

Relationship between genotype and serum levels of adipokines and bone mineral density in type 2 diabetes mellitus patients

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Abstract

Background: There are conflicting data about bone density alterations in type 2 diabetic patients. Regarding the role of adipokines in glucose metabolism, they may have metabolic effects on bone mineral density (BMD) changes. The aim of this study was to determine the relationship between circulating visfatin, adiponectin and the visfatin genotype with BMD in type 2 diabetes (T2DM).

Methods: Thirty-two patients with T2DM participated in this cross-sectional study. Laboratory measurements were included FBS, HbA1C, lipid Profile, fasting serum visfatin and adiponectin. Hip and spine BMD were measured using DEXA. Genotyping for visfatin gene SNP (rs2110385) was performed by using the PCR- RFLP method.

Results: Genotype distributions of GG, GT and TT were 37.5%, 43.8% and 18.8%, respectively. Prevalence of osteoporosis in patients with GG genotype was 33.3%; whereas, not observed in other two genotypes. Hip BMD and Z-score were significantly lower in GG genotype. We found significant correlation between circulating visfatin and hip BMD ($r=-0.31$). Circulating adiponectin and visfatin levels had significant correlation with hip BMD independent of BMI and age.

Conclusion: Our results suggest that adipokines may contribute to BMD changes in type 2 diabetes mellitus patients. Genotype variations may explain inconsistent BMD changes among these patients.

Keywords: Bone mineral density, Aipokines, Visfatin, Genotype, Adiponectin

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Introduction

Inconsistent and debatable reported data are available on bone mineral density (BMD) changes in type 2 diabetic patients (1-12). Furthermore, the role of contributory hormones in glucose metabolism also thought had metabolic effects on bone, resulting in BMD changes. Therefore, diabetes mellitus has become a topic of interest in bone research.

The main complications of diabetes include nephropathy, neuropathy, retinopathy, macrovascular and microvascular diseases, and alterations of bone and mineral metabolism (13). Diabetic osteopenia causes an increase in bone fractures (14,15) and disturbs fracture restoration (16,17) and affects quality of life. Prevalence of diabetes mellitus has been rapidly increased in recent years frequently as a result of an increase in prevalence of obesity and other metabolic risk factors (18, 19, 20). Increased central adipose tissue is a common metabolic feature of type 2 diabetes (21). Growing evidence suggests that positive associations between fat mass and BMD are mediated by not only biomechanical, but also biochemical factors (22). The release of adipokines by either adipocytes or adipose tissue-infiltrated macrophages leads to a chronic subinflammatory state that could play a central role in the development of insulin resistance and type 2 diabetes (23-26). On the other hand, some studies showed correlation between adipokines and bone mineral density (27-31) however, their findings are controversial.

Adiponectin and visfatin, as adipokines secreted by adipose tissue, are potential contributors to bone metabolism (32). Visfatin is recently described as an adipokine, previously recognized as a pre-B cell colony-enhancing factor (PBEF), comes into focus to play an important role in regulation of glycemic homeostasis (33). Recently, it has demonstrated the actions of visfatin on human primary osteoblasts (34). Accordingly, it can be supposed that this adipokine may play an important role in bone mass density.

Adiponectin is a 30-kDa adipokine that released from adipocytes. Molecular structure of this hormone is similar to collagen polypeptide and has insulin-sensitizing properties (35-37). Circulating adiponectin is reduced in patients with obesity, coronary artery disease, and type 2 diabetes (38-41). This adipokine has anti-diabetic, anti-atherogenic (42, 43), anti-inflammatory (43-46) and angiogenic (47, 48) properties.

The relationship between this adipokine and BMD has been reported in previous studies; but, there were notorious finding among individual subjects in every study (22,28-32). Genetic influences have been estimated to account for approximately 46-80% of the individual BMD variances (49). The genetic role on bone metabolism not only in the formation of the peak bone mass, but also the subsequent bone failure will be affected by genetic variations (50). Recently, genetic variations have been suggested as contributory factors in BMD diversities in different populations (51-54). Innovative studies investigated the association between single nucleotide polymorphisms (SNPs) in the adipokine gene and BMD (49,55-57). It has been reported that there are single nucleotide polymorphisms in promoter region of visfatin gene that associated with susceptibility to T2DM and regulation of glucose homeostasis (58). Polymorphism studies on the coding regions and promoter regions have declared that there are number of single nucleotide polymorphisms in promoter region that may influence plasma glucose concentration, G2h and insulin levels (59-61).

So far, there has been no report on the association of circulating visfatin and its gene polymorphism and bone mineral density (BMD). Therefore, the aim of the present study was to determine whether SNP in the visfatin gene is related to BMD in type 2 diabetes mellitus patients.

Methods

Study population

Subjects were recruited from an outpatient clinic of Dr. Shariati Hospital (an

affiliated educational hospital of Tehran University of Medical Sciences, Tehran, Iran) from January to June 2008. The diagnosis of T2DM was made based on the World Health Organization criteria (62). Inclusion criteria were age ≥ 40 years, BMI (Body Mass Index) ≥ 25 kg/m² and at least 2 years of diagnosis of type 2 diabetes. Exclusion criteria were history of type 1 diabetes, any chronic disease other than T2DM and its complications, and insulin therapy.

Informed written consent was obtained from all subjects before their participation in the study. The study protocol was approved by ethics committee of EMRC (Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences).

Laboratory measurements

The peripheral blood after 10-12 hour fasting were drawn. HbA1C measured using HPLC (High pressure liquid chromatography) exchange Ion method (DS5 England); FPG (fasting plasma glucose) was assessed by GOD /PAP method. Serum visfatin concentration was determined by ELISA method (Human visfatin ELISA kit, AdipoGen Pharm-aceuticals, Belmont, Seoul, Korea), minimum detectable concentration was 30 pg/ml, IntraCV (Coefficients of Variation) was 4.3% and Inter CV was 7.5%. Serum adiponectin concentration was determined by ELISA method (Human adiponectin ELISA kit, AdipoGen Pharmaceuticals, Belmont, Seoul, Korea), minimum detectable concentration was 100pg/ml, IntraCV was 5.15 % and InterCV was 3.82 %.

Measurement of BMD

BMD was measured by DXA using Lunar DPX-MD (Lunar Corporation, Madison, Wisconsin, 53713. USA). The DXA was calibrated daily and weekly by using appropriated phantoms methods. To assess BMD, second to fourth lumbar spine and from the femur bone (neck, trochanter and the whole femur), bone density was calculated based on gr/cm².

Extraction of genomic DNA

DNA extraction was carried out using FlexiGen Kit (QIAGEN Inc. Valencia, CA) from whole blood according standard protocol. The extracted DNA was stored at 4°C until it used for PCR and RFLP analysis.

Genotyping

Genomic DNA from all subjects was analyzed for the presence of the G or T nucleotide at -4689G/T of the visfatin gene by a designed visfatin genotyping kit.

Statistical analysis

Results are reported as mean \pm SD. All of the statistical analyses were performed using the SPSS version 15 software. Student T-test was used to compare quantitative variables. Chi-square was used for comparing of qualitative variables. Also ANOVA was used for comparing the quantitative variable in different genotypes. P-value less than 0.05 was considered as statistically significant.

Results

Thirty-two type 2 diabetic patients participated in this cross-sectional study. Table 1 demonstrates demographic and biochemical characteristics of participants. Eighty-one percents of participants were women.

The frequencies of homozygous major allele (GG), heterozygous (GT), homozygous minor allele (TT) of SNP "rs2110385" were 37.5%, 43.8% and 18.8 %, respectively. The results of Hardy-Weinberg Equilibrium test were not significant.

As shown in table 2, prevalence of osteoporosis in patients with GG genotype was 33.3%; whereas, not observed in two other genotypes (P=0.05).

As illustrated in table 3, there was not a significant difference in mean age among genotypes.

Our findings demonstrate a significant positive correlation between circulating adiponectin and spine BMD (P=0.002, r=0.519), T-score spine (P=0.001, r=0.577), hip BMD (P=0.09, r=0.301), and Z-score hip

($P=0.001$, $r=0.664$), as well as significant correlation has been found between adiponectin levels and BMI ($P=0.001$, $r=0.54$). Furthermore, there was a significant positive correlation between BMI and T-scores ($P=0.001$, $r=0.546$) and Z-scores ($P=0.02$, $r=0.388$) of spine BMD; regarding hip BMD as dependent variable

and age and BMI as fixed variables in univariate model, there was a significant correlation with hip BMD independent of age and BMI ($P=0.018$).

Moreover, adiponectin revealed significant correlation with hip BMD independent of age and BMI ($P=0.059$).

Table 1. Demographic characteristic of participants in study

variables	Values
Age (years)	56±9
Time duration of T2DM (months)	51±39
BMI (kg/m ²)	30.4±4.9
FBS (mg/dl)	164.25±64.37
Hb A1C (%)	7.58±2.00
Adiponectin (µg/ml)	5.83±2.24
Visfatin (pg/ml)	14.95±16.93
Hip BMD	0.94±0.14
Spine BMD	1.08±0.21

Data are means±SD, BMI: Body Mass Index, BMD: Bone Mineral Density, FBS: Fasting Blood Sugar

Table 2. Prevalence of osteopenia and osteoporosis in different visfatin genotypes

Genotype	Bone status		
	Normal	osteopenia	osteoporosis
TT	66.7	33.3	0
GG	50.0	16.7	33.3
GT	42.9	57.1	0

Data are %

Table 3 . Clinical characteristics and BMD of type 2 diabetic patients in various visfatin genotypes

Characteristics	Genotypes		
	TT	GG	GT
Hip BMD	0.9±0.5	0.88±0.13	1.00±0.15
T-score hip	-0.80±0.49	-1.08±1.25	-1.12±1.41
Z-score hip	-0.20±0.99	-0.66±0.95	0.27±0.94
Spine BMD	0.97±0.12	1.13±0.26	1.09±0.18
T-score spine	-1.86±1.03	-1.31±0.79	-0.95±1.49
Z-score spine	-0.96±0.91	-0.75±0.46	-0.65±1.19
BMI	32.36±4.91*	27.37±1.67	32.26±5.62
adiponectin	6.33±0.62	5.23±1.47	6.14±3.37
Visfatin(ng/ml)	28.27±23.31*	16.00±18.49	8.34±7.44

Data are mean ± SD, P-values were significant (<0.05).

Discussion

The association between diabetes and bone disorders is complex and one controversial matter. Main factor that

contributes to contradictory results is BMI. Generally, the prevalence of type 2 diabetes and BMI increase parallel to each other, makes it difficult to determine the correlation

between diabetes and osteoporosis. In addition to the mechanical effects of body fat mass on bone mineral density through a mechanical load exerted on the skeleton by fat mass, it has been suggested that different hormones releasing from adipose tissue, also have contributory effects (30). Thus, assessment of adipokines may be useful.

Finding from Peng XD et al. research on Chinese men (20-80 y) to investigate correlation between serum adipocytokines levels and BMD, in accordance with our results, revealed that adiponectin is an independent predictor of BMD in men (32).

Results from recent studies to determinate the relationship between the adiponectin levels and BMD in elderly men (28), patients with type 2 diabetes (22), healthy postmenopausal women (30) and men and postmenopausal women with type 2 diabetes (29) revealed that there is a significant correlation between adiponectin and BMD, similar to our findings. Results of our study showed significant correlation between adipokines and hip BMD. Scant findings in this field were available. Evidence of recent research by Gonnelli S et al. (31) demonstrated no significant correlations between adiponectin and BMD at all skeletal sites. It appears that controversial results from various studies emerge from varied inclusion criteria such as gender and age. As shown in recently performed study, this correlation was more noticeable in men and adiponectin, showed noteworthy negative effects on bone mineral density in men (32). Furthermore, it should be considered diversity between ethnicity as well as inheritance. Accordingly, present differences in values of BMI reported from East-South Asian population in compare to European and American population, may influence bone mineral density along with adipokines levels and their effects on bone metabolism.

Although, few studies have examined the relationship between single nucleotide polymorphism on adipokines gene and its serum levels and BMD, results of present

study suggest that genetic variation in adipokines gene may be involved in BMD values.

The effects of single nucleotide polymorphisms and haplotypes of adiponectin genes on normal BMD variation in healthy Chinese women and men was explored by Zhang ZL et al. (55). They reported no significant within-family association between each SNP in the adiponectin gene and peak BMD.

Lee WY et al. (56) investigated the correlation between single nucleotide polymorphisms in the adiponectin gene and BMD. Their findings revealed an association between T45G polymorphism in exon 2 of the adiponectin gene and lumbar spine BMD in Korean women.

Also, results of previous studies on other adipokines polymorphism such as leptin, demonstrated their correlation with BMD (49) that is in favor with our findings; however, there was controversial outcomes about this area under discussion (57).

In an experimental study by Xie et al. (34) on cultured human osteoblast-like cells, it was reported that visfatin has similar functions as insulin on glucose uptake, proliferation, and type I collagen enhancement. Moreover, they reported that osteocalcin secretion from human osteoblast-like cells downregulates by visfatin. In favor with aforementioned study, present study results showed that patients with TT genotype had higher concentration of visfatin, also, better bone status in compare to other genotypes.

Several studies have shown that myoblastic cell lines can be converted to adipocytes through the expression of PPAR γ , and ligand activation of PPAR γ derives the differentiation of multiprotein mesenchymal progenitor cells towards adipocytes over osteoblasts (63). Akune et al (64) further found the relationship between osteogenesis and adipogenesis using cells and animal deficient in PPAR γ expression. With same results, one recent study showed that homozygous PPAR γ -deficient ES cells

failed to differentiate into adipocytes, but spontaneously differentiated into osteoblasts; PPAR γ haploinsufficiency enhanced osteoblastogenesis in vitro and increased bone mass in mice in vivo (65).

Besides these evidence, other researches showed visfatin and adiponectin mRNA were increased with agonist PPAR-treat OLETF rats. So, PPAR classic pathway may explain the association between common mediators and osteogenesis and adipogenesis.

In conclusion, bone histology studies in humans indicate that bone mineral density are affected by adipokines that may be as a result of genetic variation in populations.

Serum visfatin and its genotype may be correlated with BMD, that may have had clinically application for assessing the susceptibility to osteoporosis in type 2 diabetic patients.

Nonetheless, further studies are warranted to clarify the effects of adipocytokines in bone metabolism.

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