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OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(9):166-72

Research Article Doi: 10.17546/msd.

The impact of serum c-peptide levels on bone mineral density in postmenopausal type 2 diabetic women

Berna İmge Aydoğan[1]*, Adile Begüm Bahçecioğlu Mutlu[1], Çağlar Keskin[1], Funda Seher Özalp Ateş[2], Sevim Güllü[1]

Abstract

Objective: Recent studies suggested that c-peptide had biological activity on bone metabolism. We aimed to evaluate the impact of c-peptide level on bone mineral density (BMD) in postmenopausal type 2 diabetic women.

Material and Methods: Thirty-five insulin naïve type 2 diabetic postmenopausal women were included in our prospective cohort study. Fasting and random c-peptide, parathyroid hormone (PTH), vitamin D, insulin, alkaline phosphatase level (ALP) levels and BMD were evaluated.

Results: The mean age of patients was 61.8±8.56 years. The mean fasting c-peptide, random c-peptide and insulin levels were 3.0±1.24, 7.7±3.7 and 13.9±11.2 µIU/ml, respectively. The mean femoral neck (FN-BMD) and total lumbar spine bone mineral density (L-BMD) were 0.787±0.127 and 0.919±0.122 g/cm^2, respectively. C-peptide level was associated with total hip BMD ($p<0.05$) but this relation disappeared after regression analysis adjusted for confounders. A negative correlation between PTH level and FN-BMD was found (p: 0.01). Total hip BMD and L-BMD were negatively correlated with age (p: 0.01 and p: 0.02, respectively). A positive association between DPP4 inhibitor treatment and total hip BMD was observed (p: 0.03).

Conclusions: We observed a positive association between total hip BMD and serum c-peptide level. However, this relationship disappeared in multiple linear regression analysis. Further studies are necessary to evaluate the impact of c-peptide level on the risk of osteoporosis in T2DM.

Keywords: C-peptide, osteoporosis, type 2 diabetes mellitus, bone mineral density

Introduction

Osteoporosis and osteoporotic fractures are associated with increased risk of morbidity and all-cause mortality in elderly population (1-4). Type 2 diabetes mellitus is another health concern which becomes more frequent with ageing and often coexists with osteoporosis in elderly population.

The relationship between type 1 diabetes mellitus (T1DM) and decreased bone mineral density (BMD) is well-known (5). Both T1DM and T2DM are associated with hip fracture risk, whereas the risk is greater in T1DM when compared to T2DM (5-7). However, changes in BMD in T2DM remains controversial (7, 8). A significant increase of BMD in type 2 diabetics was observed in some studies and this finding was attributable to causal relationships between T2DM, hyperinsulinemia, obesity, and protective effect of obesity from osteoporosis (7, 9, 10). Increased insulin levels and insulin resistance were reported to be positively associated with BMD in several studies (11, 12).

These findings were consistent with anabolic action of insulin in bone formation (13). Increased risk of fracture in T2DM despite increased BMD, was suggested to be caused by worsened bone quality (14). Previous studies proposed decreased cortical bone density and low bone material strength index in type 2 diabetic patients (15, 16).

C-peptide level is a reliable index of insulin secretion (17, 18). Although it was previously known as an inactive peptide, recent studies proposed that c-peptide may be involved in physiological pathways (19, 20). A possible role of c-peptide in bone metabolism was suggested in diabetic and non-diabetic patients (21-24). The mechanism in which the c-peptide affects bone mineral density has not been explained clearly. A previous study demonstrated that c-peptide activated ERK1/2 signaling in osteoblast-like cell lines and decreased receptor activator of nuclear factor kappa-B ligand (RANKL) mRNA in vivo (25).

Received 29-07-2019 **Accepted** 05-09-2019 **Available Online** 09-09-2019 **Published** 30-09-2019
1 Ankara University Faculty of Medicine, Department of Endocrinology and Metabolism Diseases, Ankara, TR
2 Ankara University Faculty of Medicine, Department of Bioistatistics, Ankara, TR
*** Corresponding Author:** Berna İmge Aydoğan **E-mail:** imgehalici@gmail.com **Phone:** +90 (312) 508 21 00

We aimed to evaluate the impact of serum c-peptide level on bone mineral density in patients with T2DM.

Material and Methods

Patients: Thirty-five postmenopausal women with insulin-naïve Type 2 DM were included in this prospective cross-sectional study. The exclusion criteria of the study were; the use of agents or substances that affect Ca+2 homeostasis or metabolism, uncontrolled diabetes (HbA1C level ≥8.5%), serum creatinine levels ≥1.5 mg/dl for men and ≥1.4 for women , patients with any kind of malignancy, other conditions that may affect vitamin D/calcium/bone metabolism (Cushing' s syndrome, active/chronic liver disease, primary hyperparathyroidism, chronic obstructive pulmonary disease, malabsorption syndromes etc.), fracture history, premature ovarian failure, current/ex-smoking, alcohol consumption and drug abuse. Body mass index was calculated by dividing the weight (in kg) by the square meters of the height. Written informed consents were obtained from all participants. This study was approved by local ethical committee of Ankara University (08-614-19).

Biochemistry: Venous blood samples were obtained after 8 hours of fasting. Fasting blood glucose was measured by using enzymatic method with Roche P800 device. Insulin and c-peptide were measured using the Cobas e411 (Roche Diagnostics, Switzerland). Serum creatinine, calcium, and phosphorus measurements were made with a Beckman Coulter DXI 800 device (Brea, California, USA). Parathyroid hormone (PTH) was measured by the chemiluminescence method with a Beckman Coulter AU5800 device (Brea, California, USA). The 25 hydroxy vitamin D (25OHD) was measured with high performance liquid chromatography method. The corrected calcium (cCa) was calculated by the equation: cCa = [(4 - albumin) × 0.8] + Ca.

Bone mineral density: Bone mineral density (g/cm2) of femoral neck, total hip and lumbal spine were measured by dual energy X-ray absorptiometry (The Hologic Discovery QDR™ series DXA systems, USA). Bone mineral density was expressed as the amount of mineral (g)/area scanned (cm2). Osteopenia was defined as a BMD between 1.0 and 2.5 SD below that of a "young normal" adult and osteoporosis was defined as femoral or lumbar density of 2.5 standard deviations below that of a young adult according to WHO criteria (26).

Statistical Analysis: All statistical analysis were performed using SPSS version 11.5 (SPSS, Chicago, IL, USA). Kolmogorov-Smirnov test was used to assess the assumption of normality. Normally distributed continuous variables were presented as mean ± standard deviation. Non-normally distributed continuous variables were presented as median (min-max). Categorical data were summarized as counts and percentages. Nominal variables were assessed with chi-square analysis/Fisher's exact tests. The associations between continuous variables were determined by Pearson/Spearman correlation analysis. For continous variables, Student's t test/Mann-Whitney U test was used to evaluate the difference between two groups (who had osteoporosis and who had not). A two-sided p-value < 0.05 was considered as statistically significant. The variables which had a significance level of p< 0.20 from the univariate analysis were identified as candidate variables for the multiple linear regression models. The multiple linear regression models was created with Backward Method. A priori power analysis was conducted and showed that at least 29 women should be included to evaluate a relationship between c-peptide level and BMD with 0.5 correlation, using a two-sided hypothesis test with a significance level of 0.05 and 0.8 power.

Results

The mean (±s.d) age of patients was 61.8 (±8.56) years. The patients were postmenopausal for median (min.-max.) 13 (2-28) years. Baseline characteristics and biochemical measurements are summarized in Table 1. The mean total lumbar spine (L-BMD), femoral neck (FN-FMD) and total hip BMD were 0.919±0.122, 0.787±0.127, 0.921±0.147 g/cm2, respectively.

In the univariate analysis, there was no significant association between osteoporosis and any of the evaluated parameters (Table 2). In adjusted model for age, diabetes duration, menopause duration, nephropathy, PTH and Ca levels; only age was positively associated with presence of osteoporosis (p: 0.01, 95% CI=1.05-1.51).

Insulin level was positively correlated with fasting and c-peptide level (r=0.42, p: 0.01 and r=0.58, p: 0.001, respectively). Random c-peptide level was negatively correlated with HbA1c level, duration of diabetes, FPG and positively correlated with insulin level (r=-0.08, p<0.001; r=-0.22, p<0.001; r=-0.29, p: 0.01, r=0.42, p<0.001, respectively) (Table 3).

Lumbar spine total BMD was higher in patients without nephropathy when compared to with nephropathy (0.930±0.117 vs 0.737±0.048, p: 0.02). Associations between L-BMD and biochemical parameters are summarized in Table 4.

Total hip BMD was positively associated with age and fasting c-peptide level (p: 0.01 and p: 0.04, respectively) (Table 4). Total hip BMD was higher in patients under DPP4 inhibitor treatment (1.021±0.120 vs 0.892±0.143, p: 0.02). Other clinical and biochemical parameters were not associated with total hip BMD. Associations between total hip BMD and biochemical parameters are summarized in Table 4.

Femoral neck BMD was positively associated with age (p: 0.006) and negatively associated with PTH level (p: 0.01). Associations between FN-BMD and biochemical parameters are summarized in Table 4.

Age was the only parameter associated with L-BMD in multiple linear regression analysis p: 0.02). Parathyroid hormone level was slightly associated with FN-BMD but this relationship was not statistically significant (p: 0.06). Total hip BMD was negatively associated with age and positively associated with DPP-4 inhibitor usage (p: 0.01 and p: 0.03, respectively). Multiple linear regression analyses are summarized in table 5.

Table 1. Baseline characteristics of study participants

Parameters	Mean (±s.d) / Median (min.-max.)
Age (years) *	61.8±8.5
Age of menopause (years) **	46 (43-55)
Menopause duration (years) **	13 (2-28)
Duration of diabetes (years) *	10.5±7.9
BMI (kg/m^2) *	31.5±4.4
Waist circumference (cm) *	107.1±12.9
Hypertension, n(%) (A/P)	25(71.4)/10(28.6)
HbA1C (%) *	7.1±0.87
FBG (70-100 mg/dl) **	122 (73-216)
Insulin (4-16 uIU/ml) **	12.1 (3.8-71)
Fasting c-peptide (RR:1.1-4.4 ng/ml) *	3.0±1.2
Random c-peptide *	7.7±3.8
cCalcium (RR:8.5-10.5 mg/dL) *	9.6±0.52
Vitamin D (RR:20-100 ng/mL) **	12.1 (5.1-35)
PTH (RR:14-72 pg/mL) **	56 (26.8-160)
TSH (RR:0.5-5.5 mIU/L) **	1.88 (1-6)
ALP (RR: 30-130 U/L)	75.4±24.5
Medications;	
Metformin, n(%) (A/P)	0(0.0)/35(100.0)
DPP4 inhibitors, n(%) (A/P)	27(77.1)/8(22.9)
Sulphonylurea, n(%) (A/P)	22(62.9)/13(37.1)
Diabetic retinopathy , n(%) (A/P)	32(91.4)/3(8.6)
Diabetic neuropathy, n(%) (A/P)	29(82.9)/6(17.1)
Diabetic nephropathy, n(%) (A/P)	33(94.3)/2(5.7)
Coronary artery disease n(%)	28(80.0)/7(20.0)

*: mean±standard deviation, **: median (min.-max.) ,A/P=absent/present, ALP:alkaline phosphatase; BMI; body mass index; cCalcium:corrected calcium; DPP4: dipeptidyl peptidase-4; FPG : fasting plasma glucose; PTH: parathormone; HbA1c: Glycosylated haemoglobin; RR=reference range, SU: sulphonylurea, TSH: thyroid stimulating hormone.

Table 2. Impact of clinical and biochemical parameters on osteoporosis

Parameters	Osteoporosis		
	Absent (n=25)	**Present (n=10)**	**p-value**
	Mean (±s.d) / Median (min.-max.)		
Age (years)	60.2±8.4	65.8±7.9	0.08
Age of menopause (years) **	46 (43-55)	48 (45-53)	0.46
Menopause duration (years)**	10 (2-25)	15.5 (5-28)	0.19
Duration of diabetes (years)*	11.1±8.6	8.6±6.1	0.17
BMI (kg/m^2)*	31.76±4.45	30.75±4.43	0.54
Waist circumference (cm)*	106.4±13.4	109.1±12.1	0.56
HbA1C (%)*	7.2±0.9	6.7±0.73	0.25
FBG (mg/dl) **	123 (73-216)	121.5 (76-206)	0.92
Insulin (µIU/mL)*	15.2±12.8	10.7±3.97	0.37
C-peptide (fasting) (ng/ml)*	2.8±1.2	3.4±1.4	0.18
C-peptide (random)(ng/ml)**	7.17 (2.1-14.9)	6.4 (3.5-20.9)	0.89
cCalcium (mg/dl)*	9.48±0.51	9.79±0.52	0.12
Vitamin D (ng/ml)**	9.9 (5.1-35)	13.7 (5.1-31)	0.36
Parathormon (pg/mL)**	55.4 (26-113)	62.7 (42-160)	0.15
ALP (U/L)*	73.8±22.8	78.9±29.1	0.63
TSH (mIU/L)**	2 (1-6)	2 (1-5)	0.92
Hypertension, n(%) (A/P)	7(28.0)/18(72.0)	3 (30.0)/7 (70.0)	0.61
Hyperlipidemia, n(%) (A/P)	4(16.0)/21(84.0)	4 (40.0)/6 (60.0)	0.14
Medications;			
Metformin, n(%) (A/P)	0(0.0)/25(100.0)	0 (0.0)/10 (100.0)	0.28
DPP4 inhibitors, n(%) (A/P)	19(76.0)/6(24.0)	8 (80.0)/2 (20.0)	0.58
Sulphonylurea, n(%) (A/P)	14(56.0)/11(44.0)	8(80.0)/2(20.0)	0.21
Diabetic retinopathy, n(%) (A/P)	23(92.0)/2(8.00)	9(90.0)/1(10.0)	0.28
Diabetic neuropathy, n(%) (A/P)	22(88.0)/3(12.0)	7(70.0)/3(30.0)	0.32
Diabetic nephropathy, n(%) (A/P)	25(100.0)/0(0.0)	8(80.0)/2(20.0)	0.07

**: median (min.-max.), A/P=absent/present, ALP:alkaline phosphatase; BMI; body mass index; cCalcium:corrected calcium; DPP4: dipeptidyl peptidase-4; FPG : fasting plasma glucose; HbA1c: Glycosylated haemoglobin; RR=reference range, SU: sulphonylurea,

Table 3. Associations between fasting/ random c-peptide levels, clinical and biochemical parameters.

Parameters	Fasting c-peptide		Random c-peptide	
	Correlation Coefficient (r)	**p-value**	**Correlation Coefficient (r)**	**p-value**
Age	0.18	0.31	0.11	0.54
Age at menopause	-0.21	0.25	-0.11	0.53
Duration of menopause	0.12	0.51	0.01	0.95
Duration of diabetes	-0.22	0.21	-0.52	**0.001**
BMI	0.27	0.11	0.24	0.16
Waist circumference	0.11	0.54	0.078	0.65
calcium	0.30	0.08	-0.04	0.79
phosphorus	-0.21	0.22	-0.52	0.02
ALP	0.22	0.22	0.17	0.43
PTH	0.08	0.64	0.29	0.11
Vitamin D	-0.14	0.43	-0.06	0.72
FPG	-0.29	0.09	-0.41	**0.01**
Insulin	0.42	**0.01**	0.58	**0.001**
HbA1c	-0.08	0.64	-0.59	**0.001**
TSH	-0.22	0.21	-0.28	0.2
Hypertension	0.04	0.79	0.19	0.26
Metformin	-0.08	0.63	0.02	0.91
DPP4 inhibitors	-0.31	0.07	-0.38	0.02
SU	0.03	0.86	-0.31	0.07
Retinopathy	-0.24	0.17	-0.19	0.27
Neuropathy	-0.10	0.54	0.06	0.72
Nephropathy	-0.11	0.55	-0.15	0.38

ALP:alkaline phosphatase; BMI; body mass index; cCalcium:corrected Calcium; DPP4: dipeptidyl peptidase-4; FPG : fasting plasma glucose; PTH: parathormone; HbA1c: Glycosylated haemoglobin; SU: sulphonylurea, TSH: thyroid stimulating hormone.

Table 4. Associations between femoral neck, total hip, lumbar spine BMD, clinical and biochemical parameters.

Parameters	FN-BMD (g/cm2)		Total hip BMD (g/cm^2)		L-BMD (g/cm^2)	
	r	**p-value**	**r**	**p-value**	**r**	**p-value**
Age	-0.45	0.006	-0.42	0.01	0.28	0.08
Age at menopause	-0.07	0.6	0.06	0.72	0.22	0.90
Duration of menopause	-0.28	0.11	-0.21	0.23	0.19	0.20
Duration of diabetes	0.13	0.43	0.25	0.13	0.17	0.32
BMI	0.02	0.88	0.12	0.48	0.14	0.40
Waist circumference	-0.01	0.91	0.16	0.33	0.02	0.87
cCalcium	0.09	0.58	-0.21	0.27	-0.01	0.91
Phosphorus	0.17	0.31	0.03	0.84	0.19	0.25
ALP	0.18	0.30	0.10	0.56	0.05	0.76
PTH	-.0.44	0.01	-0.11	0.53	-0.19	0.28
Vitamin D	-0.05	0.74	0.06	0.71	0.004	0.98
FPG	-0.05	0.74	-0.04	0.79	-0.14	0.39
Insulin	-0.20	0.26	0.20	0.25	0.10	0.56
HbA1c	0.17	0.33	0.04	0.78	0.21	0.24
c-peptide (fasting)	-0.13	0.44	-0.34	0.04	-0.33	0.85
c-peptide (random)	-0.27	0.12	-0.06	0.72	-0.14	0.40
TSH	0.10	0.54	0.03	0.85	-0.07	0.56

-r: Correlation Coefficient

Table 5. Multiple linear regression analysis of parameters related to lumbar spine BMD, femur shaft BMD and total hip BMD

Variables		Unstandardized coefficients		Standardized coefficiens	t	p-value
		B	Std. error	beta		
L-BMD*	Age	-0.007	0.002	-0.51	-3.3	0.02
FN-BMD**	PTH	-0.001	0.001	-0.30	-1.8	0.06
Total hip BMD***	Age	-0.007	0.003	-0.41	-2.6	0.01
	DPP4 inh.	0.12	0.056	0.34	2.2	0.03

*excluded variables: duration of menopause, nephropathy, sulphonylurea treatments. ** excluded variables: Age, random c-peptide level, duration of menopause, sulphonylurea treatment. *** excluded variables: Fasting c-peptide level.

Discussion

In the present study, serum c-peptide level was positively related with total hip BMD in postmenopausal women with diabetes. However, in multivariate analysis we did not observe a significant relationship between serum c-peptide levels and BMD at any sites. Total hip BMD and lumbar spine BMD (L-BMD) were both negatively associated with age. Femoral neck BMD (FN-BMD) was negatively associated with parathyroid hormone (PTH) level. Dipeptidyl peptidase-4 inhibitor treatment was associated with higher total hip BMD.

Previous studies suggested that c-peptide had multiple effects in physiological pathways. Its role in prevention of diabetic vascular complications was demonstrated in several studies (27-29). Regarding to bone metabolism, c-peptide was shown to cause stimulation of Na-K- ATPase and activate Ca2+ dependent signaling pathways which are essential for osteoblast activities (20, 30, 31). Stimulation of ERK1/2 by c-peptide via phosphoinositide 3-kinase pathway (PI3K) was demonstrated (32). In osteoblast-like cell lines, activation of ERK1/2 was shown to decrease RANKL mRNA levels (33). In the study of Russo et al., c-peptide prevented the reduction of type1 collagen mRNA/protein, in addition to activating of ERK1/2 signaling and reducing RANKL mRNA/protein levels in Saos-2 cells which represent a model for osteoblastic differentiation in human cells (25).

The association between BMD and c-peptide level was investigated in a limited number of clinical studies (21, 23, 24, 34). Results were conflicting in both diabetic and non-diabetic populations (21, 23, 34). A cross-sectional survey was conducted with 6625 participants of National Health and Nutrition Examination Survey. The study showed that there was a significant negative association between serum c-peptide level, total and most regional BMDs in both genders independent of confounding factors (24). However, this relationship was not observed among subjects older than 60 years, whose c-peptide levels were higher when compared to youngers (24). A study by Montalcini et al. reported that serum c-peptide was strongly associated with L-BMD in postmenopausal non-diabetic women independent of confounders like insulin, 25-OH-D, PTH, FGF-23 levels and BMI values (23). A recent study reported that serum c-peptide level was inversely associated with fracture risk and positively with BMD, in postmenopausal women without diabetes (35). An alternative hypothesis of this study was that c-peptide may be a marker of osteoporosis rather than a cause (35). In type 1 diabetic patients, glucagon stimulated c-peptide level was shown to be related with lumbar and femoral BMD (21). However, a recent study investigated BMD in type 1 diabetic and non-diabetic premenopausal women, and did not demonstrate its association with urinary c-peptide level (34).

In postmenopausal patients with T2DM, c-peptide level was positively associated with FN-BMD in both genders and inversely with fracture risk in women (22). Authors attributed these findings to anabolic effects of insulin; stimulation of bone formation and osteoblast proliferation. However, the impact of urinary c-peptide on fracture risk was independent of BMD in women. Authors pointed out a possible favorable effect of c-peptide on bone quality which was not reflected by BMD. In our cohort, FN-BMD was not associated with BMD. In univariate analysis, BMD was positively associated with c –peptide level, but this relationship disappeared after adjustments for confounding factors.

Substrates of DPP4 and their receptors are expressed in bone (36, 37). Glucose dependent insulinotropic peptide (GIP) administration was shown to increase bone density in rats. Glucagon-like peptide 1 receptor agonists were shown to decrease bone loss and increase bone formation in vitro and in vivo (38, 39). A previous meta-analysis suggested that DPP-4 inhibitors was related with reduced fracture risk (40). In our study, DPP4 inhibitor treatment was positively associated with the total hip BMD.

A major strength of our study was the exclusion of many factors that promote to osteoporosis, just as alcohol consumption, current-ex smoking, medications which effect calcium and bone metabolism, comorbidities related to osteoporosis. The major limitation of study was relatively small sample size.

Conclusion

We observed a positive association between total hip BMD and serum c-peptide level. However, this relationship disappeared after adjustment for confounders. A possible link between BMD of total hip may be masked by limited number of participants. Direct effect of c-peptide levels on BMD and fracture risk independent of BMD should be evaluated.

Acknowledgements: None

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: **BİA, ABBM, ÇK, SG**; Research concept and design, Patient examinations, Research the literature, preparation of the article. Chemical Analysis**. FSÖA:** Statistical analyzes **BİA;** Revision of the article.

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