ELMER PRESS

Original Article

J Endocrinol Metab. 2023;13(1):33-38

The Severity of Bone Loss: A Comparison Between Cushing's Disease and Cushing's Syndrome

Rania Naguib[a, b, e], Eman Z. Elkemary[c], Karim Mohamed Elsharkawi[d]

Abstract

Background: This study aimed to estimate the prevalence of osteoporosis among patients with adrenal Cushing's syndrome (ACS) and Cushing's disease (CD), to investigate whether there is a difference in the degree of osteoporosis between both groups; and if so, what factors may be responsible for this variation.

Methods: This is a cross-sectional study in which data from 19 patients with CD and eight patients with ACS were analyzed. Osteoporosis was assessed using the bone mineral density (BMD) of the lumbar spine using dual-energy X-ray absorptiometry (DEXA).

Results: Bone loss was present in 81.5% of the patients with Cushing's syndrome. Osteoporosis is nearly three times more common in ACS (62.5%) than in CD (26.3%). BMD at the lumbar spine was lower in patients with ACS than in patients with CD. ACS had significantly lower levels of dehydroepiandrosterone sulfate (DHEA-S) than CD. In comparison to the CD group, the serum parathyroid hormone (PTH) concentration in the ACS group was significantly higher. In the entire patient population, there was a statistically significant correlation between the DHEA-S and the lumbar BMD values.

Conclusions: Patients with ACS have more severe osteoporosis than patients with CD, and the difference in DHEA-S concentrations might be important in explaining this difference. BMD examinations should be prioritized to enable rapid recognition and intervention for osteoporosis. Measurement of bone turnover markers such as PTH may aid in the early diagnosis of the consequences of hypercortisolism on the bone.

Keywords: Bone mineral density; Bone loss; Osteoporosis; Cushing's syndrome; Cushing's disease

Manuscript submitted January 3, 2023, accepted February 2, 2023
Published online February 17, 2023

[a]Department of Clinical Science, College of Medicine, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia
[b]Internal Medicine Department, Endocrinology Unit, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[c]Clinical Pathology Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[d]Department of Radiodiagnosis, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[e]Corresponding Author: Rania Naguib, Department of Clinical Science, College of Medicine, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. Email: ranianaguib2000@yahoo.com

doi: https://doi.org/10.14740/jem857

Introduction

Cushing's syndrome (CS) is a group of symptoms and signs caused by abnormally elevated cortisol levels from a variety of causes. There are several types of CS, e.g., exogenous Cushing and ectopic adrenocorticotropic hormone (ACTH)-dependent Cushing, Cushing's disease (CD), and adrenal Cushing's syndrome (ACS) [1]. Hypercortisolism, which is the defining feature of CS is a known important cause of secondary osteoporosis characterized by a loss of bone mass and density [2]. Cushing osteopathy is one of the most severe complications. Abnormal bone turnover, decreased bone mineral density (BMD), and increased fracture risk are common effects of excessive glucocorticoids [3]. The structural and functional skeletal damage is disabling and correlates with the patients' reported low quality of life [3, 4]. According to data on bone density in endogenous hypercortisolism, patients with CS of adrenal, pituitary, or ectopic origin have marked osteopenia/osteoporosis [5, 6].

Assessment of bone derangement is based on dual-energy X-ray absorptiometry (DEXA) to measure BMD. BMD is unquestionably one of the most important determinants of bone quality and the World Health Organization (WHO) diagnostic classification of osteoporosis is based on its values as expressed in T scores [3, 7, 8]. Patients with CS are reported to have a 60% to 80% prevalence of osteopenia and a 30% to 65% prevalence of osteoporosis, respectively [1, 9, 10]. Other studies on CS patients who underwent DEXA scans reveal a BMD decline of 15-20% in comparison to normal subjects and 28% to 50% of the affected patients have osteoporosis [3, 11]. Although their phenotypes are similar, patients with primary adrenal-dependent or pituitary-dependent CS exhibit different patterns of adrenal steroid secretion. While cortical adenomas cause CS to secrete only cortisol, creating a "pure hypercortisolism", cortical adenomas can promote steroid synthesis in the zona fasciculata as well as in the zona reticularis of the adrenal cortex [9].

The rarity of CS and other endogenous hypercortisolism, such as adrenal adenomas or hyperplasia secreting cortisol independently from ACTH or ectopic ACTH-secreting tumors, helps to explain this. All of these conditions are uncommon and, as a result, are frequently diagnosed later in the course of the disease. Osteopathy in endogenous hypercortisolism is frequently disregarded due to the lengthy and challenging di-

agnostic procedures, as well as the endocrinologist's specific focus on the treatment of the underlying diseases [3].

The aim of this study is to estimate the prevalence of osteoporosis among patients with ACS and CD in Egypt, to investigate whether there is a difference in the degree of osteoporosis between pituitary and adrenal CS; and if so, what factors may be responsible for this variation.

Materials and Methods

The study was conducted in compliance with the ethical standards of the responsible institution on human subjects as well as with the Helsinki Declaration and was approved by the local Ethical Committee at Alexandria University Faculty of Medicine, Alexandria, Egypt (IRB number: 0305681). All participants provided informed consent.

A cross-sectional prospective study where data from 19 patients with CD and eight patients with ACS recruited from Alexandria Faculty of Medicine, Alexandria, Egypt, during the period from July to November 2022 were analyzed. All of the patients had manifestations of hypercortisolism. Patients who had mild autonomous cortisol secretion, other types of hypercortisolism other than CD and ACS (such as ectopic ACTH syndrome, adrenocortical hyperplasia, or adrenocortical carcinoma) or recurring diseases were excluded from the study. Since diabetes mellitus can affect the BMD and might be a confounding factor, patients diagnosed with diabetes mellitus were also excluded. Pregnancy, alcoholism, smoking, and a history of chronic disease were also among the exclusion criteria. The enrolled patients underwent evaluation while their diseases were still active, and none of them were taking any medications known to have an impact on bone metabolism or the hypothalamic-pituitary-adrenal axis. The following endocrine workup and clinical features were used to confirm the diagnosis of CS: 1) measuring serum cortisol levels at 2400 h; 2) evaluation of the 24-h excretion of urinary free cortisol (UFC) calculated as the mean value of three different samples collected on consecutive days; 3) measurement of plasma ACTH at 0800 h; 4) low-dose overnight dexamethasone suppression test (1 mg, orally, at 2300 h and measurement of serum cortisol at 0800 h in the following morning). For the diagnosis of ACTH-dependent CS, the corticotropin-releasing hormone test and the high-dose overnight dexamethasone suppression test (8 mg, orally, at 2300 h and measurement of serum cortisol at 0800 h the following morning) were used. An adrenal computed tomography (CT) scan or a pituitary magnetic resonance imaging (MRI) was used to assess the presence of a space-occupying lesion. All patients underwent surgical treatment, either resection of the pituitary adenoma or the pituitary gland using the transsphenoidal approach. Both cortisol-secreting and ACTH-secreting adenomas were identified in these surgically removed tissues.

Serum calcium and phosphorus concentrations were measured using COBAS C311 automated chemistry analyzer (Roche Diagnostics, Germany). Serum level of cortisol, dehydroepiandrosterone sulfate (DHEA-S), and 25-hydroxyvitamin D3 (25(OH)D3) concentrations were measured using COBAS E411 automated immunoassay analyzer (Roche Diagnostics, Germany) [12]. Serum ACTH and parathyroid hormone (PTH) concentrations were measured using COBAS E411 automated immunoassay analyzer (Roche Diagnostics, Germany) [12].

The 24-h UFC concentration was assessed using Immulite 2000 automated immunoassay analyzer (Siemens Healthcare Diagnostics, USA) [12]. A DEXA scan machine (Model Hologic Horizon) was used. The bone density is measured at the lumbar spine using the T-score, which represents the difference between the measured BMD and the population standard deviation (SD) for similar ages and sex. A normal result is a T-score from -1 to +1. T-score between -1 and -2.5 represents osteopenia, whereas a T-score of -2.5 or less indicates osteoporosis. The prevalence and the degree of severity of osteoporosis were compared between ACS and the CD groups.

IBM SPSS Statistics for Windows, version 20, was used to analyze the data (IBM Corp., Armonk, NY). To describe the criteria of the studied sample, descriptive statistics in terms of frequency and percentages were used. The Chi-square test was used to examine the relationship between categorical variables. A P value of less than 0.05 was deemed statistically significant. The normal distribution of all data was tested. The mean ± standard deviation of normally distributed data is reported. The Student's *t*-test was used for comparisons between the two groups. Data were presented as mean and standard error after confirming the normality distribution (all variables were not following the normal distribution) and comparison was done using the Mann-Whitney test. Pearson's test was used to assess correlations. Age, body mass index (BMI) and UFC were adjusted using linear regression analysis. To compare the correlations of categorical variables, logistic regression analysis was used.

Results

In this study, there were eight ACS patients and 19 CD patients. In patients with ACD, there were three men and five women, and the mean age was 46.5 ± 8.6 years; while in the CD group, there were five men and 14 women with a mean age of 44.3 ± 14.2 years. ACS and the CD groups did not differ significantly in terms of age, sex, and BMI (Table 1).

The 24-h UFC concentration in the ACS group was significantly higher while serum ACTH concentration was significantly lower compared to the CD group ($P < 0.05$). There were no detectable differences in serum cortisol levels between CD and ACS. ACS had significantly lower levels of DHEA-S than CD (Table 1).

In all patients, there was no significant difference in the serum concentration of calcium, phosphorus, or serum 25(OH) D3 concentrations between the ACS and CD groups. In comparison to the CD group, the serum PTH concentration in the ACS group was significantly higher ($P < 0.01$) (Table 1). The PTH, serum calcium, 25(OH)D3, urinary cortisol, and serum cortisol concentrations did not correlate with BMD, according to Pearson correlation analysis (data not shown).

The prevalence of osteopenia and osteoporosis in the entire patient population with CS (n = 27) was 44.4% and 37.1%, respectively. So, overall bone loss was found in 81.5% of our population. The prevalence of osteoporosis in individuals with

Table 1. Comparison Between ACS and CD Regarding Demographics and Clinical Characteristics, Hormonal Profile, and Other Biochemical Markers of Bone Metabolism

	ACS (n = 8)	CD (n = 19)	P value
Age (years)	46.5 ± 3.07	44.3 ± 0.34	0.48
Sex (male/female)	3/5	5/14	0.16
BMI (kg/m^2)	26.32 ± 1.5	28.41 ± 0.49	0.535
ACTH (pg/mL)	7.43 ± 1.45	93.87 ± 4.62	< 0.01*
DHEA-S (µg/L)	86.0 ± 22.96	302.9 ± 12.55	< 0.01*
UFC (µg/24 h)	647.5 ± 71.36	405.6 ± 31.54	0.01*
Serum cortisol (mmol/L)	549.5 ± 28.0	574.8 ± 15.25	0.12
25(OH)D3 (nmol/L)	39.15 ± 6.76	38.02 ± 4.43	0.578
PTH (ng/L)	49.60 ± 4.98	26.10 ± 4.896	< 0.01*
Serum Ca (mmol/L)	2.08 ± 0.25	1.97 ± 0.03	0.053
Serum P (mmol/L)	1.55 ± 0.25	1.67 ± 0.77	0.06

Data are presented as mean ± standard error. *P ≤ 0.05. ACS: adrenal-dependent Cushing's syndrome; CD: Cushing's disease; BMI: body mass index; ACTH: adrenocorticotropic hormone; DHEA-S: dehydroepiandrosterone sulfate; UFC: urinary free cortisol; 25(OH)D3: 25-hydroxyvitamin D3; PTH: parathyroid hormone: Ca: calcium; P: phosphorus.

adrenal CS was (62.5%), which is nearly as triple that in patients with pituitary CD (26.3%) (Table 2).

In comparison to the CD group, lumbar BMD was significantly lower in the ACS group (P < 0.05). Patients with ACS had significantly lower lumbar bone densitometric values (BMD: 0.83 ± 0.12 g/cm^2; T-score: -2.98 ± 0.16), whereas patients with CD had significantly higher values (BMD: 0.97 ± 0.32 g/cm^2; T-score: -1.91 ± 0.33). After accounting for the non-significant difference in age, sex, BMI and UFC values between CD and ACS, the difference in BMD and T-score at the lumber spine remained statistically significant (P = 0.012) (Table 3). In the entire patient population, there was a statistically significant correlation between the DHEA-S and the lumbar BMD values (r = 0.67, P = 0.001). No additional evidence of a significant relationship between BMD values and the assessed hormonal variables such as ACTH in both ACS and CD groups was detected.

Discussion

Osteoporosis has been acknowledged as a serious side effect of endogenous hypercortisolism. Between 50% and 59% of cases of osteoporosis have been reported to be brought on by excessive endogenous cortisol. The presenting symptom of hypercortisolism can be pathological fractures. Early detection of the typical changes in bone mass caused by hypercortisolism aids in the early diagnosis of bone mass loss and prompt treatment, reducing the likelihood of adverse events [13].

Table 2. Comparison Between ACS and CD Regarding the Prevalence of Osteoporosis

T-score values	ACS (n = 8)	CD (n = 19)	P value
Normal	1 (12.5%)	4 (21.0%)	< 0.01*
Osteopenia	2 (25%)	10 (52.6%)	< 0.001*
Osteoporosis	5 (62.5%)	5 (26.3%)	0.03*

*P ≤ 0.05. ACS: adrenal-dependent Cushing's syndrome; CD: Cushing's disease.

Table 3. Comparison Between ACS and CD Regarding Bone Densitometric Values

	ACS	CD	P value
BMD (g/cm^2)	0.83 ± 0.12	0.97 ± 0.32	0.011*
T-score	-2.98 ± 0.16	-1.91± 0.33	< 0·01*

Data are presented as mean ± standard deviation. *P ≤ 0.05. ACS: adrenal-dependent Cushing's syndrome; CD: Cushing's disease; BMD: bone mineral density.

The aim of the current study was to estimate the prevalence of osteoporosis among patients with ACS and CD, to investigate whether there is a difference in the degree of osteoporosis between them, and if so, what factors may be responsible for this variation.

The present study confirms that bone loss (osteopenia and osteoporosis) was present in 81.5% of the patients with CS. This prevalence was marginally higher than the percentage (70%) reported in a national survey conducted in Japan in 1988 and in a study conducted by Ohmori et al who reported a prevalence of 54.8% [14]. In the large ERCUSYN study [15], DEXA measurements of the spine revealed osteopenia in 43% of men and 41% of women. Men were significantly more likely than women to have osteoporosis at the spine (40% vs. 20%). The discrepancy might be explained by the fact that data from the nationwide survey conducted in Japan were based on physician responses to questionnaires rather than on specific diagnostic criteria. So, our findings are more likely to be valid and reliable. The higher percentage in our study might be also explained by cultural and religious reasons where most of the ladies are covered so they are not exposed to adequate sunlight with subsequent higher prevalence of osteoporosis. Our findings indicate that osteoporosis is nearly three times more common in ACS (62.5%) than in CD (26.3%). Ohmori et al reported similar findings with osteoporosis found in 69.6% of ACS compared to 36.8% in CD [14].

The most interesting finding of this study was that BMD at the lumbar spine was lower in patients with ACS than in patients with CD. These current findings confirm results from previous studies [9, 11, 13, 16, 17].

Excess glucocorticoids are thought to cause secondary osteoporosis through several different mechanisms. Chronic glucocorticoid overproduction inhibits bone formation by directly impairing osteoblast differentiation and function, promoting osteoblast and osteocyte apoptosis. While decreasing the production of osteoprotegerin by osteoblasts and osteocytes, excessive glucocorticoids secretion can increase bone resorption by promoting osteoclast differentiation, maturation, and survival, likely through enhancing osteoclast autophagy and increasing macrophage colony-stimulating factor and RANKL [18, 19]. Additionally, glucocorticoids excess is known to result in vi-

tamin D deficiency because it affects vitamin D metabolism and action by impairing the conversion of cholecalciferol into 25(OH)D [20, 21]. These negative actions of glucocorticoids on vitamin D may also be amplified by concomitant glucocorticoid-induced hypogonadism, obesity, and diabetes. Glucocorticoids may modify the endogenous pulsatile secretion of PTH, decreasing its secretion and potentially contributing to reducing the bone synthesis of insulin-like growth factor 1 (IGF-1). Glucocorticoids decrease enteral calcium absorption and may cause secondary compensatory hyperparathyroidism, which in turn raises the amount of bone resorption [3, 20]. Moreover, glucocorticoids have several unfavorable indirect effects on the skeleton through neuroendocrine mechanisms. When glucocorticoids are oversecreted, they reduce growth hormone (GH) secretion by increasing hypothalamic somatostatin tone. Mild hypercortisolism has been linked to blunted GH secretion. Additionally, glucocorticoids may inhibit IGF-I peripheral action and suppress liver IGF-I secretion. Inhibiting both gonadotropin and gonadal sex steroid secretion, glucocorticoids may lead to hypogonadism. The concurrent glucocorticoid-mediated suppression of the GH/IGF-I and gonadal axes may have synergistically detrimental effects [3, 22]. The glucocorticoid-induced impairment of gonadal steroid synthesis, which prevents bone resorption, is an indirect mechanism causing decreased bone mass. Additionally, because muscle contraction no longer has the same trophic effect on bone, the loss of muscle mass and power frequently seen in patients with CS or those receiving long-term exogenous corticosteroid treatment may contribute to osteoporosis. Furthermore, there are many other cells in the bone that also contain glucocorticoid receptors in addition to osteoblastic lineage cells. These cells are capable of producing and releasing growth factors and cytokines, which in turn affect bone turnover [9].

There is a difference in the degree of osteoporosis depending on the cause of hypercortisolism. Our study demonstrated that lumbar BMD values are noticeably more compromised in ACS than in CD. The remarkable decline in serum DHEA-S levels seen in ACS patients in this study may provide a convincing justification for why ACS is more detrimental to the bone than CD. DHEA-S concentrations have anabolic effects on bone [3, 9]. These results are in concordance with the results obtained by Minetto et al [9]. In another study by Guo et al on 78 patients with CS, they reported a significant difference in lumbar BMD between ACS and CD. This was explained by a difference in UFC levels but not by a difference in DHEA-S concentration [13]. Other authors found no differences in the degree of bone damage concerning the cause of hypercortisolism [23, 24]. The large amounts of DHEA-S that the adrenal glands physiologically secrete have almost no direct effects, but they are converted to DHEA, which is its biologically active counterpart. DHEA-S serves as the reservoir for DHEA as a result, and the two steroids have a linear relationship. Research studies into the anabolic effects of DHEA on bone in patients who receive pharmacological doses of the steroid are currently ongoing. In healthy older subjects of both sexes, DHEA replacement therapy had questioned effects on circulating levels of bone turnover markers, with unchanged or increased levels of bone formation markers and unchanged or decreased levels of bone resorption markers. The mechanisms underlying DHEA's osteoanabolic effect need to be clarified. DHEA may promote bone growth either directly or by converting to androgens and estrogens. DHEA sulphatase is present in osteoblasts in sufficient quantities to change inactive DHEA-S into active DHEA. Since a statistically significant correlation was discovered between BMD and serum DHEA-S concentrations, the conversion of DHEA into estrogens by osteoblasts has been proposed as a preventative measure against postmenopausal osteoporosis. Additionally, it has been shown that 1,25-dihydroxyvitamin D3 interacts with DHEA and DHEA-S to promote osteoblastic differentiation and proliferation [9].

In the current study, biochemical indicators of bone metabolism differed significantly between patients with hypercortisolism. Serum PTH level in the ACS group was significantly higher compared to the CD group. Similar higher PTH concentrations were previously reported, and the authors suggested that this indicated active bone turnover and secondary hyperparathyroidism [13, 16].

Regarding BMD-related variables, this study did not discover any evidence of a significant relationship between BMD values and the assessed hormonal variables such as ACTH in both ACS and CD groups. A recent study [13] demonstrated a significant correlation between BMD and ACTH concentration in patients with CD; as a result, this study stated that ACTH may have a protective effect on lumbar BMD in patients with CD. According to reports, ACTH stimulates osteoblast proliferation and elevates collagen I mRNA in the osteoblastic cell line SaOs2 *in vitro*. ACTH binds to the MC2R, a member of the melanocortin receptor family that is expressed in osteoblastic cells *in vivo*. Osteoblast differentiation is promoted by increased gene expression of *Osterix* and collagen type I alpha when bone marrow, stromal cells, and leptin are exposed *in vitro*. In the same study, ACTH was not associated with BMD in ACS patients, implying that ACTH may not have a protective effect on lumbar BMD in ACS patients. This finding could be attributed to the low ACTH concentration in this group. In our study, ACTH may have a bone-protective effect; however, it is insufficient to counteract the negative effects of increased cortisol levels on bone metabolism in CD patients. This similarity of lack of association between ACTH and BMD in the ACS group enforces our results. The discrepancy regarding the association in the CD group might be explained by different sample sizes and selection criteria.

The limitations of this cross-sectional study on comparatively small patient populations are disclosed. Nevertheless, it is important to keep in mind that ACS is extremely uncommon, making up only 15% of all CS cases. Another limitation is that we did not evaluate the patients for the presence of hypogonadism. This was based on the results of the binary logistic regression analysis in a previous study [13] that revealed no relationship between lumbar BMD and gonadal function. Therefore, we do not think that sex hormones affected bone density in this study as a confounding factor. Another limitation is that, for practical reasons, the duration of the disease, age of menarche, and dietary calcium intake were not analyzed and thus could not be investigated as mediating factors. Patients with ACS have more severe osteoporosis than patients with CD, and the difference in DHEA-S concentrations might be important in explaining the different effects on the bone.

Early detection and management of CS are critical for reducing bone complications. BMD examinations should be performed to enable rapid recognition and intervention for osteoporosis. Measurement of bone turnover markers such as PTH may aid in the early diagnosis of the consequences of hypercortisolism on the bone.

Further studies are recommended to evaluate the BMD of the hip and femoral neck and to assess and compare the effect of surgery as a management plan on the degree of bone loss in both ACS and CD. Studies assessing the prevalence of symptomatic and asymptomatic fractures are also recommended since these are major causes of morbidity which affects the quality of life.

Acknowledgments

None to declare.

Financial Disclosure

None to declare.

Conflict of Interest

All authors declare that there is no conflict of interest.

Informed Consent

Informed consent has been taken from all participants.

Author Contributions

Rania Naguib: concept, design, literature search, clinical studies, data acquisition, manuscript preparation, manuscript editing and manuscript review. Eman Z. Elkemary: literature search, clinical studies, and manuscript preparation. Karim Mohamed Elsharkawi: literature search, clinical studies, and manuscript preparation. This manuscript has been read and approved by all authors.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations

CS: Cushing's syndrome; ACS: adrenal Cushing's syndrome; CD: Cushing's disease; BMD: bone mineral density; DEXA: dual-energy X-ray absorptiometry; DHEA-S: dehydroepiandrosterone sulfate; PTH: parathyroid hormone; WHO: World Health Organization; ACTH: adrenocorticotropic hormone; UFC: urinary free cortisol

References

1. Wang D, Dang CX, Hao YX, Yu X, Liu PF, Li JS. Relationship between osteoporosis and Cushing syndrome based on bioinformatics. Medicine (Baltimore). 2022;101(43):e31283.
2. Alejandro P, Constantinescu F. A review of osteoporosis in the older adult: an update. Rheum Dis Clin North Am. 2018;44(3):437-451.
3. Frara S, Allora A, di Filippo L, Formenti AM, Loli P, Polizzi E, Tradati D, et al. Osteopathy in mild adrenal Cushing's syndrome and Cushing disease. Best Pract Res Clin Endocrinol Metab. 2021;35(2):101515.
4. Cusimano MD, Huang TQ, Marchie A, Smyth HS, Kovacs K. Development and validation of the disease-specific QOL-CD quality of life questionnaire for patients with Cushing's disease. Neurosurg Focus. 2020;48(6):E4.
5. Batista SL, de Araujo IM, Carvalho AL, Alencar M, Nahas AK, Elias J, Jr., Nogueira-Barbosa MH, et al. Beyond the metabolic syndrome: Visceral and marrow adipose tissues impair bone quantity and quality in Cushing's disease. PLoS One. 2019;14(10):e0223432.
6. Reimondo G, Puglisi S, Pia A, Terzolo M. Autonomous hypercortisolism: definition and clinical implications. Minerva Endocrinol. 2019;44(1):33-42.
7. Silva BC, Broy SB, Boutroy S, Schousboe JT, Shepherd JA, Leslie WD. Fracture risk prediction by non-BMD DXA measures: the 2015 ISCD official positions part 2: trabecular bone score. J Clin Densitom. 2015;18(3):309-330.
8. Ulivieri FM, Rinaudo L. Beyond bone mineral density: a new dual X-ray absorptiometry index of bone strength to predict fragility fractures, the bone strain index. Front Med (Lausanne). 2020;7:590139.
9. Minetto M, Reimondo G, Osella G, Ventura M, Angeli A, Terzolo M. Bone loss is more severe in primary adrenal than in pituitary-dependent Cushing's syndrome. Osteoporos Int. 2004;15(11):855-861.
10. van der Eerden AW, den Heijer M, Oyen WJ, Hermus AR. Cushing's syndrome and bone mineral density: lowest Z scores in young patients. Neth J Med. 2007;65(4):137-141.
11. dos Santos CV, Vieira Neto L, Madeira M, Alves Coelho MC, de Mendonca LM, Paranhos-Neto Fde P, Lima IC, et al. Bone density and microarchitecture in endogenous hypercortisolism. Clin Endocrinol (Oxf). 2015;83(4):468-474.
12. Rifai N. Tietz textbook of clinical chemistry and molecular diagnostics. Elsevier Health Sciences. 2017.
13. Guo W, Li F, Zhu C, Wang B, Wang K, Dai C, Jia H, et al. Effect of hypercortisolism on bone mineral density and bone metabolism: A potential protective effect of adrenocorticotropic hormone in patients with Cushing's disease. J Int Med Res. 2018;46(1):492-503.

14. Ohmori N, Nomura K, Ohmori K, Kato Y, Itoh T, Takano K. Osteoporosis is more prevalent in adrenal than in pituitary Cushing's syndrome. Endocr J. 2003;50(1):1-7.
15. Valassi E, Santos A, Yaneva M, Toth M, Strasburger CJ, Chanson P, Wass JA, et al. The European Registry on Cushing's syndrome: 2-year experience. Baseline demographic and clinical characteristics. Eur J Endocrinol. 2011;165(3):383-392.
16. Chiodini I, Carnevale V, Torlontano M, Fusilli S, Guglielmi G, Pileri M, Modoni S, et al. Alterations of bone turnover and bone mass at different skeletal sites due to pure glucocorticoid excess: study in eumenorrheic patients with Cushing's syndrome. J Clin Endocrinol Metab. 1998;83(6):1863-1867.
17. Francucci CM, Pantanetti P, Garrapa GG, Massi F, Arnaldi G, Mantero F. Bone metabolism and mass in women with Cushing's syndrome and adrenal incidentaloma. Clin Endocrinol (Oxf). 2002;57(5):587-593.
18. Chotiyarnwong P, McCloskey EV. Pathogenesis of glucocorticoid-induced osteoporosis and options for treatment. Nat Rev Endocrinol. 2020;16(8):437-447.
19. Fu L, Wu W, Sun X, Zhang P. Glucocorticoids enhanced osteoclast autophagy through the PI3K/Akt/mTOR signaling pathway. Calcif Tissue Int. 2020;107(1):60-71.
20. Sato AY, Richardson D, Cregor M, Davis HM, Au ED, McAndrews K, Zimmers TA, et al. Glucocorticoids induce bone and muscle atrophy by tissue-specific mechanisms upstream of E3 ubiquitin ligases. Endocrinology. 2017;158(3):664-677.
21. Sempos CT, Heijboer AC, Bikle DD, Bollerslev J, Bouillon R, Brannon PM, DeLuca HF, et al. Vitamin D assays and the definition of hypovitaminosis D: results from the First International Conference on Controversies in Vitamin D. Br J Clin Pharmacol. 2018;84(10):2194-2207.
22. Formenti AM, Maffezzoni F, Doga M, Mazziotti G, Giustina A. Growth hormone deficiency in treated acromegaly and active Cushing's syndrome. Best Pract Res Clin Endocrinol Metab. 2017;31(1):79-90.
23. Trementino L, Appolloni G, Ceccoli L, Marcelli G, Concettoni C, Boscaro M, Arnaldi G. Bone complications in patients with Cushing's syndrome: looking for clinical, biochemical, and genetic determinants. Osteoporos Int. 2014;25(3):913-921.
24. Apaydin T, Yavuz DG. Assessment of non-traumatic vertebral fractures in Cushing's syndrome patients. J Endocrinol Invest. 2021;44(8):1767-1773.

