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Association of vitamin D receptor gene polymorphisms with type 2 diabetes mellitus inKurdish postmenopausal women: a case–control study

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Abstract

Background

This study investigated the association between vitamin D receptor (**VDR**) gene polymorphisms (**ApaI, FokI, and TaqI**) and susceptibility to type 2 diabetes mellitus (**T2DM**) in Kurdish postmenopausal women, together with serum 25-hydroxyvitamin D [25(OH)D] levels.

Methods

This single-center case–control study included 197 postmenopausal women: 99 with T2DM and 98 healthy controls. Whole blood samples were collected for genomic DNA extraction and HbA1c measurement. Serum was analyzed for fasting blood sugar (**FBS**), calcium, phosphorus, and 25(OH)D. VDR polymorphisms were genotyped by polymerase chain reaction–restriction fragment length polymorphism (**PCR-RFLP**).

Results

Median age did not differ significantly between groups *(p* = 0.054). Women with T2DM had significantly higher FBS, HbA1c, and BMI, and lower serum 25(OH)D, calcium, and phosphorus levels than controls (*p < 0.001* for all). The FokI ff genotype was associated with increased odds of T2DM (OR = 2.45, 95% CI 1.18–5.10; *p* = 0.01**)**, as was the TaqI tt genotype (OR = 3.22, 95% CI 1.52–6.83; *p* = 0.004**).** No significant association was observed for **ApaI**. Serum 25(OH)D concentrations differed significantly across genotype groups in both the T2DM and control groups.

Conclusion

In this study population, FokI and TaqI polymorphisms were associated with higher odds of T2DM, whereas ApaI showed no significant association. Lower serum 25(OH)D, calcium, and phosphorus levels in women with T2DM may reflect altered vitamin D–mineral status.

1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the most common metabolic disorders worldwide and represents a major global public health challenge. It is characterized by impaired insulin secretion, decreased peripheral insulin sensitivity, and β-cell dysfunction leading to chronic hyperglycemia (1, 2). Besides persistent hyperglycemia, T2DM is strongly associated with a wide range of microvascular and macrovascular complications including neuropathy, retinopathy, nephropathy, cerebrovascular, coronary artery involvement. T2DM is global health problem, affecting an estimated approximately 589 million adults aged 20–79 years worldwide (11.1% of the adult population) (3, 4). The incidence of T2DM

increases due to obesity, sedentary lifestyles, changes in dietary pattern and population aging. Among older adults, postmenopausal women at increased risk of T2DM due to endocrine variations and metabolic alternations related to estrogen deficiency (3–5). After menopausal period Vitamin D has been indicated to play significant role in glucose homeostasis and pancreatic insulin secretion (6). The physiological effects of vitamin D are mediated primarily through the vitamin D receptor (VDR), an intracellular receptor expressed in several tissues, including pancreatic β cells, skeletal muscle, adipose tissue, and the liver (7). Among the biological pathways associated with T2DM, vitamin D–mediated signaling has been identified as an important modulator of glucose metabolism (8). Vitamin D signaling has been proposed to influence pancreatic β-cell function, peripheral insulin sensitivity, and immune-inflammatory responses. In T2DM, disease susceptibility and progression are shaped by a complex interplay of environmental and genetic factors (9). Although lifestyle behaviors, dietary habits, and physical activity levels contribute to disease risk, emerging evidence suggests that genetic polymorphisms also play an important role in interindividual susceptibility to T2DM. (9). Although environmental influences including lifestyle behaviors, dietary habits, and physical activity levels contribute to disease development, emerging evidence indicates that genetic polymorphisms substantially influences individual susceptibility to T2DM. Genetic polymorphisms in the *VDR* gene, including FokI, BsmI, ApaI, and TaqI, have been extensively investigated due to their potential functional relevance (10). These genetic variants may modulate receptor expression, alter transcriptional regulation of downstream genes, and subsequently affect metabolic pathways associated with glucose homeostasis, insulin secretion, lipid metabolism, and inflammation(11). Consequently, VDR polymorphisms may contribute to inter individual variability metabolic effects and susceptibility to metabolic disorders. However, previous studies evaluating the association between VDR polymorphisms and vulnerability to type 2 diabetes mellitus have reported heterogeneous and controversial results. These contradictory results may be attributed to variations in ethnic background, environmental diversity, gene–environment interactions, lifestyle-related factors and methodological approaches. Postmenopausal women constitute a physiologically and metabolically susceptible group due to the profound endocrine alterations estrogen decline. The menopausal period is frequently accompanied by visceral fat accumulation, chronic low-grade systemic inflammation, and impaired insulin sensitivity. These metabolic impairments may promote a dysregulated metabolic state in which genetic determinants play a role in glucose metabolic regulation. In particular, polymorphisms in the *VDR* gene may interact with menopause-related hormonal and metabolic disturbances, thereby modulating glucose homeostasis and affecting susceptibility to type 2 diabetes mellitus. To the best of our knowledge, this is one of the first studies to examine the association of VDR gene polymorphisms with type 2 diabetes mellitus in Kurdish postmenopausal women, providing novel population-specific evidence in an understudied ethnic group.

2. Materials

Genomic DNA was extracted from peripheral blood leukocytes using the standard salting-out protocol. PCR reactions were conducted using a ready-to-use 2× Super PCR Master Mix (Cat. No. YT1553; Yekta

Tajhiz Azma,Tahran, Iran). Agarose powder for electrophoretic separation was purchased from Pishgam Co. (Tehran, Iran). Restriction enzymes including ApaI, TaqI, and FokI were obtained from Thermo Scientific Fermentas. Oligonucleotide primers were synthesized by SinaClon BioScience (Tehran, Iran**)** (12).

2.1 Study design and participants

This case–control study was conducted to evaluate the association between vitamin D receptor (VDR) gene polymorphisms and susceptibility to type 2 diabetes mellitus (T2DM). A total of 197 postmenopausal women were recruited from the **Diabetes Center of Kermanshah, western Iran**, from April to August 2025. The study population consisted of 99 patients diagnosed with T2DM and 98 age - matched healthy volunteers that were available without a history of diabetes (13). Postmenopausal status was determined as amenorrhea for at least 12 consecutive months. Exclusion criteria from the study included chronic inflammatory disorders, autoimmune conditions, malignant diseases, severe hepatic or renal impairment, or those receiving vitamin D supplementation. All participants provided written informed consent; the study protocol was approved by the Ethics Committee of Kermanshah University of Medical Sciences (IR.KUMS.MED.REC.1403.086).

2.2 Biochemical measurements

Biochemical parameters, including fasting blood glucose (FBS), glycated hemoglobin (HbA1c), serum calcium, and phosphorus levels, were assessed using standardized laboratory procedures. Whole blood samples were collected after a 12–14-hour overnight fast. FBS was measured by an enzymatic colorimetric method with an automated clinical chemistry analyzer. HbA1c levels were measured using an enzymatic colorimetric assay with Diazyme HbA1c reagents (Diazyme Laboratories, Poway, CA, USA) on an automated Hitachi clinical chemistry analyzer (Hitachi Ltd., Tokyo, Japan), and results were reported as percentage of total hemoglobin(14). Serum calcium and inorganic phosphorus levels were determined using commercially diagnostic kits (Pars Azmun, Tehran, Iran) on an automated Cobas clinical chemistry analyzer (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's protocol. Serum 25(OH)D levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Pishgaman Sanjesh Isatis, Tehran, Iran) according to the manufacturer's instructions, and results were expressed in ng/mL.

2.3 DNA extraction and genotyping

After 12–14 hours of overnight fasting, peripheral venous blood samples approximately 3 mL were obtained from all participants in a physically relaxed state to minimize stress-related variability. Blood samples were collected into EDTA-anti coagulated tubes and maintained at − 20°C until further analysis. Genomic DNA was extracted from whole blood using the standard salting-out protocol, and DNA yield and purity (A260/A280 ratio) were evaluated using a NanoDrop spectrophotometer 2000c (Thermo Fisher Scientific, USA) (15).

2.4 Genotyping of VDR gene polymorphisms by PCR-RFLP

Genotyping of *VDR* gene polymorphisms ApaI (rs79785232), FokI (rs2228570), and TaqI (rs731236) was performed using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. Target gene fragments were amplified using specific primer pairs listed in Table 1. PCR was performed in a final reaction volume of 25 µL using a ready-to-use 2× PCR Master Mix (Yekta Tajhiz Azma, Tehran, Iran). The amplification protocol consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 58°C for ApaI and TaqI and 57°C for FokI for 45 s, and extension at 72°C for 60 s, with a final extension at 72°C for 10 min. The PCR products were digested with the corresponding restriction enzymes under the manufacturer's recommended conditions and separated by electrophoresis on 2% agarose gels stained with a safe DNA-binding dye.PCR products were digested with 1 µL of ApaI, FokI (at 37°C for overnight), and TaqI (at 65°C for overnight). The digested amplicons were separated by electrophoresis on 2% (w/v) agarose gels stained with GelRed (Biotium, Fremont, CA, USA). (16).

2.5 Statistical analysis

Statistical analyses were performed using GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA). The normality of continuous variables was assessed using the Shapiro–Wilk test together with visual inspection of histograms and Q–Q plots. Normally distributed continuous variables were expressed as mean ± standard deviation (SD), whereas non-normally distributed continuous variables were presented as median [interquartile range (IQR)]. Categorical variables were reported as frequencies and percentages. The genotype and allele frequencies of the analyzed VDR polymorphisms were calculated for each group. The Hardy–Weinberg equilibrium (HWE) was determined in the control group using the chi-square test. Logistic regression analysis was applied to estimate the relationship between VDR polymorphisms and susceptibility to T2DM with results described as odds ratios (ORs) with 95% confidence intervals (CIs). Statistical significance was considered a p-value < 0.05. The normality of continuous variables was evaluated using the Shapiro–Wilk test together with visual assessment of histograms and Q–Q plots. Since most variables showed non-normal distribution, continuous data were expressed as median [IQR], and comparisons between patients with T2DM and healthy controls were conducted using the Mann–Whitney U test. Crude logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between each VDR polymorphism and T2DM. In the recessive model, homozygous variant genotypes (aa, ff, or tt) were compared with the combined reference genotypes (AA + Aa, FF + Ff, or TT + Tt). No adjusted model was fitted; therefore, the reported estimates should be interpreted as unadjusted associations.

3. Results

3.1 Demographic and Biochemical characteristics

The clinical and biochemical characteristics of the study participants are summarized in **Table 2**. The median age of T2DM patients were 61.0 years [IQR: 54.0–69.0], which was comparable to the healthy control group (58.0 years [IQR: 50.0–64.0]), showing no statistically significant difference (P=0.054).

As expected, compared with healthy controls, postmenopausal women with T2DM had significantly higher levels of FBS (158.0 vs. 88.0 mg/dL; $p < 0.001$), HbA1c (8.60% vs. 5.50%; $p < 0.001$) and BMI (25.73 ± 2.09 vs. 23.84 ± 1.84 kg/m²; *p < 0.001*),.Furthermore, serum 25(OH) D levels were significantly lower in patient with T2DM than in healthy controls (24.80 vs. 33.00 ng/mL; $p < 0.001$). Similarly, T2DM patients showed significantly lower serum Calcium (8.20 vs. 8.90 mg/dL, $p<0.001$) and Phosphorus levels (2.90 vs. 3.50 mg/dL, $p<0.001$).

3.2. Distribution of VDR Gene Polymorphisms

The genotype frequencies of *VDR* ApaI, FokI, and TaqI polymorphisms were in accordance with the Hardy-Weinberg Equilibrium (HWE) in the control group (*p=0.16, p=0.13, and p=0.39*, respectively), The genotype frequencies in the control group were consistent with Hardy–Weinberg equilibrium.. The comparative analysis of genotype and allele frequencies between the two groups is presented in Table 3.

No significant association was observed for the ApaI polymorphism. In contrast, the FokI ff genotype (OR, 2.45; 95% CI, 1.18–5.10) and the TaqI tt genotype (OR, 3.22; 95% CI, 1.52–6.83) were associated with higher odds of T2DM in the crude recessive-model analysis. Similarly, the VDR *TaqI* tt genotype was significantly associated with T2DM risk (OR = 3.22, 95% CI: 1.52–6.83; $p = 0.004$).

3.3. Association between VDR Genotypes and Serum 25(OH) D Levels

To investigate whether the *VDR* variants influence the systemic bioavailability of vitamin D, serum 25(OH) D levels were compared across different genotypes within the T2DM group (Table 3).

Serum 25(OH) D levels were compared across genotype groups separately in healthy controls and participants with T2DM. For *VDR* ***TaqI*** **variant,** vitamin D levels varied significantly across **TT, Tt**, and **tt** genotypes in both controls and T2DM subjects. (*p<0.001*) . In both groups, **TT** carriers exhibited the highest vitamin D levels, whereas **tt** carriers showed the lowest levels. For *VDR* ***Apa1***, significant genotype-dependent differences in serum vitamin D levels were observed in both the control and the T2DM group *(p<0.001*). In controls, vitamin D levels decreased from **AA** to **Aa** and **aa** genotypes, while in diabetic subjects, **aa** carriers had the lowest vitamin D levels.

For *VDR* ***Fok1***, serum vitamin D concentrations demonstrated significant variation among **FF**, **Ff**, and **ff** genotypes in both controls and T2DM groups (*p<0.001*), **FF** carriers had the highest vitamin D levels, whereas **ff** carriers had the lowest levels in both groups.

Overall, serum 25(OH)D concentrations differed across VDR genotype groups in both healthy controls and participants with T2DM. Uppercase letters indicate the reference allele and lowercase letters indicate the variant allele.

Data are presented as mean ± standard deviation. The p-values indicate differences in serum vitamin D levels among genotypes of each polymorphism within the control and diabetic groups. Statistical comparisons were performed using one-way ANOVA or Kruskal–Wallis test as appropriate. A p-value <

0.05 was considered statistically significant. The reported p-values represent within-group comparisons of serum vitamin D levels among genotypes for each polymorphism.

Discussion

The present case-control study evaluated the association between *VDR* gene polymorphisms *(FokI, ApaI* and *TaqI*) and susceptibility to T2DM in Kurdish postmenopausal women. The FokI and TaqI polymorphisms were associated with higher odds of T2DM in this study, whereas no significant association was observed for the *ApaI* polymorphism. Furthermore, our results revealed that fasting blood sugar (FBS), HbA1c, and body mass index (BMI) were significantly higher in patients with T2DM compared with healthy controls, whereas serum levels of vitamin D, calcium, and phosphorus were significantly lower in the T2DM participates. Vitamin D is fat soluble steroid hormone, play an essential role in immune modulation, pancreatic β cell function, insulin secretion, tissue insulin sensitivity, glucose homeostasis and calcium and phosphor homeostasis. The vitamin D biological effects are principally mediated through the VDR. These findings add population-specific data from Kurdish postmenopausal women, a relatively understudied subgroup. The VDR is a ligand-activated nuclear transcriptional factor that mediates the expression of a wide range of target genes. The *VDR* gene plays a crucial role in vitamin D metabolism and signaling pathway (17). The vitamin D deficiency has been associated with increased susceptibility to type 2 diabetes mellitus (T2DM). In recent years, VDR gene polymorphisms have been widely investigated for their potential association with a broad range of diseases, including malignancies and diabetes (18). The *VDR* gene has approximately 60 polymorphic variants, of which *ApaI, FokI* and *TaqI* are among the most widely investigated. Several studies have reported an association between VDR and an increased risk of T2DM. The *VDR* gene has crucial role in insulin metabolism and its polymorphisms may be associated with insulin resistant (19, 20).

The ***ApaI*** *VDR* **polymorphism,** positioned in exon 9 near the 3' untranslated region (3' UTR), involves a G-to-T nucleotide substitution that has been associated with downregulate *VDR* gene expression(21). The ***FokI*** *VDR* **polymorphism** occurs at the 5' end of the *VDR* gene involves a T to C nucleotide substitution within the first codon of exon 2. This change removes the primary translation initiation site producing a mutant protein that is three amino acids shorter than wild- type form. This mutant receptor isoform exhibits elevated transcriptional activity(22). The *TaqI VDR* polymorphism located in exon 9 (codon 352) and results from a T-to-C nucleotide substitution. According to our findings *ApaI VDR* **polymorphism** did not significantly association with T2DM risk (23). The same result was reported by Alzaim et al., did not find any association between *ApaI gene* polymorphisms and T2DM risk (24). In contrast Gupta et al. showed significant association with ApaI polymorphism and Gestational diabetes mellitus (GDM) in the north Indian population (25). Han et al reported a significant association several *VDR* gene polymorphisms *ApaI, BsmI,* and *FokI* insulin- resistance –related disorders in Asian population(26). A case-control study by Melake et al. suggested that vitamin D deficiency may be associated with diabetic nephropathy disease. The study also reported that the *TaqI VDR* polymorphism was associated with elevated risk of developing this complication in the Ethiopian participants (27). Consistent with our findings, previous study in an Iranian population demonstrated a significant association between the

TaqI VDR polymorphism and the risk of T2DM suggesting that this variant may be function as a genetic risk factor for the disorder(28). A recent investigation in a Han Chinese population identified an association between the *FokI VDR* polymorphism and susceptibility to type 2 diabetic kidney disease (T2DKD), although no significant linked was found with T2DM. The authors proposed that individuals carrying the ff genotypes may increase the risk of diabetes-related complications (29).

Our finding demonstrated the *TaqI VDR* polymorphism was significantly association with susceptibility to T2DM; In addition, t allele is more frequent in T2DM patients group than control group. In contrast, Dilmec etal. observed no association between the *TaqI VDR* polymorphism and T2DM susceptibility in Turkish population. The finding also did not found association of *AqaI* genotype and T2DM risk (30). One of the most important results of the present study was significantly lower serum 25(OH) D levels in T2DM patient compare with healthy control group. This finding is consistent with previous reports indicating that vitamin D deficiency is associated with impaired glucose metabolism, insulin resistance, chronic inflammation; impaired pancreatic β-cell function (31). In postmenopausal women, vitamin D deficiency may be magnified due to estrogen decline, increased adiposity and decreased physical activity may be altered vitamin D metabolism and bioavailability. Additionally, we observed lower serum calcium and phosphorus levels between T2DM patients compared with control group. This finding suggested that alterations in calcium homeostasis may indicate disturbance in insulin secretion and intracellular signaling. A case-control study, Al-Ghafari etal, found that *ApaI* and *TaqI VDR* polymorphisms were significantly correlated with serum calcium levels in colorectal cancer patients (32). Mohammed et al. reported a significant correlation between low serum 25(OH) D levels and the development of T2DM in individuals carrying the *ApaI VDR* polymorphism whereas no significant association was observed for the *TaqI VDR* polymorphism(33). In Qatari cohort study, Ahmed and colleagues indicated that serum 25(OH) D levels were significantly associated with diabetic retinopathy particularly in female patient(34). In other study Gendy etal. found that serum 25(OH) D levels were significantly lower in Egyptian patients with T2DM carrying the *FokI VDR* polymorphism(35).

The genotype-specific variation observed in serum 25(OH) D levels among different VDR polymorphisms is an important finding. Individuals carrying the wild-type genotypes generally displayed higher vitamin D concentrations, whereas participants with homozygous variant genotypes tended to show lower levels. This pattern is consistent with an association between VDR genetic variation and circulating 25(OH) D concentrations. However, the functional basis of this association was not examined in the present study, and other determinants of vitamin D status may also have contributed to the observed variation.

Limitation

Despite the novel insights provided by the present study, several limitations should be considered. *One of the main limitations of this investigation* is the relatively small sample size, which is typical of pilot studies. This study has several limitations. First, its relatively small, single-center sample limits statistical power and generalizability. Second, the analyses were based on crude models and did not adjust for potential confounders, including BMI, medication use, season, sunlight exposure, and dietary

intake. Third, the case–control design precludes causal inference. Finally, the findings require replication in larger, independent populations. In addition, seasonal variation, sunlight exposure, dietary intake, antidiabetic medications, and other factors affecting vitamin D status were not fully controlled. Furthermore, serum 25(OH) D levels are influenced by various environmental and lifestyle factors, including sunlight exposure, dietary habits, seasonal variation, physical activity, and socioeconomic status. In addition, the study population was limited to Kurdish postmenopausal women from a single clinical center

Conclusion

In conclusion, the VDR *FokI* (rs2228570) and *TaqI* (rs731236) polymorphisms were significantly associated with higher odds of T2DM in Kurdish postmenopausal women. Participants with T2DM also had higher fasting blood glucose (FBS) and glycated hemoglobin (HbA1c) levels and lower serum 25(OH) D concentrations than controls. The associations observed for *FokI* and *TaqI* require replication in larger, independent cohorts before their potential utility as genetic risk markers for T2DM can be evaluated. Whether vitamin D supplementation can prevent or reduce T2DM risk in postmenopausal women should be investigated in adequately powered randomized clinical trials.

Declarations

Data availability

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

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Author contributions

MB, MKh, and AB contributed to data collection, data analysis, and manuscript drafting. EN and MRK contributed to study supervision and critical revision of the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethics approval and consent to participate:

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Kermanshah University of Medical Sciences (IR.KUMS.MED.REC.1403.086). Written informed consent was obtained from all participants.

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Tables

Tables 1 to 3 are available in the Supplementary Files section.

Supplementary Files

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