Association of Vitamin D Receptor gene polymorphisms with the occurrence of low bone density, osteopenia, and osteoporosis in patients with type 2 diabetes

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Abstract

Background Diabetes, osteoporosis, and Vitamin D deficiency are interrelated. The association of the Vitamin D receptor (VDR) gene with bone density is not established in different populations. This study analyze the association between five VDR gene polymorphisms (Fok1 (rs2228570), Taq1 (rs731236), Bsm1 (rs1544410), Apa1 (rs7975232), and EcoRV (rs4516035)) and the occurrence of low bone density (LBD)/osteopenia/osteoporosis in Iranian type 2 diabetics (T2D).

Methods This study contained 165 patients with T2D. Separated for gender and polymorphism, crude and adjusted (for age and BMI), logistic regression analysis provided odds ratios (ORs). The most common haplotype was used as the reference for each type of VDR gene polymorphism.

Results The occurrence of T2D was 13.03% (165/1266) for the people dwelling in the city of Sanandaj in 2012, until 2013. 13.92% and 81.29% of participants with T2D had osteoporosis and vitamin D deficiency, respectively. In women, tt genotype significantly decreased the risk of LBD/osteopenia/osteoporosis versus Tt genotype, after adjusting for BMI and age (OR=0.18, CI: 0.03-0.97). Conversely, the EE genotype increased the risk of LBD/osteopenia/osteoporosis versus Ee genotype (OR=7.64, CI: 2.03-28.72).

Conclusions Among the patients with T2D, Vitamin D deficiency and osteoporosis were prevalent, and some genetic variations in VDR were significantly associated with osteoporosis. In women, a weak protective role in LBD/osteopenia/osteoporosis was found for tt variant of TaqI polymorphism. More importantly, EE variant of EcoRV polymorphism had a strong significant association with increasing the risk of LBD/osteopenia/osteoporosis. Further ethnicity-based cohort studies based on a large study group of T2D patients are recommended.

Background

Diabetes, osteoporosis, and Vitamin D deficiency are common (1–3) and interrelated (4–6), globally. Osteoporosis and type 2 diabetics (T2D) are among the major risk factors of deaths in any population (7). The impact of genetic and environmental factors on these outcomes is demonstrated, before (8–10).

Osteoporosis is verified as a hereditary illness; evidence of 60–80% of bone density to be controlled by different genes(11). To date, a list of candidate genes is available, including the Vitamin D receptor (VDR) genes. The VDR coding gene was the first gene to be considered as a major locus for genetic effects on bone density (12, 13). Almost 50% of the population worldwide are affected by Vitamin D insufficiency (2). Vitamin D has a well-known role in regulating the normal functioning of the pancreatic beta cells and is with both types of diabetes (14, 15). The function of Vitamin D is mediated through VDR (16). Among different VDR polymorphisms, Fok1 (rs2228570), Taq1 (rs731236), Bsm1 (rs1544410), Apa1 (rs7975232), and EcoRV (rs4516035) are strongly suspected to alter the activity of the VDR protein (17).

During the last decade, multiple studies showed associations between T2D and polymorphisms of the VDR genes (BsmI (18–21), FokI (18, 19, 21) and TaqI (20)), however, studies denying such associations could also be found (22, 23). Since the reported results of VDR SNPs (Single nucleotide polymorphisms) may be population-specific, it is necessary to study genetic polymorphisms in different ethnic groups, separately.

This study aimed to analyze the association between VDR gene polymorphisms (TaqI, BsmI, ApoI, FokI, and EcoRV) and occurrence of LBD/osteopenia/osteoporosis in a group of Iranian type 2 diabetics, participated in phase III of the Iranian Multi-Center Osteoporosis Study (IMOS) in the city of Sanandaj, capital of Kurdistan Province (latitude 35.2458° N, longitudes 47.0092° E, population in 2011: 446,000) (24).

Methods

Participants

This study utilized the secondary data of phase III of IMOS. Primary participants had been selected based on a randomized clustered sampling of all regions of the city between 2012 and 2013. Then and there in 2015, a genetic study of five VDR polymorphisms was performed on remained stored blood of 1032 samples of the 1266 primary participants in the Sanandaj city (4). Questionnaire and laboratory testing data were available.

Exclusion criteria

The data records of primary participants were excluded from our study when there was no mention of diabetes in the questionnaire and laboratory data of FBS and HbA1c were both missing. Other exclusion criteria were insulin usage in participants aged less than 25 years in the absence of other oral hypoglycemic agents as well as mention of the type one diabetes in the questionnaire.

Inclusion criteria

Participants of the IMOS study were healthy subjects aged over 20 years (4). The present study included those participants who considered to be afflicted with T2D. To achieve a realistic estimation for individuals with T2D, we were inspired by the method of studies such as those conducted on the NHANES population (25, 26). According to exclusion criteria, each remained participant with self-report of diabetes in the primary questionnaire, even if they were taking no diabetes medication, were sorted out as having T2D. Among records in which there was no positive history of diabetes, participants with FBS $\geq$ 126 mg/dl or HbA1c $\geq$ 6.5% were assumed as undiagnosed T2D.

Osteoporosis status

All participants in the initial study were invited to perform bone marrow densitometry (BMD). BMD measurements at three sites (the lumbar (l_2-L_4) spine, Hip, and femoral neck) were performed using Dual-Energy X-ray Absorptiometry (Norland XR46) (4). Considering the age, gender, and menopausal status, we assessed the records of the BMD measurements (g/cm^2) based on the criteria of the World Health Organization (WHO) (27). The DEXA variables were expressed also by T-score and Z-score. We used the z-score in pre-menopausal women and men under age 50. For the rest, we used T-Score. The recruited participants were divided into two groups: normal and LBD/osteopenia/osteoporosis. It means that the second group included participants who were diagnosed with either low bone density (LBD), osteopenia or osteoporosis at any of the three mention sites (27).

Vitamin D deficiency

Considering Endocrine Society Clinical Practice Guideline (28), vitamin D deficiency was defined as 25-hydroxyvitamin D below 20 ng/ml (50 nmol/liter).

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes, and DNA extraction was performed using the standard phenol-chloroform method (29). Five site VDR SNPs including Fok1 (rs2228570), Taq1 (rs731236), Bsm1 (rs1544410), Apa1 (rs7975232) and EcoRV (rs4516035) were analyzed using Taq-Man 5'-exonuclease SNP assay (Applied Biosystems, FosteCity, CA, USA) by allelic discrimination method with the ABI 7300 Real-Time PCR System (Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA). Negative controls were included to ensure the accuracy of genotyping. Thermal cycling conditions and data analysis were according to the standard manufacturer's instructions (4).

Naming the genotypes is as follows. The first letter of the gene's name being repeatedly capitalized indicates the homozygous wild variant of the polymorphism, the first letter of the gene's name being repeatedly written in the lower case indicates the homozygous variant, and finally, the big letter followed by a small letter indicates a heterozygous variant.

Statistical analysis

Considering the SAMPLE guideline (30), in the case of continuous variables with normal distribution, the data were summarized as mean and standard deviations (SD), otherwise, the data were reported as the median and interquartile range (IQR=(Q25, Q75)). Categorical variables were expressed as numbers (percentages). The normality assumption was checked using statistical tests (Kolmogorov-Smirnov/Shapiro-Wilk test) and graphical assessments (histograms, Q–Q plots, and box-plots). Evaluating relationships between the main outcomes and explanatory variables (bivariate analysis), the following tests were performed: Chi-squared test (Pearson/Fisher test), t-test, Mann-Whitney U test, and Pearson/Spearman correlation coefficient.

Logistic regression analysis was applied to evaluate the association of osteopenia/osteoporosis occurrence with VDR gene polymorphisms. The most common haplotype was used as the reference for each type of VDR gene polymorphism. Separately for each gender and polymorphism, crude and adjusted (for age, BMI) odds ratios (ORs) were calculated by logistic regression analysis. All analyses were performed at a significance level of 5% and confidence intervals (CI) were calculated for each relevant parameter. The software Stata (ver. 11) was used to analyze the data.

Results

Among 1266 data records of Sanandaj city, 165 (13.03%) were finally selected as potential cases affected by T2D based on self-reports on the incident of the diseases, history of diabetes medication and recorded laboratory data. The self-report of T2D among participants was 27.88% (46/165), referring to the primary questionnaire.

According to the exclusion criteria, 261 of 1266 (20.62%) records were excluded because of missing necessary data for defining diabetes status. Of 1266 participants, five (3.9%), aged < 25, were excluded by diagnosing T1DM. Missing data of BMD measurements were 4.24% (7/165) among participants with T2D.

The final selected participants for this study (N = 165), aged 26–83 years, mostly female (61.82%). Their mean (SD) of age was 50 (12) years. The demographic data of the participants with T2D is illustrated in Table 1. Considering WHO osteoporosis criteria (27), 13.92% (22/158) of T2D participants have osteoporosis in any of three primary sites; the occurrence of osteoporosis was significantly higher (P = 0.043) in women than men

(15.15% (15/99) vs. 11.86% (7/59); respectively). The occurrence of vitamin D deficiency was 81.29% (126/155) among participants based on the Endocrine Society Clinical Practice Guideline (28). BMD results of hip and L_2-L_4 were significantly higher in men than women (Table 1).

Table 1
Demographic Data of Participants with type 2 diabetes enrolled in phase III of IMOS study in the city of Sanandaj

variables		**Men**	**women**	**Total**	**P-Value**
Num(%)		**63 (38.18)**	**102 (61.82)**	**165 (100)**	
		N (%)	N (%)	N (%)	
Serum Vitamin D status (nmol/l)	deficient (< 50)	49 (85.96)	77 (78.57)	126 (81.29)	0.316)
	insufficient (50-72.5)	4 (7.02)	15 (15.31)	19 (12.26)	
	sufficient to optimal (≥ 72.5)	4 (7.02)	6 (6.12)	10 (6.45)	
	Total	57 (100)	98 (100)	155 (100)	
Bone density status	Normal	23 (38.98)	55 (55.56)	78 (49.37)	.044
	LBD/osteopenia/osteoporosis	36 (61.02)	44 (44.44)	80 (50.63)	
	Total	59 (100)	99 (100)	158 (100)	
		Mean (SD)	Mean (SD)	Mean (SD)	
Age (Yrs.)		51.02 (12.85)	50.76 (10.07)	50.86 (11.17)	.889
High (cm)		169.00 (6.06)	155.43 (5.57)	160.50 (8.73)	.000
weight (kg)		79.10(12.8 5)	73.60 (11.08)	75.66 (12.03)	.005
BMI(kg/m^2)		27.63 (3.85)	30.46 (4.26)	29.40 (4.32)	.000
BMD (g/cm^2)	Hip	.97(.12)	.91 (.15)	.93 (.14)	.009
	L_2-L_4	.97(.13)	.92 (.16)	.94 (.15)	.030
	Femoral	.88 (.12)	.85 (.14)	.86(.13)	.105
Variables with normal distribution describe as mean (SD). P-Value < .05 assumed significant and bolded.					

Table 2 shows the laboratory Data of participants for the whole sample and both genders. Comparing men and women, there was no significant difference in the mean of serum vitamin D, FBS, HbA1c, P, ALKs and ALK-B levels, however, the levels of serum Ca, Alb and creatinine showed significant differences.

Table 2

Differences in laboratory data between two sexes of participants with type 2 diabetes in phase III of IMOS study in the city of Sanandaj

variables	Men		Women		Total		P-Value
FBS (mg/dl)	142	(126, 171)	132	(111,161)	137	(118,163)	.129
HbA1c (%)	6.3	(5.8,7.1)	6.6	(6.1,7.6)	7.6	(6.0,7.5)	.062
vitamin D (nmol/l)	25.9	(18.8,40.3)	22.6	(13.0,44.3)	25.1	(13.6,40.7)	.193
Ca (mg/dl)	9.7	(9.3,9.9)	9.3	(9.1,9.7)	9.5	(9.1,9.8)	.003
P (mg/dl)	3.7	(3.3,4.2)	4	(3.5,4.4)	3.9	(3.4,4.4)	.065
PTH (pmol/L)	2.5	(1.8,3.4)	3.05	(1.9,4.3)	2.8	(1.9,4)	.066
Alk (IU/L)	184	(154,227)	181.5	(159,215)	182	(156,219)	.948
Alk-B (ng/ml)	14.4	(11.2,17.3)	13.85	(11.2,17.8)	14	(11.2,17.5)	.920
Alb (g/dl)	4.8	(4.6,4.9)	4.6	(4.4,4.8)	4.7	(4.5,4.9)	.000
Creatinine (mg/dl)	.9	(.7,1.1)	.7	(.6, .8)	.8	(.7,.9)	.000

All variables had skewed distribution; the Mann-Whitney U test was performed to compare the variables in two independent groups: male and female. The data presented as Mdn (Q_1, Q_3); Mdn = median; Q_1 = 25th percentile; Q_3 = 75th percentile. P-Value < .05 were assumed as significant and bolded.

Table 3 shows the frequency of the VDR gene's polymorphism in the participants with T2D according to gender. There was no significant difference between the two genders in the frequency of each of the studied VDR gene's polymorphisms. The most common genotypes for Bsm1, Fok1, Apa1, Taq1 and EcoRV were Bb (65.13%), FF (52.29%), Aa (46.36%), Tt (46.71%) and Ee (42.38%), respectively.

Table 3
Frequency of VDR gene's polymorphisms (Bsm1, Fok1, Apa1, Taq1, EcoRV) in women versus men in participants with type 2 diabetes

Gene	polymorphism	Male		Female		Total		P-Value
Bsm1	Bb	40	(70.18)	59	(62.11)	99	(65.13)	.544[a]
	BB	14	(24.56)	31	(32.63)	46	(29.61)	
	bb	3	(5.26)	5	(5.26)	8	(5.26)	
	Total	57	(100)	95	(100)	152	(100)	
Fok1	FF	29	(50.88)	51	(53.13)	80	(52.29)	.603[a]
	Ff	25	(43.86)	36	(37.50)	61	(39.87)	
	ff	3	(5.26)	9	(9.38)	12	(7.84)	
	Total	57	(100)	96	(100)	153	(100)	
Apa1	Aa	25	(44.64)	45	(47.37)	70	(46.36)	.894[b]
	AA	21	(37.50)	32	(33.68)	53	(35.10)	
	aa	10	(17.86)	18	(18.95)	28	(18.54)	
	Total	56	(100)	95	(100)	151	(100)	
Taq1	Tt	26	(46.43)	45	(46.88)	71	(46.71)	.685[b]
	TT	19	(33.93)	37	(38.54)	56	(36.84)	
	tt	11	(19.64)	14	(14.58)	25	(16.45)	
	Total	56	(100)	96	(100)	152	(100)	
ECORV	Ee	24	(42.86)	40	(42.11)	64	(42.38)	.928[b]
	EE	25	(44.64)	41	(43.16)	66	(43.71)	
	ee	7	(12.50)	14	(14.74)	21	(13.91)	
	Total	56	(100)	95	(100)	151	(100)	

P-Value < .05 is bolded and assumed as significant. [a]: based on Fisher's exact test, b: based on Pearson Chi2 test

In participants with T2D, the association of the VDR gene's polymorphism (Bsm1, Fok1, Apa1, Taq1, and EcoRV) with osteoporosis is shown in Table 4. Models 1 and 2 show the crude association and the association adjusted

for BMI and age, respectively. In women, tt genotype significantly decreased the risk of LBD/osteopenia/osteoporosis vs. Tt genotype, after adjusting for BMI and age (OR = 0.18, CI: 0.03–0.97). Conversely, the EE genotype increased the risk of LBD/osteopenia/osteoporosis vs. Ee genotype in women, after adjusting for BMI and age (OR = 7.64, CI: 2.03–28.72).

Table 4
Association of the VDR gene's polymorphism (Bsm1, Fok1, Apa1, Taq1, and EcoRV) with LBD/osteopenia/osteoporosis in both genders, according to BMI and age in participants with

LBD/osteopenia/osteoporosis vs NL		**men**	**women**	**men**	**women**
Fok1		Ff vs FF	Ff vs FF	ff vs FF	ff vs FF
	Model 1	1.09(.36,3.29)	.98(.42,2.32)	.31(.02,3.78)	.13(.02,1.12)*
	Model2	.98(.29,3.23)	.85(.29,2.47)	.32(.02,4.76)	.29(.03,2.81)
Bsm1		BB vs Bb	BB vs Bb	bb vs Bb	bb vs Bb
	Model 1	.72(.21,2.49)	1.51(.63,3.63)	.27(.02,3.24)	2.13(.33,13.70)
	Model 2	.69(.18,2.66)	.88(.29,2.68)	.40(.03,5.78)	.77(.09,6.27)
Apa1		AA vs Aa	AA vs Aa	aa vs Aa	aa vs Aa
	Model 1	.63(.19,2.09)	.85(.34,2.13)	.71(.15,3.22)	.70(.23,2.13)
	Model2	.69(.19,2.53)	.61(.19,1.96)	1.09(.19,6.21)	.87(.22,3.47)
Taq1		TT vs Tt	TT vs Tt	tt vs Tt	tt vs Tt
	Model 1	1.26(.37,4.23)	1.41(.59,3.40)	1.96(.42,9.11)	.33(.08,1.34)
	Model2	1.80(.46,6.95)	.92(.30,2.84)	2.63(.46,14.86)	.18(.03,.97)**
EcoRV		EE vs Ee	EE vs Ee	ee vs Ee	ee vs Ee
	Model 1	1.27(.40,4.02)	3.52(1.39,8.87)***	1.79(.29,11.13)	1.25(.34,4.53)
	Model2	1.26(.36,4.41)	7.64(2.03,28.72)***	1.17(.17,8.07)	.91(.16,5.11)

Model 1: Crude model; Model 2:Adjusted for BMI and age; NC: not calculated due to empty cells; LBD: low bone density ; the data presented as OR(CI intervals) and, in significant relationship after adjusting: numbers it was bolded, *: P-value is marginally significant(< .1), **: P-value is significant(< .05); ***:p < .01;****: p < .001

Sensitivity Analysis

Among 165 selected participants, only 46 (27.88%) had mentioned being diabetic in their primary questionnaire. It was not possible to perform the same logistic analysis due to many empty cells in this group. We separately repeated the association analysis in another subgroup of participants in which $HbA_1c \geq 6.4$ and $FBS \geq 126$. The sensitivity analysis is illustrated in Table 5. Regarding the associations of EcoRV polymorphism, the sensitivity analysis was consistent with the primary analysis.

Table 5

Association of the VDR gene's polymorphism (Bsm1, Fok1, Apa1, Taq1, and EcoRV) with LBD/osteopenia/osteoporosis in both genders, according to BMI and age in the T2D participants with $HbA_1c \geq 6.4\%$ and FBS ≥ 126 mg/dl

LBD/osteopenia/osteoporosis vs NL		**men**	**women**	**men**	**women**
Fok1		Ff vs FF	Ff vs FF	ff vs FF	ff vs FF
	Model 1	8 (.66,97.31)	1.24 (.31,4.93)	NA	1(not calculated)
	Model2	15.86 (.60,422.28)*	.53(.07, 3.96)	NA	1(not calculated)
Bsm1		BB vs Bb	BB vs Bb	bb vs Bb	bb vs Bb
	Model 1	1(not calculated)	1.86(.46,7.48)	1(not calculated)	1.86 (.10, 34,44)
	Model 2	1(not calculated)	1.26 (.21,7.60)	1(not calculated)	2.35(.11,49.00)
Apa1		AA vs Aa	AA vs Aa	aa vs Aa	aa vs Aa
	Model 1	.28(.02,3.52)	.7(.17,2.91)	1(not calculated)	1(not calculated)
	Model2	.37(.02,6.00)	.58(.09,3.49)	1(not calculated)	1(not calculated)
Taq1		TT vs Tt	TT vs Tt	tt vs Tt	tt vs Tt
	Model 1	1(not calculated)	1.8(.41,7.81)	.6(.03,13.58)	.72(.10,5.17)
	Model2	1(not calculated)	1.19(.17,8.24)	1.75(.04,75.19)	.34(.03,3.77)
EcoRV		EE vs Ee	EE vs Ee	ee vs Ee	ee vs Ee
	Model 1	8(.58,110.27)	5.04(1.13,22.50)***	1(not calculated)	.93(.08,11.18)
	Model2	3.63(.17,77.74)	7.24(1.00,52.47)***	1(not calculated)	.87(.05,16.29)

LBD/osteopenia/osteoporosis vs NL	**men**	**women**	**men**	**women**

Model 1: Crude model; Model 2:Adjusted for BMI and age; NC: not calculated due to empty cells; LBD: low bone density ;the data presented as OR(CI intervals) and, in significant relationship after adjusting: numbers it was bolded, *: P-value is marginally significant(< .1), **: P-value is significant(< .05); ***:p < .01;****: p < .001, NA: data not available due to empty cells

Discussion

This population-based cross-sectional study demonstrated that the occurrence of T2D was 13.03% (165/1266) in the people who were dwelling in the city of Sanandaj in 2012 to 2013. 13.92% (22/158) and 81.29% (126/155) of the participants with T2D had osteoporosis and vitamin D deficiency, respectively (Table 1).

Among diverse ethnic groups worldwide, the reported rate of prevalence of diabetes is between 7.8% and 15.5% (31). In our study, the prevalence of T2D in people over 26 years of age was about 13%. Recently, a study on the Iranian population over the age of 30 years reported a diabetes prevalence around 14% in a population over the age of thirty (31), consistent with our findings. However, the self-report of T2D among our participants was only 27.88% (46/165). We attribute this low rate of self-report to the inadequacy of the primary questionnaire (questionnaire of IMOS III), in which the participants were not asked about the incidence of diabetes, specifically, and merely were asked to name their past and present illnesses.

A recent meta-analysis on the overall prevalence of osteoporosis in the Iranian population indicates that low bone density (LBD, osteoporosis, and osteopenia) is a common problem among individuals older than 30 years. Moreover, the prevalence becomes “much higher” when age is more than 50 (32). The occurrence of osteoporosis in our T2D participants, with a mean age of 50 years (min: 26 and max:83), was 15.15% (N = 15) in women and 11.86% (N = 7) in men. Altogether, the occurrence of LBD/osteopenia/osteoporosis was 44.44% in women and 61.02% in men (P = 0.044) (Table 1). In 2008, a study on postmenopausal women with T2D in Sanandaj showed higher prevalence rates of osteoporosis and osteopenia (33). Given that all of the study participants were menopausal, this seems to be a logical difference. Furthermore, reports of the International Osteoporosis Foundation (IOF) in 2011 shows that the prevalence of osteopenia/osteoporosis in the Iranian population aged over 50 was about 81.9% in women and 61.1% in men (24). Due to the wide range of age in our participants, and given that age is one of the most important risk factors for bone loss, we conclude that the distinct age range difference between various understudied populations can justify the apparent difference in these results.

Iran is a country with high rates of long-term sunshine duration which contributes to its geographical location; in high latitude locations, the reports often stipulate that the peak of sunshine hours exceeds 9 hours per day. This especially includes the cities of Sanandaj and Kermanshah, which even in the autumn have high sunny hours (34). Our results showed 81.29% of T2D participants suffered from vitamin D deficiency and, there was no significant difference between the two genders in this issue (Table 2). Our results have concordance with a study in the Kermanshah province in which they reported that 82.2% of patients with T2D had vitamin D deficiency (35). The Kermanshah province (provincial capital: Kermanshah) and Kurdistan province (provincial capital: Sanandaj) are adjacent to each other. The paragraph can be summarized as follows, it seems that despite a high

rate of long-term sunshine duration in Iran (34), we still have a serious problem with vitamin D deficiency among the Iranian population (36); especially in diabetic patients (34, 35).

Furthermore, vitamin D has a well-known role in regulating the normal functioning of the pancreatic beta cells, and there is a relationship between the presence of vitamin D deficiency and the incidence (37), control (38), complications and mortality due to diabetes (39). VDR gene is the most studied gene worldwide among genes implied on the osteoporosis genetics (40). Although many studies report the VDR gene as an influence on bone mineral density (41, 42), the association of the VDR gene with bone density has not been established in different ethnic populations. Results of a recent systematic review on relationships between VDR gene polymorphisms and postmenopausal osteoporosis (PMOP) susceptibility revealed that different VDR polymorphisms have dissimilar effects on the BMD or risk of postmenopausal osteoporosis (43). In our study, there was no significant difference between the two genders in the frequency of the VDR gene's polymorphism in participants with T2D (Table 3).

It is shown that FokI polymorphism (rs2228570) plays a role in increasing the risk of osteoporosis (44). Additionally, BsmI polymorphism (rs1544410) is associated with low bone mineral density and osteoporosis (45). The present study did not detect an association between LBD/osteopenia/osteoporosis and any polymorphisms of Fok1, BSMI, and ApaI in the patients who seemed to be affected by T2D. However, our result showed the TaqI variant has a weak protective role for LBD/osteopenia/osteoporosis in women with T2D (tt Vs TT; OR: 0.18; CI95%: 0.03–0.97). Likewise, Gnanaprakash et al (16) showed that TaqI Polymorphism protects the Asian Indian population against T2D, perhaps through influencing anthropometric and biochemical parameters. It has already been proven that VDR TaqI affects mRNA stability which leads to biological functions of Vitamin D (43). Moreover, we found a strong significant association between a variant of EcoRV polymorphism and an increase in the risk of LBD/osteopenia/osteoporosis in women with T2D (Table 4 and Table 5). Interestingly the possibility of LBD/osteopenia/osteoporosis in women with EE genotype was 7.6 times higher than the women with Ee genotype (EE Vs Ee; adjusted OR: 7.64; CI95%:2.03–28.72). Although the significant association persisted after adjustment for age and BMI, it was not independent of BMI and age. There was no significant relationship between LBD/osteopenia/osteoporosis and types of EcoRV genotypes in men. To our knowledge, EcoRV SNP has never been studied in connection with osteoporosis in patients with T2D and, it is the first study in which the association of EcoRV polymorphism with LBD/osteopenia/osteoporosis is investigated in T2D participants.

Unlike other studies, we found no significant relationship between low bone density (LBD/osteopenia/osteoporosis) and types of ApoI, FokI and BsmI genotypes. Although, the sensitivity analysis showed that, only in men, the FF genotype increases the risk of LBD/osteopenia/osteoporosis in comparison to Ff with a significant effect size (FF Vs Ff; adjusted OR:15.86; CI95%: 0.60-422.28). There may be possible reasons for such discrepancies, including heterogeneity between different ethnicity, environmental factors, and diversity in a combination of study populations, small sample sizes, different diabetes duration, the different mean age of population study and any other factors which may mask/exaggerate the genetic effects. Due to the main reason for the discrepancies in the results of different studies, further ethnicity-based cohort studies in which a large study group of T2D patients, with a comprehensive questionnaire and medical report, is available, are highly recommended.

The present findings should be put in the context of strengths and weaknesses. Observational controlled researches have their deficiency, and cohort design is a better way to overcome such deficiencies (4). The current study arose from the IMOS study (phase III) (24). The participants of phase III had been selected based on a randomized clustered sampling of all regions of the city between 2012 and 2013. To design a cohort study, the prevalence of osteoporosis from the previous phases and the design effect were taken into account while the sample size for the third phase of the IMOS study was calculating. Therefore, the results of our study could be generalizable to the general population. Besides, the statistical results of the prevalence of T2D and its related osteoporosis in our study were consistent with other conducted population-based studies on Iranian people, at the same time interval. Therefore, we concluded that this consistency was a confirmation for the representativeness of the current study for the whole population. This enabled us to perform multivariate analysis of different predictive factors of LBD/osteopenia/osteoporosis. However, the identified associations were difficult to interpret because of the limitations of the study including cross-sectional design from which causation cannot be established. Moreover, the inevitable limitation of this study was the inability to accurately select the population with T2D due to the non-availability of precise data of diabetes diagnosing of participants, especially previous diagnose of diabetes and duration of diabetes. Using sensitivity analysis, we tried to address this limitation. Another drawback was the lack of access to information about the duration of diabetes, as a factor influencing the fracture risk.

Conclusions

To sum up, vitamin D deficiency and LBD/osteopenia/osteoporosis are highly prevalent among patients with T2D who lived in Sanandaj city of Iran. TaqI variant had a weak protective role for LBD/osteopenia/osteoporosis in women with T2D. Therefore, a strong significant association between a variant of EcoRV polymorphism and an increase in the risk of LBD/osteopenia/osteoporosis in women with T2D was found.

Due to high discrepancies among different studies, performing ethnicity-based cohort studies based on a large study group of T2D patients, benefitting from the comprehensive questionnaires and medical reports, are highly recommendable.

Abbreviations

T2D
Type 2 diabetics; VDR:Vitamin D receptor; SNP:Single nucleotide polymorphisms; LBD:Low bone density; IMOS:Iranian Multi-Center Osteoporosis Study; EMRI:Metabolism Clinical Sciences Institute; BMD:Bone marrow densitometry; SD:Standard deviation; IQR:Inter quartile range; OR:Odds ratio; CI:Confidence interval.

Declarations

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Authors' contributions

MG, AAK, BL, and MRM conceived and designed the study. MG, FR, MMA and ENE wrote the manuscript. MG, FZ, PK, and AK analyzed and interpreted the data. All authors have approved the final submission.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the Endocrinology and Metabolism Clinical Sciences Institute (EMRI); the authors' affiliated institution. Given the retrospective nature of this study, patient consent was not required.

Consent for publication

Not applicable.

Competing interests

The authors declare to have no conflict of interest.

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