Original Research
ISSN (Print): 2455-1244
ISSN (Online): 0971-913X
Journal of Endocrinology and Reproduction, *Vol 24(2), 105-119, December 2020, DOI: 10.18311/jer/2020/27844*

An Investigation on Bone Mineral Density in Hyperprolactinemia

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Abstract

Hyperprolactinemia patients have been reported to have low Bone Mineral Density (BMD). This study aimed to compare bone mineral density and associated factors in hyperprolactinemia. A total of 35 hyperprolactinemia patients (>100ng/mL serum prolactin levels) and 10 controls participated in study. Hyperprolactinemia cases were classified into macroprolactinemia and true hyperprolactinemia as determined by Poly-Ethylene Glycol precipitation. Serum levels of Prolactin, Estradiol, Calcium, Phosphate or Alkaline Phosphatase were measured. BMD was measured at lumbar vertebrae, left femur, and left forearm by dual energy x-ray absorptiometry (DXA) scan. The prevalence of osteopenia/osteoporosis was 50% in macroprolactinemia, 70% in true hyperprolactinemia and 60% in controls (statistical differences were insignificant; p = 0.517). Pearson correlation analysis did not find any significant correlation of Prolactin, Estradiol, Calcium, Phosphate or Alkaline Phosphatase with T score or Z score at lumbar spine, femur or forearm (wrist) region in hyperprolactinemia patients and controls. The only significant correlations were found between body mass index (BMI) and prolactin levels (r = 0.473, p = 0.003); and between BMI and total femur T score (r = 0.360, p = 0.015) and Z score (r = 0.362, p = 0.015). Mean BMI was also significantly high (p = 0.029) in hyperprolactinemia patients with normal DXA (28.7±5.3 kg/m^2) compared to those with Osteopenia/Osteoporosis (24.7±4.8 kg/m^2). There was no significant difference in incidence of osteopenia/osteoporosis between macroprolactinemia and true hyperprolactinemia patients. The only significant correlation of BMD was found with BMI suggesting high BMI to be a protective factor against osteoporosis in hyperprolactinemia patients.

Keywords: Body Mass Index, Bone Mineral Density, Dual-Energy X-ray Absorptiometry, Hyperprolactinemia, Macroprolactinemia

1. Introduction

Hyperprolactinemia is the condition where serum prolactin levels rise above the normal range. The condition occurs physiologically during pregnancy and lactation, but can also occur pathologically due to pituitary micro-/macro-adenoma, drugs with dopamine inhibiting components such as antipsychotic drugs or other secondary causes such as chronic kidney diseases, in addition to 16-35% hyperprolactinemia cases which remain idiopathic[1,2]. When large immune-complex molecules of prolactin are the major form of prolactin in sera of hyperprolactinemia patients, the condition is called macroprolactinemia[3]. Pathological hyperprolactinemia may result in galactorrhoea and/or menstrual irregularities in females and erectile dysfunction and/or loss of libido in males and may also cause infertility in both sexes[4].

Osteoporosis is the condition where bone density decreases leading to microarchitectural deterioration of bone tissue making bones more fragile and prone to fracture[5]. According to WHO criteria, osteoporosis is defined as a BMD of 2.5 SD or more below the average value of young healthy women. There is an estimated 30 to 40% lifetime risk of having wrist, hip or vertebral fracture[6]. Hyperprolactinemia is known to increase

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bone turnover and fracture risk, and decrease BMD due to its osteoclast effect on bones[7]. BMD analysis in hyperprolactinemic males showed that 80% patients had low BMD at lumbar spine, while 30% patients had low BMD at femoral neck[8]. Studies have shown that prolactinoma patients also present with osteopenia and osteoporosis[9]. High prevalence of radiological vertebral fractures was found in male patients with prolactin – secreting adenomas as compared to controls[10,11]. Patients on prolactin-raising anti-psychotics have also been found to have significantly increased risk of low BMD compared to healthy controls[12].

The effects of hyperprolactinemia on bone metabolism may be attributed to GnRH dysregulation and subsequent estrogen deficiency or by hyperprolactinemia itself[13]. Dopamine agonist drugs along with transsphenoidal surgery were reported to reverse the symptoms of multiple osteoporotic vertebral fractures in a male patient with long-lasting hypogonadism due to prolactinoma[14]. Other risk factors associated with reduced BMD as per WHO include lower BMI[15] smoking[16] and alcohol intake[17]. Disease duration was also suggested to be associated with vertebral fractures in hyperprolactinemia patients[10]. Serum levels of calcium, phosphate and Alkaline Phosphatase (ALP) are important markers for bone metabolism having major role in bone formation and resorption. Calcium deficiency or malabsorption due to the effect of estradiol on calcium transport or other hormonal imbalance may be responsible for osteopenia or osteoporosis resulting in bone loss[18].

No data is available on BMD analysis in macroprolactinemia patients. We suggest osteoporosis as more likely a manifestation of hyperprolactinemia but not macroprolactinemia as true hyperprolactinemia cases more commonly present the related symptoms. Thus, the present study was aimed to compare bone mineral density and factors associated with it in hyperprolactinemia patients.

2. Materials and Methods

The study was approved by Institutional Ethics Committee. A total of 91 hyperprolactinemia patients (prolactin level >100 ng/mLin two occasions of >1month interval) aged 19-48 years were initially enrolled in the study from Department of Reproductive Biology, AIIMS, New Delhi. Physiological hyperprolactinemia cases and cases with other secondary causes of hyperprolactinemia (chronic kidney disease, liver cirrhosis etc) were excluded from the study. Written informed consent was obtained from each participant before inclusion in the study. Detailed clinical and medical history was taken as per pre-determined proforma. The minimum initial evaluation included complete medical history, physical examination, hormone measurements and CT/MRI for prolactinoma or other pituitary pathology. About 35 hyperprolactinemia patients out of 91 initially enrolled agreed to get BMD analysis done. Remaining 56 patients either denied follow-up due to high cost of test or were restricted by treating doctor for BMD analysis. So, further study was done with 35 hyperprolactinemia patients along with 10 age-matched healthy women having normal prolactin level and regular menstrual cycle recruited as controls. Prolactin assays were done on highly specific Chemiluminescence Microparticle Immunoassay (CMIA) (7K76 G6-5314/R06 B7K760) using ARCHITECT PLUS i2000SR automated immunoassay system (Abbott Laboratories, USA).

About 5 mL peripheral blood was collected from each participant in plain vial and was allowed to coagulate at room temperature for 30 min. Serum samples were obtained by centrifuging blood sample at 5000 rpm for 5 min at room temperature and were stored at -80°C until further analysis. Serum prolactin levels were estimated for all patients and controls. Poly Ethylene Glycol (PEG) precipitation was done with PEG 6000 (Catalogue# SC- 302016) to classify macroprolactinemia patients having post PEG recovery of prolactin <25% from true hyperprolactinemia patients having post PEG recovery of prolactin >25%. Serum levels of estradiol, calcium, phosphate and alkaline phosphatase were also determined for all the 35 hyperprolactinemia patients.

BMD was measured in three major sites: lumbar vertebrae (L1–L4), left femur (femoral neck, trochanteric, intertrochanteric and ward's regions), and left forearm (UD, MID and 1/3 region) by dual energy x-ray absorptiometry (DXA) scan using Hologic Discovery DXA system (Discovery A (S/N) 84023). According to WHO, BMD predicts fracture with an increase in fracture risk of approximately 1.5/SD decrease in bone mineral density (termed the gradient of risk).

Two sets of values were generated by the DXA scanner to measure bone density: T score (between -1.0 and -2.5 define osteopenia and less than -2.5 define osteoporosis) used for the measurement of BMD in postmenopausal women and men ≥50 year age based upon comparison

with healthy adult population; and Z score used to compare BMD in pre-menopausal women and men younger than 50 years with reference values of the same age, sex and ethnicity[19]. Since Z score lacks ethnicity matched reference population as well as standardization and calculation techniques[20], both T scores and Z scores were reported in this paper for BMD measurements.

2.1 Statistical Analysis

Descriptive statistics were expressed as n (%), mean ± SD and median [interquartile range]. Continuous variables were compared by Wilcoxon rank-sum (Mann-Whitney) test and KruskelWallis test with Bonferroni correction. Categorical variables were compared using Chi-square test. Correlations between measures of bone mineral density and factors influencing bone mineral density were tested by Pearson's correlation test. Statistics was performed using StataCorp. 2015. Stata Statistical Software: Release 14.2. College Station, TX: StataCorp LP and IBM Statistical Package for the Social Sciences (SPSS) software, version 21.

3. Results

The demographic and clinical profile of 35 hyperprolactinemia patients and 10 controls is presented in Table 1. There was no significant difference (p = 0.953) in mean BMI of hyperprolactinemia patients (26.2 ± 5.3 kg/m^2) and controls (26.3 ± 3.7 kg/m^2). Out of the 35 hyperprolactinemia cases, there were 33 (94.3%) females and 2 (5.7%) males, both the males being alcoholic and one of them was also a smoker. Overall DXA report was considered "Normal" when all the three sites had normal DXA, "Osteopenia" when one or more of the three sites studied had osteopenia but no site had osteoporosis, and "Osteoporosis" when one or more of the three sites studied had osteoporosis. BMD analysis results in hyperprolactinemia patients showed that 22 out of the 35 cases (62.9%) had osteopenia or osteoporosis at one or more sites studied, while 6 out of the 10 controls (60%) had osteopenia at one or more sites, and the differences were not significant (p = 0.531). In hyperprolactinemia patients, 18 cases (51.4%) had osteopenia and 4 cases (11.4%) had osteoporosis. No control had osteoporosis at any of the three sites studied. Comparison of the number of subjects with osteopenia or osteoporosis at each site separately, as well as comparison of median T score/ Z score between patients and controls per site did not show any significant difference between hyperprolactinemia patients and controls at any site (Table 1).

The 35 hyperprolactinemia cases classified based upon the cause of high prolactin had 9 (25.7%) pituitary adenoma, 15 (42.9%) drugs induced and 11 (31.4%) idiopathic hyperprolactinemia cases. Low

Table 1. Demographic, clinical profile and bone mineral density analysis in hyperprolactinemia patients and controls

	Hyper-prolactinemia Patients (N = 35)	**Controls** (N = 10)	***p Value***
BMI(kg/m^2) mean±SD	26.2 ±5.3	26.3 ±3.7	0.953
Gender Females, n (%) Males, n (%)	 33 (94.3) 2 (5.7)	 10 (100) 0 (0)	0.439
Alcoholic Yes, n (%) No, n (%)	 2 (5.7) 33 (94.3)	 0 (0) 10 (100)	0.439
Smoker Yes, n (%) No, n (%)	 1 (2.9) 34 (97.1)	 0 (0) 10 (100)	0.589
DXA[a] Normal, n (%) Osteopenia, n (%) Osteoporosis,n (%)	 13 (37.1) 18 (51.4) 4 (11.4)	 4 (40) 6 (60) 0	0.531

Table 1. Cont

	Hyper-prolactinemia Patients (N = 35)	Controls (N = 10)	*p Value*
Lumber 1st			
T score, median[IQR]	-0.8 [-1.7,-0.3]	-0.6 [-1.0,-0.2]	0.353
Normal, n (%)	21 (60.0)	8 (80)	
Osteopenia, n (%)	11 (31.4)	2 (20)	0.429
Osteoporosis, n (%)	3 (8.6)	0 (0)	
Z score, median[IQR]	-0.7 [-1.6,-0.2]	-0.4 [-0.8,-0.1]	
Normal, n (%)	21 (60.0)	8 (80)	0.274
Osteopenia, n (%)	11 (31.4)	2 (20)	
Osteoporosis, n (%)	3 (8.6)	0 (0)	0.429
Lumber 2nd			
T score, median [IQR]	-0.9 [-1.8, -0.2]	-0.3 [-0.6, 0.1]	0.140
Normal, n (%)	19 (54.3)	8 (80)	
Osteopenia, n (%)	14 (40.0)	2 (20)	0.316
Osteoporosis, n (%)	2 (5.7)	0	
Z score, median [IQR]	-0.8 [-1.8, -0.1]	-0.2 [-0.6, 0.3]	0.140
Normal, n (%)	20 (57.1)	8 (80)	
Osteopenia, n (%)	14 (40.0)	2 (20)	0.404
Osteoporosis, n (%)	1 (2.9)	0	
Lumber 3rd			
T score, median [IQR]	-1.1 [-1.7, -0.4]	-0.9 [-1.3, -0.7]	0.712
Normal, n (%)	19 (54.3)	6 (60)	
Osteopenia, n (%)	14 (40.0)	4 (40)	0.734
Osteoporosis, n (%)	2 (5.7)	0	
Z score, median [IQR]	-1 [-1.6, -0.2]	-0.85 [-1.2, -0.7]	0.702
Normal, n (%)	19 (54.3)	7 (70)	
Osteopenia, n (%)	14 (40.0)	3 (30)	0.574
Osteoporosis, n (%)	2 (5.7)	0	
Lumber 4th			
T score, median [IQR]	-1.1 [-1.8, -0.1]	-0.8 [-1.3, -0.6]	0.436
Normal, n (%)	18 (51.4)	7 (70)	
Osteopenia, n (%)	13 (37.1)	3 (30)	0.420
Osteoporosis, n (%)	4 (11.4)	0	
Z score, median [IQR]	-1.1 [-1.8, -0.1]	-0.8 [-1.2, -0.4]	0.412
Normal, n (%)	21 (55.3)	7 (70)	
Osteopenia, n (%)	12 (31.6)	3 (30)	0.446
Osteoporosis, n (%)	5 (13.2)	0	
Lumbar spine total			
T score, median [IQR]	-1 [-1.7, -0.3]	-0.7 [-0.9, -0.6]	0.338
Normal, n (%)	19 (54.3)	8 (80)	
Osteopenia, n (%)	13 (37.1)	2 (20)	0.301
Osteoporosis, n (%)	3 (8.6)	0	
Z score, median [IQR]	-1.0 [-1.7, -0.2]	-0.6 [-0.8, -0.5]	0.250
Normal, n (%)	21 (60.0)	8 (80)	
Osteopenia, n (%)	12 (34.3)	2 (20)	0.457
Osteoporosis, n (%)	2 (5.7)	0	

Table 1. Cont

	Hyper-prolactinemia Patients (N = 35)	Controls (N = 10)	*p Value*
Femur neck			
T score, median [IQR]	-0.7 [-1.6, 0.0]	-1.2 [-1.5, -0.6]	0.447
Normal, n (%)	23 (65.7)	5 (50)	
Osteopenia, n (%)	10 (28.6)	5 (50)	0.377
Osteoporosis, n (%)	2 (5.7)	0	
Z score, median [IQR]	-0.6 [-1.4, 0.0]	-1.2 [-1.5, -0.6]	0.293
Normal, n (%)	23 (65.7)	5 (50)	
Osteopenia, n (%)	10 (28.6)	5 (50)	0.377
Osteoporosis, n (%)	2 (5.7)	0	
Left Femur Trochanter			
T score, median [IQR]	-0.5 [-0.9, 0.0]	-0.7 [-1.0, -0.2]	0.436
Normal, n (%)	27 (77.1)	8 (80)	
Osteopenia, n (%)	8 (22.9)	2 (20)	0.848
Osteoporosis, n (%)	0	0	
Z score, median [IQR]	-0.4 [-0.9, -0.1]	-0.7 [-1.0, -0.2]	0.420
Normal, n (%)	29 (82.9)	8 (80)	
Osteopenia, n (%)	6 (17.1)	2 (20)	0.835
Osteoporosis, n (%)	0	0	
Left Femur Inter			
T score, median [IQR]	-0.2 [-0.6, 0.3]	-0.4 [-0.6, -0.3]	0.373
Normal, n (%)	31 (88.6)	8 (80)	
Osteopenia, n (%)	4 (11.4)	2 (20)	0.482
Osteoporosis, n (%)	0	0	
Z score, median [IQR]	-0.1 [-0.6, 0.4]	-0.4 [-0.5, -0.2]	0.452
Normal, n (%)	32 (91.4)	8 (80)	
Osteopenia, n (%)	3 (8.6)	2 (20)	0.310
Osteoporosis, n (%)	0	0	
Left Femur total			
T score, median [IQR]	-0.3 [-0.9, 0.2]	-0.7 [-1.0, -0.4]	0.360
Normal, n (%)	30 (85.7)	8 (80)	
Osteopenia, n (%)	5 (14.3)	2 (20)	0.942
Osteoporosis, n (%)	0	0	
Z score, median [IQR]	-0.3 [-0.8, 0.2]	-0.7 [-1.0, -0.4]	0.234
Normal, n (%)	31 (88.6)	8 (80)	
Osteopenia, n (%)	4 (11.4)	2 (20)	0.482
Osteoporosis, n (%)	0	0	
Left Femur ward's			
T score, median [IQR]	-0.6 [-1.2, 0.3]	-1.0 [-1.2, 0.2]	0.913
Normal, n (%)	25 (71.4)	7 (70)	
Osteopenia, n (%)	9 (25.7)	3 (30)	0.843
Osteoporosis, n (%)	1 (2.9)	0	
Z score, median [IQR]	-0.4 [-0.8, 0.4]	-0.9 [-1.2, 0.2]	0.444
Normal, n (%)	29 (82.9)	7 (70)	
Osteopenia, n (%)	5 (14.3)	3 (30)	0.466
Osteoporosis, n (%)	1 (2.9)	0	

Table 1. Cont

	Hyper-prolactinemia Patients (N = 35)	Controls (N = 10)	*p Value*
Left Forearm UD			
T score, median [IQR]	-0.2 [-1.1, 0.2]	-0.3 [-0.9, 0.2]	0.913
Normal, n (%)	27 (77.1)	9 (90)	
Osteopenia, n (%)	8 (22.9)	1 (10)	0.370
Osteoporosis, n (%)	0	0	
Z score, median [IQR]	-0.1 [-1.1, 0.3]	-0.3 [-0.9, 0.2]	0.891
Normal, n (%)	27 (77.1)	9 (90)	
Osteopenia, n (%)	8 (22.9)	1 (10)	0.370
Osteoporosis, n (%)	0	0	
Left Forearm MID			
T score, median [IQR]	-0.6 [-1.2, -0.1]	-0.3 [-0.7, 0.5]	0.136
Normal, n (%)	24 (68.6)	9 (90)	
Osteopenia, n (%)	11 (31.4)	1 (10)	0.177
Osteoporosis, n (%)	0	0	
Z score, median [IQR]	-0.5 [-1.1, -0.1]	-0.3 [-0.6, 0.5]	0.274
Normal, n (%)	27 (77.1)	9 (90)	
Osteopenia, n (%)	8 (22.9)	1 (10)	0.370
Osteoporosis, n (%)	0	0	
Left Forearm 1/3			
T score, median [IQR]	-0.5 [-1.2, 0.0]	-0.4 [-0.9, 0.0]	0.538
Normal, n (%)	26 (74.3)	9 (90)	
Osteopenia, n (%)	9 (25.7)	1 (10)	0.292
Osteoporosis, n (%)	0	0	
Z score, median [IQR]	-0.3 [-1.1, 0.2]	-0.4 [-0.9, 0.1]	0.870
Normal, n (%)	27 (77.1)	9 (90)	
Osteopenia, n (%)	8 (22.9)	1 (10)	0.370
Osteoporosis, n (%)	0	0	
L. Forearm total			
T score, median [IQR]	-0.7 [-1.4, 0.1]	-0.4 [-0.6, 0.5]	0.286
Normal, n (%)	25 (71.4)	8 (80)	
Osteopenia, n (%)	10 (28.6)	2 (20)	0.589
Osteoporosis, n (%)	0	0	
Z score, median [IQR]	-0.5 [-1.1, 0.3]	-0.4 [-0.5, 0.5]	0.632
Normal, n (%)	27 (77.8)	9 (90)	
Osteopenia, n (%)	8 (22.9)	1 (10)	0.370
Osteoporosis, n (%)	0	0	

[a] Overall DXA report considered "Normal" when all three sites had normal DXA report;"Osteopenia" when any of the three sites studied had osteopenia but no site had osteoporosis; and "Osteoporosis" when any of the three sites studied had osteoporosis.

BMD was observed in 7 (77.8%) pituitary adenoma cases, 11 (73.3%) drug induced cases and 4 (36.4%) idiopathic hyperprolactinemia cases (Table 2). There was no significant difference in number of subjects with normal DXA, osteopenia or osteoporosis among hyperprolactinemia patients with pituitary adenoma, drug-induced cause, idiopathic cause and controls (p = 0.250). Median T score and Z score at each site studied in pituitary adenoma, drug induced and idiopathic hyperprolactinemia cases as well as controls are shown in

Table 2. There was no significant difference in T score or Z score at any of the sites studied among patients with pituitary adenoma, drug-induced hyperprolactinemia, idiopathic hyperprolactinemia and controls except for the marginally significant (p = 0.044) difference in femur neck Z score (Table 2).

The post PEG precipitation estimation of prolactin revealed that out of the 35 hyperprolactinemia cases, 8 (22.9%) were macroprolactinemia, while 27 (77.1%) were true hyperprolactinemia cases. There were 4 macroprolactinemia cases (50%) and 18 (66.7%) true hyperprolactinemia cases with osteopenia or osteoporosis at one or more sites studied compared to 6 (60%) controls with osteopenia at one or more sites (Table 3). Thus, prevalence of osteopenia/ osteoporosis was 50% in macroprolactinemia cases, 70% in true hyperprolactinemia cases and 60% in controls, but the differences were not significant (p = 0.517). Comparison of median T score and Z score for each site between macroprolactinemia, true hyperprolactinemia and controls are shown in Table 3. Neither T score nor Z score varied significantly at any of the sites studied between macroprolactinemia, true hyperprolactinemia and controls.

Comparison between hyperprolactinemia patients with normal DXA (T score and Z score > -1.0 at all sites) and those with Osteopenia/Osteoporosis (T score or Z score < -1.0 at one or more sites) is presented in Table 4. Serum prolactin levels in hyperprolactinemia patients with Normal DXA and those with Osteopenia/ Osteoporosis did not show any significant difference (178.1 [151.6, 224.6] and 160.9 [122.93, 340.4] ng/mL respectively; p = 0.562). Estradiol levels in cases with Normal DXA was 44 [28, 64] pg/mL, and in cases with Osteopenia/Osteoporosis was 36.5 [25,67] pg/mL; the differences were not significant (p = 0.838). This suggests

Table 2. Comparison of BMD between different groups of hyperprolactinemia classified based on cause of hyperprolactinemia

Site of DXA scan	Pituitary adenoma (N = 9)	Drug induced (N = 15)	Idiopathic (N = 11)	Controls (N = 10)	*P value*
Overall DXA Report[a]					
Normal, n (%)	2 (22.2)	4 (26.7)	7 (63.6)	4 (40)	0.25
Osteopenia, n (%)	5 (55.6)	9 (60.0)	4 (36.4)	6 (60)	
Osteoporosis, n (%)	2 (22.2)	2 (13.3)	0 (0.0)	0	
L1					
T score	-1.5 [-2, -0.2]	-1.0 [-1.9, -0.3]	-0.8 [-1.1, 0.6]	-0.6 [-1.0, -0.2]	0.616
Z score	-1.4 [-1.8, -0.2]	-1.0 [-1.7, -0.3]	-0.7 [-1.1, -0.6]	-0.4 [-0.8, -0.1]	0.518
L2					
T score	-1.5 [-2.0, 0]	-1.3 [-2.0, 0]	-0.4 [-1.2, 0.8]	-0.3 [-0.6, 0.1]	0.139
Z score	-1.4 [-2.0, 0]	-1.2 [-1.8, -0.5]	-0.3 [-1.1, 0.8]	-0.2 [-0.6, 0.3]	0.139
L3					
T score	-1.4 [-1.8, 0.4]	-1.4 [-2.1, -0.5]	-0.9 [-1.3, 0.5]	-0.9 [-1.3, -0.7]	0.532
Z score	-1.2 [-1.8, 0.5]	-1.4 [-2.0, -0.5]	-0.8 [-1.2, 0.6]	-0.85 [-1.2, -0.7]	0.472
L4					
T score	-1.7 [-2.3, -0.1]	-1.2 [-2.3, -0.6]	-1.0 [-1.2, 0.9]	-0.8 [-1.3, -0.6]	0.358
Z score	-1.5 [-2.2, -0.1]	-1.1 [-2.1, -0.6]	-0.8 [-1.1, 0.8]	-0.8 [-1.2, -0.4]	0.340
Lumbar spine total					
T score	-1.7 [-1.9, 0]	-1.2 [-1.9, -0.6]	-0.8 [-1.2, 0.7]	-0.7 [-0.9, -0.6]	0.410
Z score	-1.5 [-1.9, 0]	-1.1 [-1.9, -0.6]	-0.8 [-1.0, 0.7]	-0.6 [-0.8, -0.5]	0.361
Femur neck					
T score	0 [-0.9, 0.4]	-1.3 [-1.9, -0.5]	-0.6 [-1.1, -0.2]	-1.2 [-1.5, -0.6]	0.093
Z score	0 [-00.6, 0.5]	-1.2 [-1.8, -0.4]	-0.5 [-1.1, -0.1]	-1.1 [-1.5, -0.6]	0.044*
L. Femur Trochanter					
T score	-0.2 [-1.2, -0.1]	-0.8 [-1.3, -0.2]	-0.1 [-0.5, 0.3]	-0.6 [-1.0, -0.2]	0.105
Z score	-0.2 [-1.0, -0.1]	-0.8 [-1.3, -0.2]	-0.1 [-0.4, 0.3]	-0.6 [-1.0, -0.2]	0.098

Table 2. Cont

Site of DXA scan	Pituitary adenoma (N = 9)	Drug induced (N = 15)	Idiopathic (N = 11)	Controls (N = 10)	*P value*
L. Femur Inter					
T score	0.1 [-00.7, 0.5]	-0.5 [-1.2, 0.1]	0 [-0.5, 0.7]	-0.4 [-0.6, -0.3]	0.186
Z score	0.2 [-0.7, 0.6]	-0.4 [-1.1, 0.2]	0.1 [-0.4, 0.7]	-0.3 [-0.5, -0.2]	0.206
L. Femur total					
T score	-0.3 [-0.6, 0.2]	-0.7 [-1.3, -0.2]	-0.2 [-0.7, 0.6]	-0.6 [-1.0, -0.4]	0.119
Z score	-0.3 [-0.5, 0.2]	-0.7 [-1.3, -0.1]	-0.1 [-0.6, 0.7]	-0.6 [-1.0, -0.4]	0.087
L. Femur ward's					
T score	0.3 [-1.2, 1.0]	-0.8 [-1.5, -0.3]	-0.1 [-0.9, 0.4]	-0.9 [-1.2, 0.2]	0.290
Z score	0.3 [-0.7, 1.1]	-0.6 [-1.3, -0.3]	-0.1 [-0.5, 0.8]	-0.8 [-1.2, 0.2]	0.146
L. Forearm UD					
T score	-0.2 [-0.5, 0.2]	-0.5 [-1.2, 0.1]	0.2 [-1.1, 0.8]	-0.2 [-0.9, 0.2]	0.746
Z score	-0.1 [-0.4, 0.2]	-0.4 [-1.2, 00.2]	0.3 [-1.1, 1.0]	-0.2 [-0.9, 0.2]	0.769
L. Forearm MID					
T score	-1.0 [-1.2, 0.3]	-0.6 [-1.4, -0.1]	-0.5 [-1.4, -0.1]	-0.2 [-0.7, 0.5]	0.509
Z score	-0.8 [-0.9, 0.5]	-0.5 [-1.2, -0.1	-0.3 [-1.1, -0.1]	-0.2 [-0.6, 0.5]	0.712
L. Forearm 1/3					
T score	-0.2 [-1.5, -0.2]	-1 [-1.4, 0.2]	-0.6 [-0.8, -0.4]	-0.4 [-0.9, 0]	0.927
Z score	-0.1 [-1.4, 0.2]	-0.8 [-1.4, 0.2]	-0.5 [-0.7, -0.2]	-0.3 [-0.9, 0.1]	0.992
L. Forearm total					
T score	-0.7 [-1.2, 0.1]	-0.6 [-1.5, 0.4]	-0.7 [-1.0, 0.2]	-0.4 [-0.6, 0.5]	0.735
Z score	-0.2 [-1.1, 0.2]	-0.5 [-1.4, 0.6]	-0.7 [-1.0, 0.3]	-0.4 [-0.5, 0.5]	0.955

Table 3. Comparison of BMD between macroprolactinemia and true hyperprolactinemia cases

Site of DXA scan	Macro-prolactinemia (N = 8)	Hyper-prolactinemia (N = 27)	Controls (N = 10)	*p value*
Overall DXA Report[a]				
Normal, n (%)	4 (50)	9 (33.3)	4 (40)	0.517
Osteopenia, n (%)	4 (50)	14 (51.9)	6 (60)	
Osteoporosis, n (%)	0 (0.0)	4 (14.8)	0	
L1				
T score	-0.6 [-1.1, 1.4]	-1.0 [-2.0, -0.3]	-0.6 [-1.0, -0.2]	0.162
Z score	-0.6 [-1.0, 1.4]	-1.0 [-1.8, -0.2]	-0.4 [-0.8, -0.1]	0.142
L2				
T score	-0.4 [-1.2, 0.9]	-1.2 [-2.0, -0.3]	-0.3 [-0.6, 0.1]	0.097
Z score	-0.3 [-1.2, 1.0]	-1.1 [-1.8, -0.2]	-0.2 [-0.6, 0.3]	0.110
L3				
T score	-1.0 [-1.7, 0.6]	-1.1 [-1.7, -0.4]	-0.9 [-1.3, -0.7]	0.750
Z score	-0.8 [-1.6, 0.6]	-1.1 [-1.6, -0.3]	-0.85 [-1.2,-0.7]	0.737
L4				
T score	-1.0 [-1.5, 0.6]	-1.2 [-2.3, -0.1]	-0.8 [-1.3, -0.6]	0.388
Z score	-0.9 [-1.5, 0.7]	-1.1 [-2.1, -0.1]	-0.8 [-1.2, -0.4]	0.404
Lumbar spine total				
T score	-0.9 [-1.3, 0.8]	-1.0 [-1.9, -0.6]	-0.7 [-0.9, -0.6]	0.326
Z score	-0.8 [-1.3, 0.9]	-1.0 [-1.9, -0.5]	-0.6 [-0.8, -0.5]	0.284

Table 3. Cont

Site of DXA scan	Macro-prolactinemia (N = 8)	Hyper-prolactinemia (N = 27)	Controls (N = 10)	*p value*
Femur neck				
T score	0 [-0.7, 0.6]	-0.9 [-1.7, -0.4]	-1.2 [-1.5, -0.6]	0.085
Z score	-0.9 [-1.5, -0.4]	-0.6 [-1.4, -0.4]	-1.1 [-1.5, -0.6]	0.120
L. Femur Troch				
T score	-0.2 [-0.7, 0.5]	-0.5 [-1.2, -0.1]	-0.6 [-1.0, -0.2]	0.408
Z score	-0.2 [-00.7, 0.5]	-0.4 [-1.0, -0.1]	-0.6 [-1.0, -0.2]	0.422
L. Femur Inter				
T score	0.4 [-0.4, 1.0]	-0.3 [-0.7, 0.1]	-0.4 [-0.6, -0.3]	0.182
Z score	0.5 [-0.4, 1.0]	-0.2 [-0.7, 0.2]	-0.3 [-0.5, -0.2]	0.224
L. Femur total				
T score	0.4 [-0.5, 0.7]	-0.5 [-0.9, -0.2]	-0.6 [-1.0, -0.4]	0.181
Z score	0.4 [-0.5, 0.8]	-0.4 [-0.9, -0.1]	-0.6 [-1.0, -0.4]	0.148
L. Femur ward's				
T score	0.4 [-0.8, 1.1]	-0.7 [-1.2, 0.1]	-0.9 [-1.2, 0.2]	0.291
Z score	0.5 [-0.6, 1.4]	-0.5 [-0.8, 0.2]	-0.8 [-1.2, 0.2]	0.281
L. Forearm UD				
T score	0.2 [0, 1.0]	-0.5 [-1.4, 0.1]	-0.2 [-0.9, 0.2]	0.076
Z score	0.2 [0, 1.1]	-0.4 [-1.4, 0.3]	-0.2 [-0.9, 0.2]	0.117
L. Forearm MID				
T score	-0.2 [-1.0, 0.3]	-1.0 [-1.2, -0.2]	-0.2 [-0.7, 0.5]	0.203
Z score	-0.2 [-1.0, 0.5]	-0.7 [-1.1, -0.1]	-0.2 [-0.6, 0.5]	0.383
L. Forearm 1/3				
T score	-0.5 [-1.5, 0.1]	-0.5 [-1.1, -0.1]	-0.4 [-0.9, 0]	0.825
Z score	-0.3 [-1.4, 0.2]	-0.3 [-0.9, 0.2]	-0.3 [-0.9, 0.1]	0.956
L. Forearm total				
T score	-0.1 [-0.9, 0.4]	-0.8 [-1.4, 0]	-0.4 [-0.6, 0.5]	0.293
Z score	0 [-0.8, 0.5]	-0.5 [-1.2, 0.2]	-0.4 [-0.5, 0.5]	0.672

that neither prolactin, nor estradiol had a role in BMD in hyperprolactinemia patients. However, mean BMI in hyperprolactinemia patients with normal DXA (28.7 ± 5.3) kg/m^2 was significantly high (p = 0.029) compared to mean BMI in hyperprolactinemia patients with Osteopenia/Osteoporosis (24.7 ± 4.8) kg/m^2. Hyperprolactinemia patients with normal DXA surprisingly had significantly longer duration of hyperprolactinemia (median 72 [14, 84] months, range 6-120 months), compared to those with Osteopenia/Osteoporosis (median 18 [6.5, 54] months, range 6-120 months). However, case-wise observation of duration of hyperprolactinemia and DXA results found no particular trend in duration of hyperprolactinemia and DXA results (Supplementary Table S1). Also, duration of hyperprolactinemia could not be determined in three cases with Osteopenia/Osteoporosis as they were lost to follow up. Other factors suggested to be associated with BMD such as age, gender, smoking, alcohol intake did not vary significantly between the two groups (p = 0.764 for age; p = 0.263 for gender; p = 0.435 for number of smokers; p = 0.263 for number of alcoholics). Hyperprolactinemia patients with normal DXA had mean serum levels of Calcium 9.0 ± 0.4 mg%, Phosphate 3.6 ± 0.9 mg%, and ALP 197.1 ± 87.1 IU, while those with Osteopenia/Osteoporosis had serum levels of Calcium 9.3 ± 0.9 mg%, Phosphate 4.2 ± 2.1 mg% and ALP 179 ± 50.8 IU. No significant difference in any of these parameters was found between the two groups (p = 0.246 for calcium, p = 0.298 for Phosphate and p = 0.442 for ALP).

Correlation analysis between serum levels of prolactin, estradiol, calcium, phosphate, ALP, BMI and T score and Z score at lumbar, left femur and left forearm region done by Pearson correlation test are presented in Table 5. The only significant correlations were found between BMI

Table 4. Comparison between hyperprolactinemia patients with Normal DXA (T score and Z score > -1.0 at all three sites) and Osteopenia/Osteoporosis (T score or Z score < -1.0 at one or more sites)

	Normal DXA N=13 (37.1%)	**Osteopenia/ Osteoporosis** N=22 (62.9%)	***p Value***
Cause of hyperprolactinemia Pituitary Adenoma, n (%) Drug induced, n (%) Idiopathic, n (%)	 2 (15.4) 4 (30.8) 7 (53.8)	 7 (31.8) 11 (50.0) 4 (18.2)	0.088
Post PEG prolactin recovery Macroprolactinemia, n (%) True Hyperprolactinemia, n (%)	 4 (30.8) 9 (69.2)	 4 (18.2) 18 (81.8)	0.289
Prolactin (ng/ml),median [IQR]	178.1 [151.6, 224.6]	160.9 [122.93, 340.4]	0.562
Estradiol (pg/ml), median [IQR]	44 [28, 64]	36.5 [25, 67]	0.838
BMI (kg/m2), mean ± SD	28.7 ± 5.3	24.7 ± 4.8	0.029*
Duration of Hyper-prolactinemia (months), median [IQR]	72 [24, 84]	18 [6.5, 54][a]	0.029*
Age (years), mean ± SD	29.5 ± 4.7	30.1 ± 6.1	0.764
Gender Male, n (%) Female, n (%)	 0 (0) 13 (100)	 2 (9.1) 20 (90.9)	0.263
Smoker Yes, n (%) No, n (%)	 0 (0) 13 (100)	 1 (4.6) 21 (95.4)	0.435
Alcoholic Yes, n (%) No, n (%)	 0 (0) 13 (100)	 2 (9.1) 20 (90.9)	0.263
Calcium (mg%), mean ± SD	9.0 ± 0.4	9.3 ± 0.9	0.246
Phosphate (mg%), mean ± SD	3.6 ± 0.9	4.2 ± 2.1	0.298
Alkaline phosphate (IU), mean ± SD	197.1 ± 87.1	179 ± 50.8	0.442

[a] Duration of hyperprolactinemia could not be determined in three cases with Osteopenia/Osteoporosis as they were lost to follow up.

and prolactin levels (r = 0.473, p = 0.003); and between BMI and total femur T score (r = 0.360, p = 0.015) and Z score (r = 0.362, p = 0.015). Pearson correlation analysis did not find any significant correlation of Prolactin, Estradiol, Calcium, Phosphate or ALP with T score or Z score at lumbar spine, femur or forearm region in hyperprolactinemia patients.

4. Discussion

Many studies have associated bone loss with hyperprolactinemia[7-12]. The present study is unique as it compares bone mineral density (T score as well as Z score) in macroprolactinemia and true hyperprolactinemia patients. We found that the percentage of cases with osteopenia/osteoporosis in macroprolactinemia (50%) were low compared to true hyperprolactinemia (66.7%) and controls (60%), but the differences were not significant (p = 0.517). This suggests that BMD may not vary significantly in hyperprolactinemia or macroprolactinemia patients compared to controls.

Unlike the studies reporting significant association of osteoporosis with hyperprolactinemia and prolactinoma[7-12] the present study did not find any significant difference in BMD (T score or Z score) in hyperprolactinemia patients compared to controls at lumbar spine, left femur or left forearm except for the significant difference in femur neck Z score (p = 0.044) between pituitary adenoma, drug induced hyperprolactinemia, idiopathic hyperprolactinemia and controls. Neither prolactin nor estradiol had any significant association with BMD in hyperprolactinemia

Table 5. Correlation analysis of serum levels of prolactin, estradiol, Calcium, Phosphate, alkaline phosphatase, BMI and BMD at lumbar spine, hip and forearm (with respect to T score as well as Z score)

	Prolactin	BMI	Estradiol	Calcium	Phosphate	AP
BMI						
N	45					
Pearson Correlation	0.473*					
Significance (2-tailed)	0.003					
Estradiol						
N	35	35				
Pearson Correlation	0.113	-0.052				
Significance (2-tailed)	0.517	0.767				
Calcium						
N	35	35	35			
Pearson Correlation	0.248	-0.100	0.088			
Significance (2-tailed)	0.151	0.568	0.617			
Phosphate						
N	35	35	35	35		
Pearson Correlation	-0.033	-0.139	-0.132	0.099		
Significance (2-tailed)	0.852	0.425	0.450	0.573		
Alkaline Phosphatase						
N						
Pearson Correlation	35	35	35	35	35	
Significance (2-tailed)	-0.220	0.127	-0.208	-0.069	0.075	
	0.205	0.469	0.232	0.692	0.669	
Lumbar T score						
N	45	45	35	35	35	35
Pearson Correlation	-0.188	0.177	0.049	-0.151	-0.093	-0.190
Significance (2-tailed)	0.217	0.246	0.780	0.388	0.597	0.275
Lumbar Z score						
N	45	45	35	35	35	35
Pearson Correlation	-0.203	0.168	0.048	-0.163	-0.068	-0.186
Significance (2-tailed)	0.182	0.269	0.784	0.350	0.696	0.283
Hip T score						
N	45	45	35	35	35	35
Pearson Correlation	-0.070	0.360*	-0.008	0.092	-0.127	-0.053
Significance (2-tailed)	0.648	0.015	0.964	0.597	0.467	0.762
Hip Z score						
N	45	45	35	35	35	35
Pearson Correlation	-0.068	0.362*	0.012	0.085	-0.146	-0.064
Significance (2-tailed)	0.656	0.015	0.945	0.625	0.403	0.715
Forearm T score						
N	45	45	35	35	35	35
Pearson Correlation	-0.040	0.104	-0.064	0.095	-0.225	-0.122
Significance (2-tailed)	0.792	0.496	0.715	00.586	0.193	0.486
Forearm Z score						
N	45	45	35	35	35	35
Pearson Correlation	-0.044	0.115	0.044	0.086	-0.220	-0.166
Significance (2-tailed)	0.773	0.450	0.802	0.623	0.203	0.340

* Correlation is significant at the 0.05 level (2-tailed)

patients in this study. These findings were consistent with earlier studies which also did not find any correlation between BMD and serum prolactin levels[10,11]. Clement-Lacroix *et al.*, suggested that prolactin might have a role in normal bone development, but not in bone loss as prolactin-receptors are expressed by osteoblasts, and not osteoclasts[21]. However, there are others reporting an effect of prolactin on both osteoblast and osteoclast cells suggesting a direct effect of hyperprolactinemia on bone resorption which occur as a result of increased RNKL and reduced OPG expression by osteoblast cells[22].

Comparison between hyperprolactinemia patients with normal DXA and those with osteopenia/osteoporosis showed significantly high BMI in the group with normal DXA compared to the latter (p = 0.029). BMI was also significantly positively correlated with femur T score and Z score (p = 0.015 for each) as well as with serum prolactin levels (p = 0.003). Several studies have linked obesity with increased bone strength and lower fracture risk. Meta-analysis on 14,887 men and 44,757 women found 17% reduced risk of hip fracture in obese individuals when compared to those with normal weight. There was an overall increased risk of any type of fracture in both men and women with low BMI when adjusted for age. When adjusted for BMD, BMI was associated only with hip fracture risk in underweight subjects[23] Haffner and Bauer also found femoral neck and lumbar spine BMD positively correlated with BMI[24]. These are similar to our findings where BMI was significantly associated with femur T score and Z score. A study on 472 adolescents reported obese individuals to have greater total BMD compared to those with normal weight[25]. This strengthens our study findings which suggest that obesity has a protective role against osteoporosis.

BMI was also significantly correlated with prolactin levels in the present study. Ferdinand Roelfsena *et al.*, also suggested effect of BMI on prolactin dynamics[26]. However, Khatoon and Badawy did not find any correlation between prolactin and BMI in male infertile patients[27].

Factors regulating the obesity-osteoporosis relationship may be mechanical load on bones which then stimulates bone formation[28] increased serum levels of leptin[29] sex hormone binding globulin[30] increased production of insulin growth factor, hyperinsulinemia[24] and conversion of androgen to estrogen in adipose tissues[30]. Increased estrogen production by aromatization in fat tissues may explain protective effect of obesity on bones[31]. No significant difference in estradiol levels in the present study is justified by more estrone levels in obese or fatty women rather than estradiol[32]. Leptin has been associated both positively and negatively with BMD. Elefteiou et al. presented increased osteoblast differentiation with leptin[33] while Ducy *et al.*, showed inhibition of bone formation by leptin acting through sympathetic nervous system[34].

In contrast to above reports, Cohen *et al.*, reported inverse association of trunk fat with bone volume and bone formation rate at trabecular region in pre-menopausal women[35]. Ishii *et al.,* although found linear association between BMD and BMI in premenopausal women, but the association between BMI and composite strength indexes was inverse, suggesting even if the association between BMD and BMI is linear, it does not compensate for increased impact forces during fall. They suggested that rather than a linear effect of BMI on fracture risk, the relationship between obesity and osteoporosis is U-shaped[36]. Palermo et al. inferred that BMI above eutrophic ranges may be weakly protective against bone fractures, but the effect no more stands true in case of morbid obesity[37].

Variations in relationship between obesity and osteoporosis may arise due to differences in fat tissue location and distribution[38]. Studies have suggested that visceral adipose tissues, adipose fat and bone marrow adipose tissue are associated with lower BMD, while subcutaneous adipose tissue seems protective or neutral towards bone health. This suggests that neither all fats, nor all fractures are alike, and so comorbidities of obesity must be considered to explain the complete picture[37].

The present study showed no significant difference in serum levels of Ca, P and ALP between groups of hyperprolactinemia patients with normal BMD and those with low BMD. These results agree with the studies which found no association of Ca, P or ALP with bone loss[39-41] but are against the findings of Sumanthy and Shanthi who reported significantly reduced serum Ca, and significantly increased serum ALP in osteopenia subjects compared to controls. Serum prolactin was also found to be inversely correlated with BMD, serum Ca and serum P in subjects with osteopenia and osteoporosis in the study[42].

Studies suggest BMD to be associated with longer duration of prolactinoma independent of serum prolactin levels and other confounding factors[11]. In hyperprolactinemic women also, vertebral fractures condition was associated with disease duration independent of prolactin values, age, BMD,

hypopituitarism or dopaminergic drug treatment[10]. But the present study found no particular trend in duration of hyperprolactinemia and DXA results.

5. Conclusion

The present study suggests that levels of prolactin, estradiol, Calcium, Phosphate, alkaline phosphatase or duration of hyperprolactinemia are not associated with bone mineral density in hyperprolactinemia patients. There was no significant difference of cases with osteopenia/osteoporosis in macroprolactinemia and true hyperprolactinemia patients. The only significant correlation of bone mineral density was found with body mass index suggesting high BMI to be a protective factor against osteoporosis in hyperprolactinemia patients which is more likely regulated by estrone levels rather than estradiol. Due to limited number of subjects in this study, further prospective studies with large number cases using estrone as marker in place of estradiol are required to better know the relationship of hyperprolactinemia with bone mineral density, estrone and body mass index.

6. Acknowledgments

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The PhD student was receiving Junior Research Fellowship (Ref. No: 23/06/2013(i)EU-V) from Council of Scientific and Industrial Research (CSIR) - University Grant Commission (UGC). We also acknowledge contribution of Dr. Nikhil Tandon, Head, Departmentof Endocrinology, AIIMS, New Delhi, for DXA test and Mr. Anurag Bajpai, Scientist-C, ICMRHQ, New Delhi, for statistical analysis.

7. References

1. Adra A, El Zibdeh MY, Abdul Malek AMM, Hamrahian AH, Abdelhamid AMS, Colao A, Anastasiades E, Ahmed EMAF, Ezzeddine JI, El Sattar MIA, Dabit ST, Ghanameh W, Nedjatian N, El-Kak F. Differential diagnosis and management of abnormal uterine bleeding due to hyperprolactinemia. Middle East Fertil Soc J. 2016; 21(3):137-147. https://doi.org/10.1016/j.mefs.2016.02.001.
2. Kalsi AK, Halder A, Jain M, Chaturvedi PK, Sharma JB. Prevalence and reproductive manifestations of macroprolactinemia. Endocrine. 2019;63(2):332-340.https://doi.org/10.1007/s12020-018-1770-6. PMid:30269265.
3. Shimatsu A, Hattori N. Macroprolactinemia: Diagnostic, clinical, and pathogenic significance. Clin Dev Immunol. 2012; 2012:167132. https://doi.org/10.1155/2012/167132. PMid:23304187 PMCid:PMC3529459.
4. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: Structure, function, and regulation of secretion. Physiol Rev. 2000; 80(4):1523-1631. https://doi.org/10.1152/physrev.2000.80.4.1523. PMid:11015620.
5. Simon JA, Mack CJ. Prevention and management of osteoporosis. Clin Cornerstone. Suppl 2003; 2:S5-12. https://doi.org/10.1016/S1098-3597(03)90042-1.
6. WHO Scientific group on the assessment of osteoporosis at primary health care level. World Health Organization, Summary Meeting Report. Brussels, Belgium; 2004. p. 5-7. https://www.who.int/chp/topics/Osteoporosis.pdf.
7. Bolanowski M, Halupczok J, Jawiarczyk-Przybyłowska A. Pituitary disorders and osteoporosis. Int J Endocrinol. 2015; 2015:206853. https://doi.org/10.1155/2015/206853. PMid:25873948 PMCid:PMC4383139.
8. Di Somma C, Colao A, Di Sarno A, Klain M, Landi ML, Facciolli G, Pivonello R, Panza N, Salvatore M, Lombardi G. Bone marker and bone density responses to dopamine agonist therapy in hyperprolactinemic males. J Clin Endocrinol Metab. 1998; 83(3):807-813. https://doi.org/10.1210/jcem.83.3.4674. PMid:9506732.
9. Zhao Y, Gan X, Luo P, He Q, Guo Q, Zhang L, Zhang X, Zhang X, Fei Z. The risk of osteopenia in premenopausal women with various sellar tumors. Gynecol Endocrinol. 2012; 28(12):945-948. https://doi.org/10.3109/09513590.2012.683080. PMid:22553947.
10. Mazziotti G, Mancini T, Mormando M, De Menis E, Bianchi A, Doga M, Porcelli T, Vescovi PP, De Marinis L, Giustina A. High prevalence of radiological vertebral fractures in women with prolactin-secreting pituitary adenomas. Pituitary. 2011; 14(4):299-306. https://doi.org/10.1007/s11102-011-0293-4. PMid:21301967.
11. Mazziotti G, Porcelli T, Mormando M, De Menis E, Bianchi A, Mejia C, Mancini T, De Marinis L, Giustina A. Vertebral fractures in males with prolactinoma. Endocrine. 2011; 39(3):288-293. https://doi.org/10.1007/s12020-011-9462-5. PMid:21479837.
12. Meaney AM, Smith S, Howes OD, O'Brien M, Murray RM, O'Keane V. Effects of long-term prolactin-raising antipsychotic medication on bone mineral density in patients with schizophrenia. Br J Psychiatry. 2004; 184:503-508. https://doi.org/10.1192/bjp.184.6.503. PMid:15172944.
13. Vestergaard P, Jørgensen JO, Hagen C, Hoeck HC, Laurberg P, Rejnmark L, Brixen K, Weeke J, Andersen M, Conceicao FL,

Nielsen TL, Mosekilde L. Fracture risk is increased in patients with GH deficiency or untreated prolactinomas--a case-control study. Clin Endocrinol (Oxf). 2002; 56(2):159-167. https://doi.org/10.1046/j.0300-0664.2001.01464.x. PMid:11874406.

14. Magaš S, Ćaćić M, Magaš ZS, Kruljac I. Multiple vertebral fractures in an elderly male with macroprolactinoma. Endocr Oncol Metab. 2016; 2:226-230. https://doi.org/10.21040/eom/2016.2.3.7.
15. Seriwatanachai D, Krishnamra N, van Leeuwen JP. Evidence for direct effects of prolactin on human osteoblasts: Inhibition of cell growth and mineralization. J Cell Biochem. 2009; 107(4):677-685. https://doi.org/10.1002/jcb.22161. PMid:19365811.
16. Yoon V, Maalouf NM, Sakhaee K. The effects of smoking on bone metabolism. Osteoporos Int. 2012; 23(8):2081-2092. https://doi.org/10.1007/s00198-012-1940-y. PMid: 22349964.
17. Sampson HW. Alcohol's harmful effects on bone. Alcohol Health Res World. 1998; 22(3):190-194. https://doi.org/10.1111/j.1530-0277.1998.tb05912.x.
18. Khatake PD, Jadhav SS, Afroz S. Relation between Serum Calcium Level, Bone Mineral Density and Blood Pressure in Postmenopausal Women. Int J Rec TrSci Tech. 2013; 7:86-88.
19. Johnell O. WHO scientific group on the assessment of osteoporosis at primary health care level, in Proceedings of the Summary Meeting Report. World Health Organization, Brussels, Belgium; 2004.
20. Chen CY, Lane HY, Lin CH. Effects of Antipsychotics on Bone Mineral Density in Patients with Schizophrenia: Gender Differences. Clin Psychopharmacol Neurosci. 2016; 14(3):238-249. https://doi.org/10.9758/cpn.2016.14.3.238. PMid:27489377 PMCid:PMC4977815.
21. Clément-Lacroix P, Ormandy C, Lepescheux L, Ammann P, Damotte D, Goffin V, Bouchard B, Amling M, Gaillard-Kelly M, Binart N, Baron R, Kelly PA. Osteoblasts are a new target for prolactin: Analysis of bone formation in prolactin receptor knockout mice. Endocrinology. 1999; 140(1):96-105. https://doi.org/10.1210/endo.140.1.6436. PMid:9886812.
22. Seriwatanachai D, Thongchote K, Charoenphandhu N, Pandaranandaka J, Tudpor K, Teerapornpuntakit J, Suthiphongchai T, Krishnamra N. Prolactin directly enhances bone turnover by raising osteoblast-expressed receptor activator of nuclear factor kappa B ligand/osteoprotegerin ratio. Bone. 2008; 42(3):535-546. https://doi.org/10.1016/j.bone.2007.11.008. PMid:18166509.
23. De Laet C, Kanis JA, Odén A, Johanson H, Johnell O, Delmas P, Eisman JA, Kroger H, Fujiwara S, Garnero P, McCloskey EV, Mellstrom D, Melton LJ 3rd, Meunier PJ, Pols HA, Reeve J, Silman A, Tenenhouse A. Body mass index as a predictor of fracture risk: a meta-analysis. Osteoporos Int. 2005; 16(11):1330-1338. https://doi.org/10.1007/s00198-005-1863-y. PMid:15928804.
24. Haffner SM, Bauer RL. The association of obesity and glucose and insulin concentrations with bone density in premenopausal and postmenopausal women. Metabolism. 1993; 42(6):735-738. https://doi.org/10.1016/0026-0495(93)90241-F.
25. Jeddi M, Dabbaghmanesh MH, Ranjbar Omrani G, Ayatollahi SM, Bagheri Z, Bakhshayeshkaram M. Relative Importance of Lean and Fat Mass on Bone Mineral Density in Iranian Children and Adolescents. Int J Endocrinol Metab. 2015; 13(3):e25542. https://doi.org/10.5812/ijem.25542v2.
26. Roelfsema F, Pijl H, Keenan DM, Veldhuis JD. Prolactin secretion in healthy adults is determined by gender, age and body mass index. PLoS One. 2012; 7(2):e31305. https://doi.org/10.1371/journal.pone.0031305. PMid:22363612 PMCid:PMC3281966.
27. Khatoon F, Badawy A-E (2013) Association between BMI and Prolactin Levels in Male Patients. Sci J Med Clin Trials. 2012; 2013: 259:6.
28. Michel BA, Bloch DA, Fries JF. Weight-bearing exercise, overexercise, and lumbar bone density over age 50 years. Arch Intern Med. 1989; 149(10):2325-2329. https://doi.org/10.1001/archinte.1989.00390100127027. PMid:2802897.
29. Goulding A, Taylor RW. Plasma leptin values in relation to bone mass and density and to dynamic biochemical markers of bone resorption and formation in postmenopausal women. Calcif Tissue Int. 1998; 63(6):456-458. https://doi.org/10.1007/s002239900557. PMid:9817937.
30. Albala C, Yáñez M, Devoto E, Sostin C, Zeballos L, Santos JL. Obesity as a protective factor for postmenopausal osteoporosis. Int J Obes Relat Metab Disord. 1996; 20(11):1027-1032.
31. Cleland WH, Mendelson CR, Simpson ER. Effects of aging and obesity on aromatase activity of human adipose cells. J Clin Endocrinol Metab. 1985; 60(1):174-177. https://doi.org/10.1210/jcem-60-1-174. PMid:3964790.
32. Ricci TA, Heymsfield SB, Pierson RN Jr, Stahl T, Chowdhury HA, Shapses SA. Moderate energy restriction increases bone resorption in obese postmenopausal women. Am J Clin Nutr. 2001; 73(2):347-352. https://doi.org/10.1093/ajcn/73.2.347. PMid:11157334.
33. Elefteriou F, Takeda S, Ebihara K, Magre J, Patano N, Kim CA, Ogawa Y, Liu X, Ware SM, Craigen WJ, Robert JJ, Vinson C, Nakao K, Capeau J, Karsenty G. Serum leptin level is a regulator of bone mass. Proc Natl Acad Sci USA.

2004; 101(9):3258-3263. https://doi.org/10.1073/pnas.0308744101. PMid:14978271 PMCid:PMC365777.

34. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen J, Vinson C, Rueger JM, Karsenty G. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell. 2000; 100(2):197-207. https://doi.org/10.1016/S0092-8674(00)81558-5.
35. Cohen A, Dempster DW, Recker RR, Lappe JM, Zhou H, Zwahlen A, Müller R, Zhao B, Guo X, Lang T, Saeed I, Liu XS, Guo XE, Cremers S, Rosen CJ, Stein EM, Nickolas TL, McMahon DJ, Young P, Shane E. Abdominal fat is associated with lower bone formation and inferior bone quality in healthy premenopausal women: A transiliac bone biopsy study. J Clin Endocrinol Metab. 2013; 98(6):2562-2572. https://doi.org/10.1210/jc.2013-1047. PMid:23515452 PMCid:PMC3667251.
36. Ishii S, Cauley JA, Greendale GA, Nielsen C, Karvonen-Gutierrez C, Ruppert K, Karlamangla AS. Pleiotropic effects of obesity on fracture risk: the Study of Women's Health Across the Nation. J Bone Miner Res. 2014; 29(12):2561-2570. https://doi.org/10.1002/jbmr.2303. PMid:24986773 PMCid:PMC4403760.
37. Palermo A, Tuccinardi D, Defeudis G, Watanabe M, D'Onofrio L, Lauria Pantano A, Napoli N, Pozzilli P, Manfrini S. BMI and BMD: The Potential Interplay between Obesity and Bone Fragility. Int J Environ Res Public Health. 2016; 13(6):544. https://doi.org/10.3390/ijerph13060544. PMid:27240395 PMCid:PMC4924001.
38. Ng AC, Melton LJ 3rd, Atkinson EJ, Achenbach SJ, Holets MF, Peterson JM, Khosla S, Drake MT. Relationship of adiposity to bone volumetric density and microstructure in men and women across the adult lifespan. Bone. 2013 Jul; 55(1):119-125. https://doi.org/10.1016/j.bone.2013.02.006. PMid:23428401 PMCid:PMC3650114.
39. Hamdi RA. Evaluation of Serum Osteocalcin Level in Iraqi Postmenopausal Women with Primary Osteoporosis. J Fac Med. 2013; 55:166-169.
40. Ali NK. Estimation of Some Mineral (Calcium, Phosphorous, Vitamin 25 (OH) D and Alkaline Phosphatase) in Osteoporosis Patients in Kirkuk City. Osteopor Phys Act. 2018; 6:215. https://doi.org/10.4172/2329-9509.1000215.
41. Jayaram N, Bijoor AR, Rajagopalan N, Venkatesh T. The value of serum and urinary n-Telopeptide in the diagnosis of osteoporosis. Indian J Orthopaed. 2002; 36:9.
42. Sumanthy S, Shanthi G. A Study of Serum Prolactin in Reduced Bone mineral Density. J Indian Acad Geriatr. 2016; 12:10-13.