusion of autonomous cortisol secretion was met in 66 out of 105 patients with UAI (63.5%), and 18 out of 47 patients with BAI (38.3%) (p=0.014). 'Possible autonomous cortisol secretion' was found in 30 (28.8%) patients with UAI, and 24 (51.1%) patients with BAI, and evidence of 'autonomous cortisol secretion' was found in 8 (7.7%) with UAI and 5 (10.6%) with BAI. Inadequate cortisol suppression after LDDST was found in 27 (60%) patients with BAI and 36 (34.3%) with UAI (p=0.013). Both tests were carried out in all patients with the exception of 1 mg-DST in a single patient with unilateral incidentaloma, and LDDST in two patients with bilateral incidentaloma. Results of serum cortisol suppression after 1 mg-DST and LDDST are shown in *Table III*.

Table II Comparison of hormonal parameters in patients with UAI and BAI.

Characteristics	Unilateral	Bilateral	p value
ACTH, ng/L	23.9±19.0	17.1±10.9	0.023
Cortisol 08 h, nmol/L	474.8±326.2	486.9±133.2	N.S.
Cortisol 24 h, nmol/L	123.2±81.2	127.6±74.0	N.S.
UFC, nmol/24 h	120.3±20.6	145.4±26.4	N.S.
Cortisol post-DST 1 mg, nmol/L	59.2±55.0	91.9±82.5	0.005
Cortisol post-LDDST, nmol/L	54.5±5.3	87.8±11.9	0.040
Patients with SH, n (%) (based on post-DST 1 mg cortisol)	17/104 (16.3)	14/47 (29.8)	N.S.
Patients with SH, n (%) (based on post-LDDST cortisol)	16/105 (15.2)	14/45 (31.1)	0.026
ACTH < 10 ng/L, n (%)	19/104 (18.3)	8/46 (17.4)	N.S.
Cortisol 24 h > 208 nmol/L, n (%)	10/102 (9.8)	7/43 (16.3)	N.S.
UFC > 245 nmol/24 h, n (%)	2/35 (5.7)	4/20 (16.7)	N.S.

Data are expressed as mean±standard deviation (X±SD), or percentage.

ACTH, Adrenocorticotropin hormone; UFC, 24-h urinary free cortisol; DST, dexamethasone suppression test; LDDST, low-dose dexamethasone suppression test; SH, subclinical hypercortisolism; N.S. – not significant.

Table III Comparison of serum cortisol levels after dexamethasone suppression tests between patients with UAI and BAI.

	Post-1 mg-DST, n=151			Post-LDDST, n=150		
Cortisol, nmol/L	Unilateral n (%)	Bilateral n (%)	p value	Unilateral	Bilateral n (%)	p value
≤ 50	66 (63.5)	18 (38.3)	0.014	69 (65.7)	18 (40.0)	0.013
50.1–137.9	30 (28.8)	24 (51.1)		27 (25.7)	19 (42.2)	
≥ 138	8 (7.7)	5 (10.6)		9 (8.6)	8 (17.8)	

1 mg-DST, serum cortisol levels after 1-mg overnight dexamethasone suppression test; post-LDDST, serum cortisol levels after low-dose dexamethasone suppression test; we used Pearson Chi-Square test.

When the diagnosis of SH was based on the post-1 mg-DST cortisol level, in combination with one of the following parameters: ACTH below cut-off level, UFC and midnight cortisol above cut-off levels, in the whole group SH was diagnosed in 31 (20.5%) patients. The SH tended to be more frequent in the BAI group compared to UAI group (29.8 *vs* 16.3, respectively, p=0.058). When we used post-LDDST cortisol level instead of post-1 mg-DST, the SH was diagnosed in 30 patients (20.0%) in the whole group and there was statistical significance between BAI and UAI groups (31.1% *vs* 15.2%, respectively, p=0.026). Results are presented in *Table II*.

Bone mineral density

Patients with BAI had significantly lower BMD at lumbar spine than patients with UAI (0.87±0.15 *vs* 0.96±0.14, p=0.002). Higher prevalence of normal T scores for the spine were found in UAI, and higher prevalence of osteoporosis was found in patients with BAI (p=0.004).

The BMD of the total hip tended to be lower in BAI than in UAI (0.85±0.13 *vs* 0.89±0.13, respectively, p=0.053), with also higher prevalence of normal T scores in UAI than BAI, and osteoporosis in BAI (p=0.003). There were no differences between two groups in BMD, and prevalence of osteoporosis and osteopenia of the femoral neck. Based on femoral neck and/or the lumbar spine DXA-scan, the prevalence of osteoporosis was 15.9% in patients with UAI and 37.1% in BAI (p=0.011). No difference was noted in the percent of postmenopausal women and years since menopause between groups. Bone mineral density and prevalence of osteoporosis are shown in *Table IV*.

Table IV Bone mineral density and prevalence of osteoporosis in UAI and BAI patients.

Characteristics	Unilateral	Bilateral	p value
DXA lumbar spine, n BMD, g/cm^2	80/105 0.96±0.14	33/47 0.87±0.15	 0.002
T score Z score Normal BMD, n (%) Osteopenia, n (%) Osteoporosis, n (%)	-1.08±1.23 0.06±1.25 33 (41.2) 39 (48.8) 8 (10.0)	-1.98±1.23 -0.36±1.27 9 (27.3) 12 (36.4) 12 (36.4)	0.001 N.S. 0.004
DXA hip, n BMD, g/cm^2	78/105 0.89±0.13	33/47 0.85±0.13	 N.S.
T score Z score Normal BMD, n (%) Osteopenia, n (%) Osteoporosis, n (%)	-0.74±0.90 0.14±0.91 46 (59.0) 32 (41.0) 0 (0.0)	-1.03±1.04 0.04±1.03 11 (33.3) 19 (57.6) 3 (9.1)	N.S. N.S. 0.003
DXA femoral neck, n BMD, g/cm^2	79/105 0.74±0.11	35/47 0.70±0.12	 N.S.
T score Z score Normal BMD, n (%) Osteopenia, n (%) Osteoporosis, n (%)	-1.33±0.94 -0.08±0.90 26 (32.9) 47 (59.5) 6 (7.6)	-1.54±0.96 -0.13±0.99 9 (25.7) 22 (62.9) 4 (11.4)	N.S. N.S. N.S.
Postmenopausal women, n (%) Years since menopause (range)	50 (73.5) 11.8±7.8 (1–31)	29 (76.3) 15.5 ±9.3 (2–33)	N.S. N.S.

Data are expressed as mean±standard deviation (X±SD), or percentage.
DXA, dual-energy X-ray absorptiometry; BMD, bone mineral density; N.S. – not significant.

Discussion

The present study shows significant difference in biochemical markers of hypercoticism between patients with UAI and BAI. Patients with BAI have lower ACTH values, and higher post 1 mg-DST and post-LDDST cortisol levels. They also have higher values of UFC, but this did not reach statistical significance. These results suggest more suppressed activity of the hypothalamic-pituitary-adrenal axis due to higher degree of autonomous cortisol secretion in patients with BAI. In the absence of overt Cushing's syndrome, the diagnosis of subclinical hypercortisolism is based on biochemical tests that include increased midnight serum or salivary and 24 h urinary free cortisol levels, suppressed plasma ACTH level, altered cortisol and ACTH response to corticotropin-releasing hormone (CRH) stimulation, low DHEAS concentration, and the lack of cortisol suppression after dexamethasone administration (20). The post 1 mg-DST is the most widely used test to diagnose subclinical hypercortisolism. However, its sensitivity and specificity vary according to the selected cut-off level. Our results showed high frequency of inadequate cortisol suppression after DST. Even more, after LDDST, the frequency of reduced suppressibility was more prevalent in the BAI group, irrespective of the different criteria used for cortisol suppression. In our study, the overall prevalence of SH was 20% which is in line with other recent studies showing prevalence of SH between 19.2 and 23.9% (21). When we evaluated SH based on post-1 mg-DST cortisol in combination with at least one abnormal result of other hormonal analyses (ACTH and UFC), the prevalence of SH only tended to be higher in patients with BAI, but it reached statistical significance when the LDDST was used as criterion. This is expected due to greater sensitivity of LDDST as a confirmatory test. Our results are consistent with results of other studies in which similar criteria for defining SH were applied (22–27). Tumor mass could be of importance for the amount of cortisol production in these patients. In our study, the largest tumor diameter was similar in patients with UAI and BAI, but with higher prevalence of SH among patients with BAI. This is in complete agreement with the results of Vasilatou et al. (22). However, in some other reports, both dimensions and prevalence of SH were similar in patients with UAI and BAI (25) or similar SH prevalence with larger tumor mass in patients with BAI was found (26). It appears that critical tumor mass is

required to produce sufficient cortisol for the induction of SH, as it has been shown that the size of the adrenal mass positively correlates with probability for SH (27). In spite of this, different pathogeneses are probably involved in the tumorigenesis of UAI and BAI.

As in our study, low serum ACTH levels are frequently found in AI patients. In patients with AI cortisol secretion varies from normal to clearly increased levels (29). Therefore, it is expected that ACTH travels with cortisol concentrations from nonsuppressed to suppressed levels reflecting the extent of autonomous cortisol secretion. Some of the studies (22, 23, 26) demonstrated higher ACTH concentration in UAI than BAI patients, but this was not the case with others (25, 27). Studies that have reported information about midnight serum cortisol and UFC showed no significant difference between the two groups (22, 23). Several reports have demonstrated an increased prevalence of metabolic and cardiovascular diseases in patients with AI regardless of the presence of SH (7, 9, 29–31). In our study, patients with BAI and UAI had similar anthropometric characteristics (age, BMI, waist circumference, the prevalence of central adiposity). Diabetes, hypertension and dyslipidemia occurred with similar frequency as shown before (22, 26). It has been shown that hypercortisolism, even if mild, is an important risk factor for osteoporosis and vertebral fractures (32). In this study, patients with BAI had significantly lower lumbar spine mineral density and higher prevalence of osteoporosis than patients with UAI. The BMD of the total hip tended to be lower in patients with BAI, and these patients had significantly higher prevalence of reduced total hip T scores. There were no differences in the prevalence of osteopenia and osteoporosis of the femoral neck between patients with UAI and BAI. Only one study assessed bone involvement in patients with BAI and UAI. Despite the similar prevalence of SH and metabolic complications, patients with BAI had lower femoral neck BMD and higher prevalence of vertebral fractures (26). It is generally accepted that trabecular bone is predominantly affected by glucocorticoid excess; however, several studies have shown that cortical bone is also affected in conditions with chronic exposure to high levels of cortisol in the blood, either endogenous or exogenous (33, 34).

Our patients with BAI had higher prevalence of SH and osteoporosis, but these were not accompanied by higher frequency of metabolic complications. This could be due to the different duration of hypercortisolism and interindividual variations in glucocorticoid sensitivity, which depends on the polymorphism of both glucocorticoid receptor (GR) and 11-hydroxysteroid-dehydrogenase type 1 genes (35, 36). In our previous study we have shown that GR gene variants, larger allele of BclI and minor allele of 9 β, were associated with predisposition to the development of UAI and reduced sensitivity to glucocorticoids (37). Increased prevalence of vertebral fractures was found in patients carriers of heterozygous N363S and homozygous BclI polymorphism of GR regardless of the degree of dysfunctional cortisol secretion (38). Majnik et al. showed that the N363S variant of the GR gene was more frequent in BAI than in UAI patients, but they did not find a difference in hormone secretion between the two groups (39). Whether N363S variant of the GR gene is involved in the development of AI and glucocorticoid induced osteoporosis needs further investigation.

A limitation of this study is its cross-sectional design. Thus, variable duration of subtle cortisol autonomous secretion may influence the clinical features (40). Therefore, long-term follow up of patients would allow a better assessment of the clinical implications of subclinical hypercortisolism.

In conclusion, this study suggests significantly higher prevalence of SH in patients with BAI than in UAI patients, irrespective of the different criteria for cortisol suppression. In spite of the similar frequency of diabetes, hypertension and dyslipidemia, patients with BAI had reduced bone mineral density, suggesting different organ sensitivity to glucocorticoids.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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