

Association between vitamin D receptor BsmI (rs1544410) gene polymorphism and serum vitamin D, serum calcium, and osteoporosis risk in women over 40 years

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Abstract

Objectives: We investigated the relationships between the vitamin D receptor BsmI (rs1544410) gene polymorphism, serum vitamin D and calcium levels, and the risk of osteoporosis in women aged 40 years and older.

Methods: This case-control investigation enrolled participants (30 cases and 30 controls) classified into normal and osteoporotic groups using a dual-energy X-ray absorptiometry (DEXA) scan. Vitamin D and calcium levels were obtained from peripheral blood samples. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed to detect the BsmI polymorphism. Differences between groups were analyzed using an analysis of variance (ANOVA) test.

Results: 60 individuals (30 cases and 30 age-matched controls) were enrolled. The lumbar and femoral T-scores, as well as calcium levels, were significantly lower in the cases compared to the controls ($P < 0.005$). Among postmenopausal women, the lumbar T-scores significantly differed between cases with VDR polymorphisms ($P = 0.003$) and controls ($P = 0.001$). Among premenopausal women, there was only a significant difference in VDR polymorphisms in the control group ($P = 0.006$). Calcium and vitamin D levels were significantly different between the VDR polymorphisms in cases and control groups, but only among postmenopausal women ($P < 0.005$ for all comparisons). No significant differences were observed between the VDR polymorphism in terms of femoral T-scores, neither in the cases nor the controls (P for all > 0.005).

Conclusion: This study found no differences in VDR polymorphism between individuals with and without osteoporosis. Additionally, the lumbar T-scores, calcium, and vitamin D levels were significantly different between the VDR polymorphisms in the case and control groups among postmenopausal women.

Keywords: BsmI gene polymorphism, vitamin D receptor, osteoporosis, post-menopause women.

1. Background

Osteoporosis, recognized as the most

prevalent chronic metabolic bone disorder, is defined by a widespread deterioration of bone mass and microarchitecture, leading to an

increased susceptibility to fragility fractures (1). In 2024, the prevalence of osteoporosis among the Iranians was reported as 38% and 25% for women and men, respectively (2). Due to the silent nature of the disease, osteoporosis remains a significant secondary health challenge until fractures occur (3). In post-menopause women, the risk of bone density reduction and osteoporosis increases due to the lack of estrogen (4).

Osteoporosis is a multifactorial condition with genetic and acquired risk factors. Aging, Caucasian race, and post-menopause are some of the crucial risk factors (5). Moreover, an insufficiency of calcium and vitamin D is strongly associated with the development of osteoporosis (6). Vitamin D levels play a crucial role in regulating mineral homeostasis and bone metabolism (7). Vitamin D is also essential in bone metabolism and induces bone synthesis by binding to its receptor, the vitamin D receptor (VDR), which stimulates skeletal tissue and regulates bone turnover (8). VDR affects the expression and transcription of genes that are involved in the formation of bone mineral density (BMD) and calcium absorption, including osteocalcin and calcium-binding proteins (9, 10).

Based on previous investigations, the VDR gene and its polymorphisms is correlated with the development and progression of osteoporosis (11-13). Morrison and colleagues were the first to indicate that genetic variations in the VDR gene could serve as predictors for spinal and femoral BMD in Caucasians (14, 15). Thereafter, numerous studies have found that VDR genetic variants, including FokI (rs10735810), ApaI (rs7975232), and BsmI (rs1544410), are associated with BMD and osteoporosis across various ethnicities (16-19). However, the relationship between a specific VDR genotype and its potential association with reduced BMD and risk of fractures remains unclear (20, 21). Furthermore, to our knowledge, there have been no investigations into the association of VDR gene polymorphisms in the Mashhad, Iran, population.

Hence, we assessed the association between

the BsmI variant and serum vitamin D and calcium levels, as well as the risk of osteoporosis among women in Mashhad City who were over 40 years old.

2. Objectives

We investigated the relationships between the vitamin D receptor BsmI (rs1544410) gene polymorphism, serum vitamin D and calcium levels, and the risk of osteoporosis in women aged 40 years and older.

3. Methods

Study participants

This case-control investigation was conducted between September 2019 and 2021, and 60 women aged over 40 years, including 30 subjects with osteoporosis (cases) and 30 age-matched subjects without osteoporosis (control group), were enrolled. Demographic data and clinical risk factors, including age, menopausal status, past medical history, and underlying diseases, were collected using a standardized questionnaire. The control group was selected from subjects referred to the hospital or private clinics in Mashhad, Iran, due to musculoskeletal complaints. The exclusion criteria for both groups were as follows: using oral or injectable corticosteroids, history of hysterectomy and/or bilateral salpingo-oophorectomy, chronic diseases, and pregnancy (Figure 1).

BMD assessment and osteoporosis definition:

BMD was assessed at the lumbar spine (L1–L4) and femoral neck using dual-energy X-ray absorptiometry (DEXA) scanning. Women exhibiting femoral neck or spinal T-scores of -2.5 or lower were classified as osteoporotic women (cases) (22).

Biochemical measurements:

A 10 mL blood sample was drawn from the antecubital vein following 12 hours of fasting. Calcium levels (mg/dL) were measured using a Prestige Chemistry Analyzer (Bionik kit, Germany) and a Roche Hitachi 704 (Germany). Vitamin D levels (IU) were measured using the

Cobas e 411 Analyzer (Roche kit, Germany) using standard protocols.

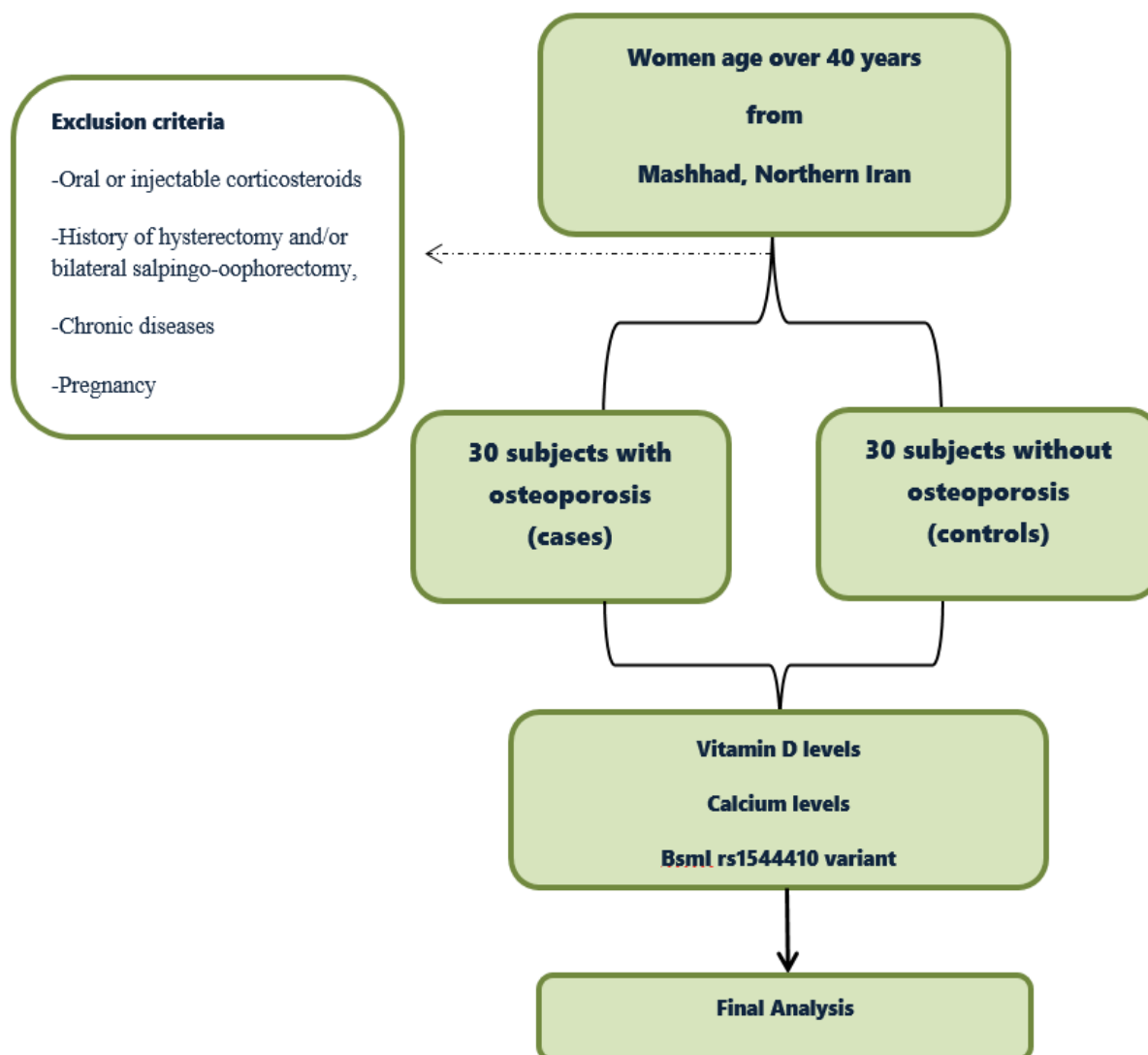


Figure 1. Flowchart of the study

DNA extraction VDR genotyping:

After using the Vacutainer system blood collection method, DNA was isolated from bloodspots dried on special NucleoSafe cards (Macherey-Nagel, Germany) utilizing standard proteinase K (GenFanAvaran, Iran) digestion, phenol-chloroform (Merck, Darmstadt, Germany) extraction, and ethanol precipitation (23). The quality and purity of DNA samples were checked using the spectrophotometric method (24). The BsmI rs1544410 polymorphism was determined using polymerase chain reaction and

restriction fragment length polymorphism (PCR-RFLP) analysis with specially designed primers generated using Gene Runner v5.3 software. The PCR reaction system contains 10 × buffer = 2.5 µL, 1.5 µL MgCl₂ (50 mM), one µL dNTPs (40 mM), 10 forward (GGGAGACGTAGCAAAAGG) and reverse primers (AGAGGTCAAGGGTCACTG) (60 pmol), 2.3 µL DNA template (100 ng), 0.15 µL Taq polymerase (5 U/ µL), and 16.35 2.3 µL sterile water. All PCR reactions were conducted at 95° C for 15 minutes, followed by 30 cycles consisting of 99°C for 1 second,

62°C for 10 seconds, and an extension phase at 72°C for 20 seconds. The final extension step was performed at 72°C for 2 minutes using an automated thermal cycler (ASTEC, PC-808, Japan). The amplified products were analyzed by electrophoresis on an 8% polyacrylamide gel. *BsmI* genotypes were analyzed in the vicinity of the restriction enzyme *Apal* (Thermo Scientific, Ref. ER1411) for 16 hours at 37°C.

Ethical consideration:

All subjects provided written informed consent before their inclusion. Moreover, the study protocols were approved by the Ethics Committee of Islamic Azad University, Mashhad, Iran, with the ethics code IR.IAU.MSHD.REC.1399.053.

Statistical analysis:

The assessment of data normality was conducted using the Kolmogorov–Smirnov test. For comparing normally distributed data, the Student's t-test was employed; for

non-normally distributed data, the Mann–Whitney U-test was used. Categorical data are expressed as counts (%) and analyzed using the chi-square test. The variable differences in VDR polymorphisms between the case and control groups were tested using the ANOVA test. All data were analyzed using SPSS version 26, with P-values < 0.005 considered statistically significant.

4. Results

A total of 60 participants (30 cases and 30 age-matched controls) were enrolled. Table 1 depicts the differences in characteristics between the cases and controls. The lumbar (-3.13 ± 0.32 vs. -0.08 ± 1.34) and femoral T-scores (-2.39 ± 0.84 vs. -0.44 ± 1.52) and calcium levels (9.30 ± 0.50 vs. 9.56 ± 0.46) were significantly lower in the cases compared to the controls ($P < 0.005$). No significant differences were found between the groups in terms of age, menopausal status, vitamin D, and VDR polymorphism.

Table 1. Characteristics of the study population

Characteristics	Case (n= 30 (50%))	Control (n= 30 (50%))	P-value
Age (year)	60.86±8.45	58.96±8.00	0.375
Menopausal status			
Post menopause	25 (83.30%)	22 (73.30%)	0.347
Pre menopause	5 (16.70%)	8 (26.70%)	
Lumbar T-score	-3.13±0.32	-0.08±1.34	0.001
Femoral T-score	-2.39±0.84	-0.44±1.52	0.001
Calcium levels	9.30±0.50	9.56±0.46	0.033
Vitamin D levels	21.35±12.99	25.49±11.76	0.094
VDR polymorphism			0.291
(AA)	11 (36.70%)	7 (23.30%)	
(AG)	14 (46.70%)	20 (66.70%)	
(GG)	5 (16.70%)	3 (10.00%)	

Abbreviation: VDR: vitamin D receptor

As depicted in Table 2, the lumbar T-scores were significantly different between the VDR polymorphisms in the cases ($P=0.003$) and control groups ($P=0.001$) among the postmenopausal women. Among premenopausal women, a significant difference was observed only in VDR polymorphisms in the control group ($P = 0.006$). Calcium and vitamin D levels

exhibited notable differences between the VDR polymorphisms in cases and control groups, but only among postmenopausal women ($P < 0.005$ for all comparisons). No significant differences were observed between the VDR polymorphism and femoral T-scores in either group, whether in cases or controls.

Table 2. Comparison between the variables stratified by menopause status

Characteristics	Post menopause								Pre menopause							
	Case			P-value	Control			P-value	Case			P-value	Control			P-value
	(AA)	(AG)	(GG)		(AA)	(AG)	(GG)		(AA)	(AG)	(GG)		(AA)	(AG)	(GG)	
Lumbar T-score	- 3.37±0.29	- 3.12±0.27	- 2.77±0.06	0.003	- 1.69±0.27	- 0.11±1.02	2.45±0.49	0.001	- 2.92±0.12	0	- 2.90±0	0.897	- 1.39±0.41	0.52±0.77	1.70±0	0.060
Femoral T-score	- 2.44±0.96	- 2.38±0.86	- 2.38±0.90	0.250	- 1.08±0.71	- 0.30±1.62	2.45±0.21	0.250	- 2.31±0.47	0	- 2.90±0	0.312	- 0.89±1.11	1.25±0.79	1.20±0	0.881
Calcium levels	8.89±0.23	9.41±0.39	9.87±0.63	0.002	9.06±0.23	9.79±0.39	10.10±0.14	0.008	9.40±0.38	0	10±0	0.147	9.30±0.42	9.38±0.35	9.32±0	0.874
Vitamin D levels	12.31±5.37	24.70±5.29	30.00±3.95	0.007	12.86±3.45	25.36±8.58	45.40±4.80	0.003	32.50±5.06	0	44.00±0	0.157	18.30±3.81	27.72±9.53	54.00±0	0.122

5. Discussion

This study investigated the association between the rs1455510 gene polymorphism of VDR and serum vitamin D, calcium, and osteoporosis in women aged 40 years and older. We enrolled 60 individuals (30 with osteoporosis (cases) and 30 without osteoporosis (controls)), and the results showed that the lumbar and femoral T-scores and calcium levels were significantly lower in the cases compared to the controls. Then, we divided the participants according to menopausal status and found that among postmenopausal women, the lumbar T-scores were significantly different between the VDR polymorphisms in both the case and control groups. Among the pre-menopauses, there was only a significant difference between the VDR polymorphisms in the control group. Additionally, calcium and vitamin D levels were significantly different between the VDR polymorphisms in cases and control groups, but only among postmenopausal women. There were no significant differences in the VDR polymorphism in terms of femoral T-scores among any of the groups, either in the cases or the controls.

The VDR gene functions as a nuclear

transcription factor facilitating the effects of 1, 25(OH)₂D₃. This action influences calcium absorption, bone remodeling, and the rate of mineralization. The gene is situated on the long arm of chromosome 12, specifically at the 13q11 locus (12q13.11), comprising 11 exons, with exons 2 through 9 being actively transcribed (25, 26). We found that the AG-genotype of VDR gene polymorphisms was a more prevalent variant among both cases and controls. Similarly, Marozik *et al.* demonstrated that the AG genotype had the highest rate of VDR BsmI rs1544410 in postmenopausal osteoporotic women and asymptomatic controls among Lithuanian and Belarusian populations (27). Furthermore, we observed no notable differences in genotype distributions between the groups, which is consistent with the results of investigations by Tamulaitienė *et al.* (21) and Uitterlinden *et al.* (28).

The BsmI polymorphism is a single nucleotide polymorphism located in the VDR gene (29). Its influence on bone health has garnered significant attention, as vitamin D is essential for the absorption of calcium and the metabolism of bone. The discovery of the VDR gene and its polymorphism has led to numerous investigations that have revealed an association with BMD, resulting in diverse

findings. Banjabi reported that *Apal* and *TaqI* were significantly related to the osteoporosis risk in the Saudi population (30). Mondockova *et al.* reported that four VDR gene polymorphisms, including *BsmI*, were associated with markers of bone turnover and an increased risk of spinal, radial, or overall fractures (31). A recent meta-analysis of 73 studies demonstrated that the *Apal* rs7975232, *BsmI* rs1544410, and *TaqI* rs731236 polymorphisms influenced the risk of osteoporosis in Caucasians. In contrast, the *BsmI* rs1544410 and *TaqI* rs731236 polymorphisms were found to influence the risk of osteoporosis in the Asian population from China, Japan, Korea, and Thailand (32). Another meta-analysis suggested that *BsmI* polymorphism is a potential biomarker for lower BMD among postmenopausal subjects from North Africa and the Middle East (33). On the other hand, *et al.* found no relationship between the *BsmI* polymorphism and diminished axial BMD among Caucasians with systemic sclerosis (34).

These inconsistent findings are attributed to differences in populations and geographic regions, which result from variations in genetic, environmental, dietary, and lifestyle factors.

Menopause is a significant contributor to the development of osteoporosis. Reports indicate that women who have undergone menopause experience a loss of bone mass ranging from 3% to 5% annually due to the decrease in estrogen production by the ovaries. Therefore, they are at a higher risk for osteoporosis and bone fractures (35). We found that the lumbar and spinal T-scores, as well as vitamin D and calcium, were improved in the GG-genotype compared to the other genotypes among both postmenopausal osteoporotic women (cases) and healthy women (controls), which is comparable with other investigations. A weak, non-significant correlation was observed between the BMD of the lumbar spine and total hip, as well as

biochemical bone markers, in females with *BsmI* genotypes who suffered from severe osteoporosis (21). Pedrera-Cana and colleagues found no significant associations between the rs1544410 genotype and bone loss among post-menopause Spanish women after five years (36). A study in Iran involving 146 women discovered that the GG genotype demonstrated a significant correlation with elevated BMD in the lumbar spine; however, this association was not significant in the femoral neck (37). Another study from India demonstrated that post-menopause women possessing the GG genotype exhibited the highest BMD in the femoral neck (38), which is similar to our findings.

Strengths and limitations:

This study focused on a specific VDR genetic polymorphism that could shed light on the relationship between genetic factors and the risk of osteoporosis among women over 40 years old. Additionally, the study's inclusion of serum vitamin D and calcium levels enhances its robustness, as these are critical factors in bone health. However, this study has limitations. Firstly, the sample size was limited due to the scope of this study, as the data and results were extracted from a thesis, and increasing the sample size was not feasible within the given constraints. The COVID-19 pandemic also affected the sample size and patient recruitment. Secondly, due to limitations in our dataset and sample size, we were initially unable to perform the Hardy-Weinberg equilibrium analysis. Thirdly, we did not account for potential confounding variables, including lifestyle factors (diet, physical activity, and sun exposure), within the population studied, which may affect the generalizability of the findings. Furthermore, a cross-sectional design could limit causal inferences about the relationship between the *BsmI* polymorphism and osteoporosis, emphasizing the need for longitudinal, large-scale, and multi-ethnic studies to establish temporal associations.

6. Conclusion

In our study, individuals with and without osteoporosis were not different in terms of VDR polymorphism. Additionally, the lumbar T-scores, calcium, and vitamin D levels were significantly different between the VDR polymorphisms in the case and control groups among postmenopausal women.

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Availability of data and materials: The datasets used in this study are available from the corresponding author upon reasonable request.

Conflicts of interests: All authors declared that they have no competing interests.

Consent for publication: Not applicable.

Ethics approval and consent to participate: The study protocols were approved by the Ethics Committee of Islamic Azad University, Mashhad, Iran, with the ethics code IR.IAU.MSHD.REC.1399.053. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

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