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Research Article

The Severity of Bone Loss in Cushing's Diseases

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Abstract

Introduction: Endogenous Cushing's syndrome (CS) is a known cause of secondary osteoporosis, characterized by a loss of bone mass and density. Cushing's osteopathy is one of it's most severe complications. Abnormal bone turnover, decreased bone mineral density (BMD), and increased fracture risk are common effects of glucocorticoid excess. *Objectives:* The aim of our work is to determine the bone densitometric profile of patients followed for Cushing's disease, to define the characteristics of osteoporosis and osteopenia in these patients, and to analyze the factors influencing BMD. *Materials and methods:* This is a retrospective descriptive study involving patients followed for Cushing's disease in the endocrinology-diabetology department of the public hospital establishment (EPH) of Bologhine-Algiers, during a period of 10 years, going from the January 2013 to January 2023. *Results:* 58 patients were followed during this period with an average age was 34 years, with a clear female predominance and a sex ratio of ≈ 4. Average Z score was (-1.6) at the vertebral level and (-1.09) at the femoral level. Osteoporosis was found in 44.8% (n = 26) patients and osteopenia in 37.9% (n = 22), while normal BMD was found in 17.24% (n = 10). Note that Z-score values were significantly lower at the lumbar spine than at the femoral neck. No significant difference in BMD was found between eumenorrheic and hypo-/amenorrheic females with Cushing's disease. Our study demonstrated a significant negative correlation between morning plasma cortisol and BMD. While there is a significant positive correlation between BMD and ACTH concentration in CD patients, BMD at the lumbar spine and femoral neck also had a significant positive correlation with weight and BMI. *Conclusion:* Early detection and management of CS are essential to reduce bone complications. BMD examinations should be performed to enable rapid recognition and intervention for osteoporosis. Lumbar bone loss occurs earlier and more extensively.

Keywords

Bone Mineral Density, Cushing's Disease, Fracture, Osteopenia, Osteoporosis

1. Introduction

Cushing's disease is characterized by the presence of a tumor, usually benign and very small, located in the pituitary gland, which causes the abnormal secretion of a hormone (ACTH) and stimulates excessive production of cortisol by the two adrenal glands. Potentially serious, Cushing's disease can have multiple consequences: obesity, facial swelling, high

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blood pressure, osteoporosis, psychological disorders, amenorrhea, frequent infections, and thromboses.

Osteopathy in endogenous hypercortisolism is often ignored due to the long and difficult diagnostic procedures, as well as the special emphasis placed by the endocrinologist on the treatment of underlying diseases.

Osteoporosis is a well-recognized complication of Cushing's disease (CD). It results from a set of systemic and local effects that glucocorticoids have on bone and mineral metabolism.

Excess in cortisol results in a shift of mesenchymal stem cells toward adipogenesis rather than osteoblastogenesis, an increased RANK-L expression with increased osteoclastic bone resorption, and an osteoblast autophagy [1].

The prevalence of osteoporosis due to excess endogenous cortisol has been reported to be up to 59% [2, 3] of the patients, and approximately one-third to one-half of patients with hypercortisolism-induced osteoporosis experience a fragility fracture [4].

To date, the diagnosis of osteoporosis is based on the measurement of surface bone mineral density (BMD).

BMD represents the addition of the density of cortical bone and trabecular bone on a column crossed by X-rays. The vertebral body are mainly made up of trabecular bone and the femoral necks of cortical bone. Thus, spinal BMD reflects more trabecular bone, while femoral neck BMD reflects more cortical bone.

We decided to evaluate the bone impact of our patients suffering from Cushing's disease.

2. The Aims of the Study

To assess the frequency of bone damage in patients with Cushing's disease and analyze the factors influencing BMD.

3. Materials and Methods

This is a retrospective descriptive study of patients followed for ACTH-dependent Cushing's disease, collected between January 1999 and June 2023, in the endocrinology-diabetology department of the public hospital establishment (EPH) of Bologhine-Algiers.

We excluded patients with exogenous Cushing's syndrome and a history of metabolic bone diseases and systemic diseases such as rheumatoid arthritis, bronchial asthma, or a history of antiepileptic drugs medication affecting the bone.

We retained the diagnosis of endogenous CS by: the presence of two of the following screening tests that were positive: midnight serum cortisol (between 23.00 and 24.00 h), 24-h urinary free cortisol, an overnight dexamethasone suppression test (ONDST) (by administering 1 mg dexamethasone between 23.00 and 24.00 h), followed by measurement of serum cortisol the next morning between 08.00 and 09.00 h. A cut-off of 50 nmol/L was considered, above which ONDST was non-suppressed. The low-dose dexamethasone suppression test (LDDST) was done by administering dexamethasone 0.5 mg every 6 h for 48 h, followed by measurement of serum cortisol at 09.00 h, after 6 h of the last dose. A value ≥50 nmol/L was considered a non-suppressed LDDST [5].

Patients were classified as having ACTH-dependent Cushing's syndrome based on an ACTH threshold of 20 pg/mL (4 pmol/l), followed by a high-dose dexamethasone suppression test (HDDST), which was performed by administering 2 mg of dexamethasone orally every 6 h for 48 h, followed by measurement of serum cortisol at 09.00h, 6 hours after the last dose. The percentage of cortiso suppression during HDDST was calculated. A reduction greater than 50% is in favor of Cushing's disease [5].

Were analyzed the anamnestic investigation, the clinical, biological, radiological, therapeutic, and progressive parameters of each patient.

Basic analyses, including serum calcium, phosphate, albumin, creatinine, alkaline phosphatase, 24-hour urinary calcium and 24-hour urinary phosphate, were performed by the colorimetric method, as were serum sodium and serum potassium, measured by potentiometry (ISE electrodes for Na+ and K+).

Cortisol was measured using a conventional radio-immunoassay RIA (Immunotech IM1841).

Adrenocorticotropin (ACTH) was determined using an IRMA immunoradiometric assay (Immunotech IM2030, B89463).

BMD was measured by dual-energy X-ray absorptiometry (DXA), Discovery Tm QDR series, Hologic, at the lumbar spine (L1–L4, AP) and femoral head, recorded in terms of absolute mineral content in g/cm^2 and *Z* -score, T-score at both sites [6].

The results were presented as BMD (g/cm^2), T-score and Z-score. In postmenopausal women and men aged over 50 years, the following criteria for BMD loss were used: T score ≥ -1 standard deviation (SD): normal; T-score between -1 and -2.5 SD: osteopenia; T-score ≤ -2.5 SD: osteoporosis. In premenopausal patients or subjects aged less than 50 years, the BMD value was considered a Z score: Z score values of -2.0 SD or less are indicated "below the expected range for the 'age' and those above -2.0 SD “within the expected age range” [7].

Osteoporosis is considered severe when the score is ≤ -2.5 with, in addition, a history of at least one bone fragility fracture [8].

In our study, the patient population was quite young and included mainly premenopausal women. Therefore, the T score for BMD was not considered, but the Z score was used to define low BMD for age.

Statistical analysis was carried out using Microsoft Office Excel software for Windows 10, version 2021.

Our results are expressed as numbers (n), percentages (%), and mean ± SD standard deviation.

BMD values at the two sites were compared using the Student's *t test*.

For correlations, the coefficient function Correlation in Excel was used to examine the relationship between variables.

A P value less than 0.05 (P < 0.05) was considered significant.

4. Results

We describe a cohort of 58 patients which demographic and biological characteristics are summarized in Table 1, the average age is 34 years and with a clear female predominance.

The most frequent comorbidities are represented by arterial hypertension (65.5%), diabetes (56.8%), and dyslipidemia (41.3%).

Table 1. *Demographic and biological characteristics of our cohort.*

	Mean ± SD	Percentage (%)
Sex (Female/male)	-	79.3/20.6
Age (years)	34 ±13.46	
BMI (kg/m$^{2)}$	31.06 ±5.48	
ACTH (pg/ml)	93.7 ±71.05	
8 am serum cortisol (nmol/l)	917.2 ±535.98	
midnight serum cortisol at (nmol/l)	556.8 ±313.5	
24-h UFC (nmol/24H)	1187 ±1294.7	
Serum Calcium (N: 81 -104) (mg/dl)	91.4 ±10.75	
Phosphoremia (N: 40 -70) (mg/dl)	33.7 ±9.71	
ALP (N: <275 IU/L)	123.2 ±84.39	

Cushingoid features were reported by the vast majority of patients; facio-trunk obesity was the most common presentation (86.2%) (Table 2). In our cohort, 17.24% of patients did not present signs of hypercatabolism. Furthermore, on a morphological aspect, 60.3% of patients presented a micro-adenoma (< 1 cm), 25.8% a macropituitary adenoma, and a normal imaging in 13.7% of patients.

Table 2. *Clinical manifestations and frequency of comorbidities in our cohort.*

Clinical signs and comorbidities	Effective n=58	Percentage (%)
Facio-truncular obesity	50	86.2
Large, purple stretch marks	41	70.6
Easy bruising	28	48.2
Proximal muscle wasting	34	58
Stool sign	25	43.1
Bone pain	22	38.59
Psychiatric disorders	61	10.9
Melanoderma	18	62.2
Hirsutism	28	17.24
HTA	38	65.5
Diabetes	33	56.8
Dyslipidemia	24	41.3

5. Bone Impact

Osteoporosis was found in 44.8% of our patients, osteopenia in 37.9%, and only 17.24% of the cohort had a normal BMD (Figure 1). Severe osteoporosis (associated with a bone fracture) is present in three cases and one case of vertebral compression.

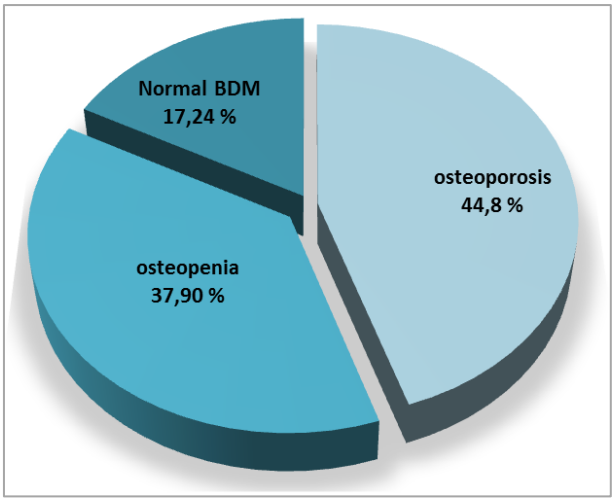

Figure 1. *Distribution of bone damage in our cohort.*

In our study, there was no significant difference between clinical characteristics in osteoporotic and non-osteoporotic patients (Table 3).

Table 3. *Comparison of clinical characteristics of osteoporotic and non-osteoporotic patients.*

Clinical signs	Osteoporotic patients n=26 Workforce (%)	Non-osteoporotic patients n=32 Workforce (%)	P-value
Facio-truncular obesity	22 (84.6)	28 (87.5)	0.25
Large, purple stretch marks	16 (61.5)	25 (78.1)	0.28
Easy bruising	13 (50)	15 (46.8)	0.25
Proximal muscle wasting	18 (69.2)	16 (50)	0.25
Stool sign	11 (42.3)	14 (43.7)	0.25
Bone pain	13 (50)	09 (28.1)	0.31
Melanoderma	11 (42.3)	07 (21.8)	0.37
Hirsutism	06 (23)	22 (68.7)	0.22

Table 4. *BMD results in our cohort.*

	Mean ±SD	95% CI for mean
Lumbar Spine T-score	-1.88 ±1.32	(-2.22 to -1.54)
Lumbar spine Z-score	-1.60 ±1.27	(-1.92 to -1.28)
Femoral neck T-score	-1.25 ±1.16	(-1.55 to -0.95)
Femoral neck Z-score	-1.09 ±1.18	(-1.39 to -0.79)

The Z-score values were significantly lower at the vertebral level with an average of (- 1.60) than at the femoral level with an average of (- 1.09) ($P< 10^{-3}$) (Table 4).

The difference in BMD was not significant between eumenorrheic and hypo-/amenorrheic females with Cushing's disease. (P-value: 0.39).

We found a significant negative correlation with morning plasma cortisol and BMD at the lumbar spine ($r^2 = 0.09$, $P< 10^{-3}$) and at the femoral neck ($r^2 = 0.04$, $P< 10^{-3}$) (Figures 2 and 3).

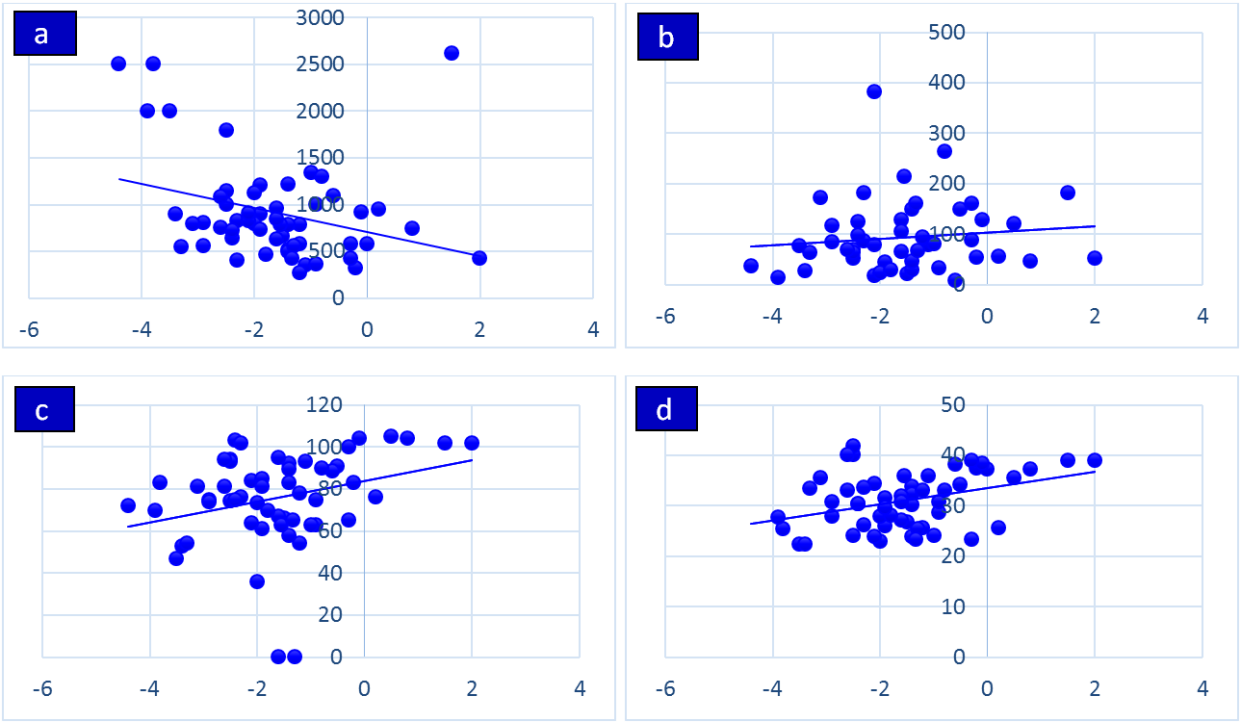

Figure 2. *Correlation of lumbar BMD and morning serum cortisol (a), adrenocorticotropic hormone (ACTH) (b), weight (c), BMI (d).*

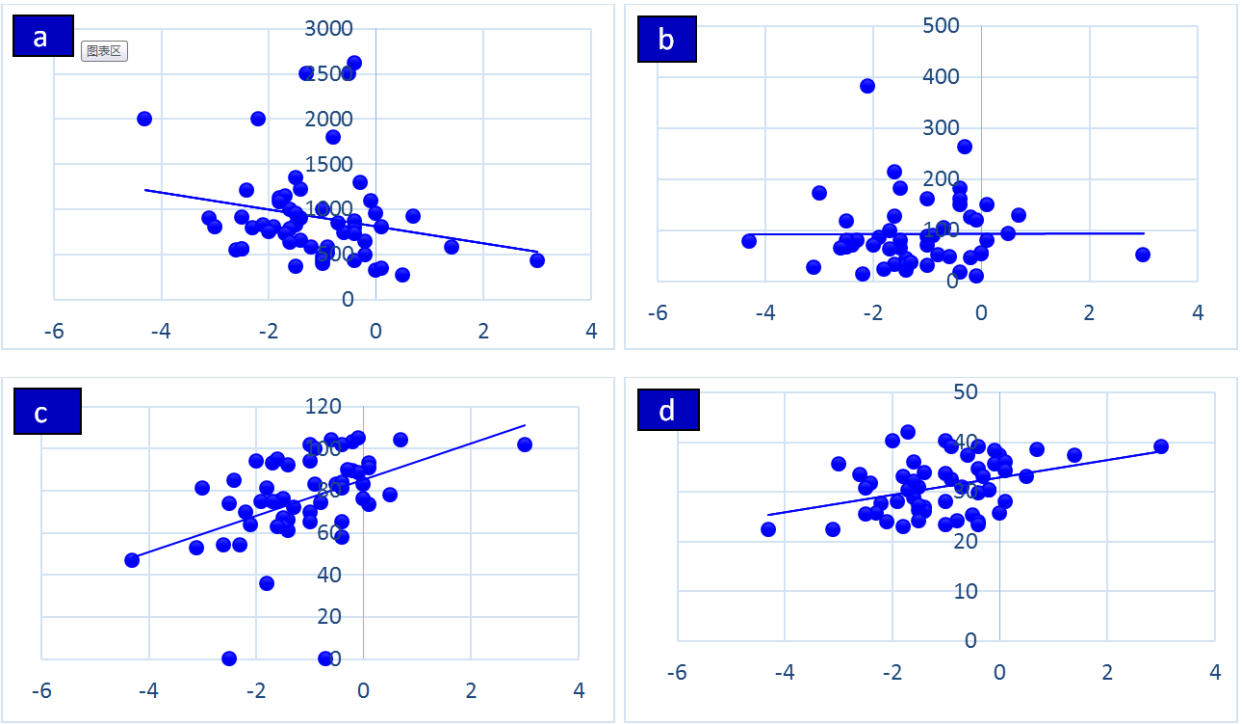

Figure 3. *Correlation of hip BMD and morning serum cortisol (a), adrenocorticotropic hormone (ACTH) (b), weight (c), BMI (d).*

While a significant positive correlation was found between serum ACTH concentration and spinal BMD ($r^2 = 0.01$, $P < 10^{-3}$), as well as femoral neck BMD ($r^2 = 1.3\ 10^{-5}$, $P < 10^{-3}$) (Figures 2 and 3).

BMD at the lumbar spine also had a significant correlation with weight ($r^2 = 0.17$, $P < 10^{-3)}$ and BMI ($r^2 = 0.13$, $P < 10^{-3}$). Similarly, BMD of the femoral neck also had a significant positive correlation with weight and BMI (BMD of the femoral neck; $r^2 = 0.29$, $P < 10^{-3}$ for weight and $r^2 = 0.14$, $P < 10^{-3}$ for BMI) (Figures 2 and 3).

6. Discussion

Osteoporosis is recognized as a serious side effect of endogenous hypercortisolism. Between 50% and 59% of cases of osteoporosis have been reported to be caused by excess endogenous cortisol. The telltale symptom of hypercortisolism can be pathological fractures. Early detection of typical bone mass changes caused by hypercortisolism facilitates early diagnosis of bone mass loss and prompt treatment, thereby reducing the likelihood of adverse events [9].

Our study confirms the fact that patients with Cushing's disease have a very high risk of osteoporosis, with an average of 44.8%. This prevalence was slightly higher than the percentage (26,3-36,8%) [10-12].

We observed significantly lower BMD and Z scores at the vertebrae level (Z-score -1.6) compared to the femur level (Z-score -1.09). Bone loss was more severe in the lumbar spine; this can be explained by the hypercortisolism, which affects the trabecular bone more than the cortical bone. [13-15] Francucci et al suggested that trabecular bone was more rapidly destroyed due to a more intense rate of bone remodeling following a greater surface-to-volume ratio and the greater sensitivity of trabecular bone than cortical bone to cortisol [16].

Our results are comparable to some previous studies, such as the study by Kawamata et al. (spine T-score at -3.53 (0.75) versus -1.5 (0.22) in femoral) [17], from Francucci et al. (spine Z-score at -1.44 (1.5) versus 1.07 (1) in femoral) [16] and a strong trend was identified in Van der Eerden's study (spine Z-score: -1.08 [-1.52; -0.63] versus -0.66 [-0.99;0.33] in femoral) [10].

Rahaman et al. also found more severe bone loss in the lumbar spine (Z score -2.50 ± 1.54) than in the hip (Z score -1.35 ± 0.98) [18].

Another study conducted by Boro et al. found a significantly lower BMD at the lumbar level (Z-score at -2.2 [IQR -2.7 to -0.7]) than at the femoral neck (Z-score at -1.7 [IQR -2.4 to -0.6]) in patients with endogenous Cushing's syndrome [1].

Hypercortisolism may explain osteoporosis by several different mechanisms. O'Brien et al. suggested that glucocorticoids directly or indirectly accelerate the apoptosis of osteoblasts and osteocytes and reduce the apoptosis of osteoclasts, causing bone mass loss [19].

Hypercortisolism leads to a reduction in bone formation, the main characteristic of corticosteroid-induced osteoporosis. Indeed, cortisol inhibits the replication of cells of the osteoblastic lineage, reduces their differentiation and maturation and induces their apoptosis, leading to a reduction in the final number of mature osteoblasts [20]. Concerning the reduction in the reserve of osteoblasts, it is known that the precursors of osteoblasts from the bone marrow and adipose tissue are common and that these are directed towards the adipocyte lineage under the influence of cortisol via induction nuclear factors activating CCAAT (protein transcription factor) and PPARγ2 (peroxisome proliferator-activated receptor γ2, major nuclear receptor for adipogenesis) [21].

Osteocytes, through their dendritic form, constitute a canalicular network transmitting information to the surface of the bone. They play a vital role in repairing bone damage. GCs impair their function by increasing the size of lacunae (cells where osteocyte cell bodies are located) and by decreasing the rate of bone mineralization around the lacuna. This leads to a reduction in the elasticity coefficient of the area surrounding the osteocyte, altering the biomechanical properties of the bone [22]. GCs also induce apoptosis of osteocytes via activation of caspase 3 (the same mechanism as for osteoblasts) [23].

Osteoclasts belong to the monocyte/macrophage family. Their differentiation requires the expression of two cytokines: the macrophage colony stimulating factor (M-CSF) and receptor activator of NF-kappa B ligand (RANK-L). GCs increase the expression of M-CSF and RANK-L, therefore promoting osteoclastogenesis [24]. Additionally, GCs cause overexpression of interleukin 6, an osteoclastogenic cytokine, and lower the expression of interferon β, an inhibitor of osteoclastogenesis. Finally, GCs reduce the apoptosis of mature osteoclasts. This results in increased bone resorption [25].

The lumbar vertebrae contain more spongy bone (trabecular bone) than the femurs; therefore, the lumbar vertebrae are more vulnerable to endogenous glucocorticoid injury. This is one of the possible reasons for the differences between different regions of BMD in patients with hypercortisolism [9].

This preferential attack of cancellous bone by cortisol partly explains the occurrence of fractures while BMD is significantly higher than in post-menopausal osteoporosis [20, 26].

Lumbar spine BMD was found to be the best predictor of vertebral fractures and was the only statistically significant predictor [27].

Furthermore, patients with endogenous CS have an increased risk of fragility fractures, despite normal or slightly reduced bone mineral density (BMD). This could be explained by a decrease in bone strength due to the qualitative deterioration of bone structure [28-30].

Other specific cases described in the literature also illustrate the occurrence of multiple severe fractures in patients

with CS in the absence of densitometric osteoporosis [28, 29]. Indeed, osteoporosis is characterized by altered bone quantity and quality, but BMD very well assesses bone quantity and not bone quality. This highlights the fact that altered micro-architecture, independent of BMD, appears to be associated with a greater risk of fractures [31]. It therefore seems important to be able to directly evaluate this MAO using a reliable tool: the TBS.

In our cohort, we found a significant negative correlation with morning plasma cortisol and BMD at the lumbar spine ($r^2 = 0.09$, $P < 10^{-3}$) and at the femoral neck ($r^2 = 0.04$, $P < 10^{-3}$). These results are consistent with other previous studies. Rahaman et al [18] found a negative correlation with morning serum cortisol and BMD at LS ($r^2 = 0.09$, P = 0.08) and hip ($r^2 = 0.09$, P = 0.09), although this is not statistically significant. Likewise, Boro et al [1] reported that BMD at the L1-L4 level showed significant negative correlations with plasma cortisol at 8 a.m. (Spearman's rho ρ = -.397, p =.011).

In addition to cortisol excess, with regard to other variables related to BMD, this study revealed a significant relationship between BMD values and ACTH in CD patients (r = 0.115, $P < 10^{-3}$) (Figures 2 and 3). This is consistent with a recent study (r = 0.388, p = 0.023) [9], which also demonstrated a significant correlation between lumbar BMD and ACTH concentration in patients with CD.

This link could be explained by the protective effect of ACTH on lumbar BMD in patients with CD. ACTH is reported to stimulate osteoblast proliferation and elevate collagen I mRNA in the osteoblastic cell line SaOs2 in vitro. ACTH binds to 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. However, it is insufficient to counteract the negative effects of increased cortisol levels on bone metabolism in CD patients [9].

We observed a positive correlation between BMI and BMD in patients with Cushing's disease (Figures 2 and 3), which suggests that obesity has a protective effect on bone impact. Boro et al. found that lumbar BMD had a significant correlation with weight (Spearman's rho [ρ] =.649, p <.001) and BMI (ρ =.586, p <.001). Similarly, femoral neck BMD also had significant positive correlations with weight and BMI (femoral neck BMD; ρ =.590, p <.001 for weight and ρ =.503, p <.001 for BMI) [1].

However, a low body mass index (BMI<21 kg/m^2) has been shown to be a significant risk factor for hip fracture [32]. Furthermore, in our study, BMD parameters were not significantly different between male or female patients with Cushing's disease and did not vary significantly between eumenorrheic and hypo-/amenorrheic women. This is in concordance with other studies that showed that neither sex nor gonadal status were significantly related to BMD in Cushing's disease [18, 33].

7. Conclusion

Osteoporosis is common in Cushing's disease due to a late diagnosis of hyperadrenocorticism and bone damage.

Patients have an increased risk of fracture secondary to osteopenia and osteoporosis, which has significant consequences in terms of mortality and morbidity. For this reason, the prevention of fractures due to osteoporosis was declared a "priority cause" by the WHO in 2000 [34]. Our study clearly showed that BMD at the vertebrae in patients with Cushing's disease is more affected than BMD at the femur and that the decrease in BMD is independent of menstrual status.

ACTH may have a protective effect on bones; however, it is not sufficient to act against the harmful effects of high cortisol levels on bone metabolism.

Osteoporosis must therefore be systematically investigated and treated in order to avoid these complications, which can compromise the prognosis.

Abbreviations

BMI: Body Mass Index
ACTH: Adrenocorticotropic Hormone
24-h UFC: 24-h Urinary Free Cortisol
ALP: Alkaline Phosphatase
HTA: Hypertension

Author Contributions

Wissame Debbah: Software, Investigation, Methodology, Writing – original draft

Mouna Mezoued: Conceptualization, Supervision, Project administration, Writing – review & editing

Aicha Bouzid: Resources, Investigation

Randa Talhi: Methodology

Khadidja Bessaid: Investigation

Malha Azzouz: Supervision, Project administration, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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