

Vitamin D receptor gene polymorphisms in patients with relapsing multiple sclerosis

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Abstract

The relationship between the Vitamin D Receptor (VDR) gene and many pathogenic pathways in Relapsing-Remitting Multiple Sclerosis (RRMS) remains unclear. Given the significance of the topic, we conducted this study to explore the correlation between vitamin D receptor gene polymorphisms and clinical and inflammatory factors in patients suffering from relapsing-remitting multiple sclerosis. The current research is a case/control study conducted based on the Helsinki Ethical Principles. RRMS disease was confirmed based on history, clinical symptoms, radiological signs and neurologist diagnosis. The research population consisted of healthy people and patients with RRMS who were referred to Hazrat Rasool Akram Hospital between 2021 and 2023. For each person participating in the study (RRMS patient and healthy), five milliliters of peripheral blood containing the anticoagulant EDTA was collected. Polymerase chain reaction was performed using two specific and appropriate oligonucleotide primers. The restriction fragment length polymorphism technique was used, one of the standard methods for identifying polymorphisms. Statistical analysis was performed using SPSS software version 23. The odds ratio and 95% confidence limits were calculated. The SNP Analyzer software was used to analyze the allele frequency of each polymorphism in healthy and RRMS individuals and compare the values. Prism version 5 software was used to draw diagrams. In the present study, a statistically significant difference was observed between the percentage of FokI genotypes in RRMS patients and healthy individuals. FokI polymorphism showed a significantly increased risk with an odds ratio of 7.28 in patients with the FF genotype compared to healthy individuals. ApaI, TaqI, and BsmI were not significantly different between the two groups. Based on the findings of the present study, FokI polymorphism showed a significant risk increase in RRMS patients with FF genotype compared to healthy individuals.

Key Words: relapsing multiple sclerosis, vitamin D, gene polymorphisms.

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Multiple Sclerosis (MS) is the predominant non-traumatic debilitating condition that impacts young adults.¹ The incidence and prevalence of MS are increasing in developed and developing countries, the main cause of which is unclear.² According to the speed of disease progression, there are four clinical stages of MS, which are: RRMS, Secondary Progressive Type, relapsing-progressive type, and primary progressive type. RRMS is the most common clinical type of MS and is characterized by many

symptoms in attacks and complete recovery between attacks.³ The most significant risk factors for MS may be a combination of genetic and environmental factors. Moreover, vitamin D deficiency is a determining risk factor as well as a frequently reported environmental factor in the etiology of MS, regardless of latitude and viral infections.⁴ In recent years, there has been a lot of evidence that shows that vitamin D can play a role in the pathogenesis of MS, disease courses, and duration.⁵

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The relationship between the Vitamin D Receptor (VDR) gene and many pathogenic pathways in RRMS remains unclear. Insufficient vitamin D and issues with VDR gene expression can decrease kisspeptin 10 levels in the blood and inhibit the maturation of T lymphocytes, increasing the likelihood of an MS recurrence.⁶ All known biological effects of vitamin D require VDR, which is present in macrophages, monocytes, T/B cells, and dendritic cells. Structurally, this receptor is placed in the family of steroid receptors, which exerts the effect of vitamin D by intracellularly regulating several genes.⁷ There are 9 exons in the VDR gene, and each one has several polymorphisms. FOKI RFLP is in exon 2, BSMI and ApaI are in intron 8, and TaqI RFLP is in exon 9. So, the 4 known polymorphisms of vitamin D receptor (VDR) are: BSMI (A/G), TaqI(T/C), APaI (A/C) in the 3' region of the VDR gene, and FOKI(A) polymorphism (C) in exon 2.⁸ The vitamin D receptor gene is located on the long arm of chromosome 12. Vitamin D's main function is to regulate calcium homeostasis, bone formation, and resorption, but it also has other effects on the immune system. This vitamin suppresses lymphocyte proliferation and immunoglobulin production.⁹ This vitamin also inhibits the activity of the pro-inflammatory factor NF-KaPPaB as well as the production of various cytokines, such as interleukin 2 and 12 and interferon gamma.¹⁰ The presence of nuclear and membrane VDRs in fat cells suggests that extra fat may change the VDR, making it harder for the body to absorb vitamin D and raising the risk of MS. This is still being studied. On the other hand, vitamin D has an anti-inflammatory effect on fat cells.^{11,12} Obesity in early life is associated with an increased risk of developing Multiple Sclerosis (MS).¹³ Given the significance of the topic, we conducted this study to explore the correlation between vitamin D receptor gene polymorphisms and clinical and inflammatory factors in patients suffering from relapsing-remitting multiple sclerosis.

Materials and Methods

The current research is a case/control study that was conducted based on the ethical principles of Helsinki¹⁴ and was approved by the Medical Ethics Department of Iran University of Medical Sciences with no. IR.IUMS.FMD.REC.1401.429. RRMS disease was confirmed based on history, clinical signs, radiological signs, and neurologist's diagnosis. The research population consisted of healthy people and patients with RRMS referring to Hazrat Rasool Akram Hospital between 2021 and 2023 who met the criteria for entering the research.

Inclusion criteria

The study included patients with relapsing-remitting multiple sclerosis (RRMS) who have been diagnosed with McDonald's 2017 criteria,¹⁵ both female and male gender, age group from 18 to 65 years. We also included newly admitted patients using no drugs in the relapse phase and patients only treated with intron beta and with a gap of at least 3 months from the relapse phase were evaluated. Patients were examined by a specialist in terms of plaques in MRI,

disability progress status, and blood markers at least in two four-month intervals. The participants showed an EDSS between 1 and 3. The patients did not take vitamin D in the preceding 6 months.

Exclusion criteria

We included the following categories: i) patients in other phases of RRMS except relapse; ii) patients with diabetes, liver disorders (cirrhosis and types of hepatitis), kidney disorders (kidney failure), congestive heart failure, high blood pressure, and cancer; iii) patients taking other drugs.

Sample size determination

The required sample size for the present study was calculated through the ratio comparison formula of two independent groups. Considering the confidence level of 95% (significance level of 5%), the power of the study was estimated to be 80%, according to the almost similar study of 21 people for each study group. In order to increase the power of the study and also to consider the possibility of dropping out, the minimum sample size required for the study group was 25 RRMS patients and 12 Healthy controls.

$$n \geq \frac{(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2 (\sigma_1^2 + \frac{\sigma_2^2}{r})}{(\mu_1 - \mu_2)^2}$$

$\alpha=0.05$; $\beta=0.2$; $Z_{\alpha} = 1.95$; $Z_{\beta} = 0.84$; Mean in group 1 (μ_1) = 2.77; Standard deviation in group 1 (σ_1) = 1.45; Mean in group 2 (μ_2) = 4.11; Standard deviation in group 2 (σ_2) = 1.41; $r=1$.

In this case/control study, 25 patients with RRMS (test group) and 12 healthy people (control group) were evaluated between 2021 and 2023. A written consent was obtained from all the people participating in the study, stating that this study did not impose any additional costs, threats, or problems for them, and whenever they were unable to cooperate and continue to participate, they were excluded from the study, and there was no negligence. It does not take place when handling them. We fully explained the situation to the patient's legal representative, child, or first-degree relative if he did not understand or was not sufficiently literate, and obtained consent in their presence and with their approval. The project manager fully communicated all the study results, including genetic test results and clinical and inflammatory factor examination results, to the patients through phone calls or face-to-face interactions, significantly aiding in their disease treatment process.

The following clinical variables were considered for analysis at sample collection: age of onset (years), duration of MS disease (months), Extended Disability Status Scale Score (EDSS/1-3), Multiple Sclerosis Severity Score (MSSS), annual relapse The rate since the onset of the disease, the number of relapses two years ago and the doctors at the time of the examination, gender, disease and smoking history, vitamin D consumption in the last 6 months, behavioral tests and cognitive disorders were evaluated.

The EDSS provides a total score on a scale of 0 to 10. EDSS levels 1.0 to 4.5 refer to fully ambulatory patients and the

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exact step count is defined by the functional system score(s). EDSS stages 5.0 to 9.5 are defined by movement disorders and common equivalents are provided in the functional systems scores.

MSSS is a useful measure of MS severity that includes EDSS and disease duration. Achieving an MSSS score ≥ 4.8 indicates a severe phase of the disease and an MSSS < 4.8 indicates a mild phase of the disease.

For each person participating in the study (RRMS patient and healthy), five milliliters of peripheral blood with EDTA anticoagulant were taken. All samples were collected from 8 am to 11 am. Two tubes of cell preparation were collected with sodium citrate for cell and plasma separation. Peripheral blood mononuclear cells were isolated using gradient centrifugation (920 g, 30 min). They were frozen in serum with DMSO (10%) and stored in liquid nitrogen (-196°C). Serum was separated by centrifugation (920 g, 15 min, room temperature) in serum separator tubes. After centrifugation, plasma and serum samples were divided into each part and stored at -80° temperature. DNA of blood samples was extracted using a genomic DNA column extraction kit manufactured by MBST company in Iran. The DNA purification method in this kit is based on the specific binding of nucleic acids to the silica column in the presence of chemotropic salts. The non-nucleic acid materials that could bind to the column membrane were washed from the column during different washing steps and by centrifugation. Then, the DNA attached to the column was dissolved and separated by adding an elution buffer, and after checking the purity and quality, it was frozen until used for the next steps. In the next step, a Polymerase Chain Reaction (PCR) was performed using two specific and appropriate oligonucleotide primers.

We designed the back-and-forth primers using Beacon software, confirmed their correct connection to their own sequences in the NCBI nucleotide database, and ordered the synthesis from Tekapo Bio Company. The primers were lyophilized from the delivery company and according to the manufacturer's protocol, they were diluted with DNase/RNase-free distilled water to a concentration of 100 pmol/ μL . Amplification of Taq DNA polymerase and dNTPs was done by ABI thermocycler (Veriti 96 Well Thermal Cycle device) (Applied Biosystem, ABI, company, USA) in three successive temperature steps. This operation was done for each of the enzymes separately, and the used items were optimized, and then four PCR variants were placed for each sample. To check the size of the amplified fragments and the specificity of the products, their electrophoresis was performed on agarose gel, and after the completion of the PCR reaction, the products were electrophoresed on 2% agarose gel. Then, the PCR-RFLP (Restriction Fragment Length Polymorphism) technique, which is one of the standard methods for identifying polymorphisms, was used. To achieve this, we mixed the PCR product with restriction enzymes as per the manufacturer's instructions, and selected the optimal cutting temperature based on the enzyme's cutting time specified in the recipe.

After the incubation time of the product, electrophoresis was performed on a 2% agarose gel, and by using the pattern obtained from restriction enzyme cleavage, the differ-

ent polymorphisms of the vitamin D receptor gene studied in each participant were separated and the gels electrophoresed with the Gel machine. Figures 1 to 4 display the genotype of VDR gene polymorphisms based on PCR-RFLP. Table 1 summarizes the visualization of RFLP bands using 3% agarose gel electrophoresis. Table 1 summarizes the SNP primer sequences and amplicon sizes.

Statistical analysis was performed using SPSS software version 23. Differences with a p value < 0.05 were considered significant. To check the normality of the data, the Kolmogorov-Smirnov test was used. Two study groups were examined for demographic variables. The Mann-Whitney U test was used to evaluate the differences between case and control groups. Spearman's rank correlation coefficient was used to determine continuous correlation. To measure the relationship between the factors and MS risk, odds ratio and 95% confidence limits were calculated. The relationship between MS patients and clinical factors was evaluated by the chi-square test and Fisher's exact test. An odds ratio and 95% confidence limits are calculated. The SNP Analyzer software was used to analyze the allele frequency of each

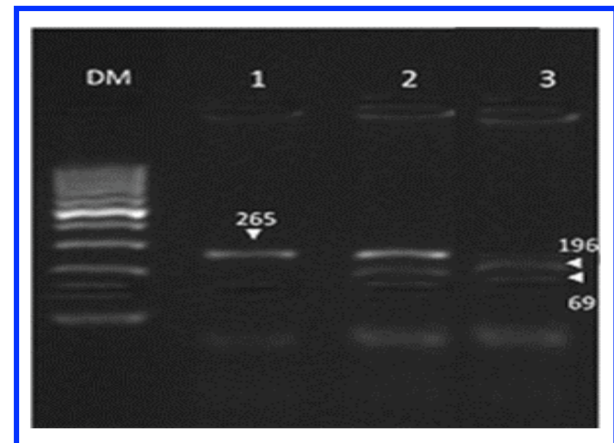


Figure 1. *FokI*: lane 1: FF, lane 2: FF, and lane 3: ff.

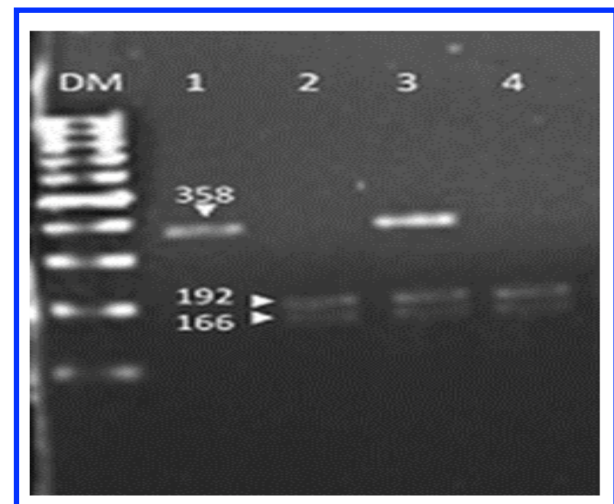


Figure 2. *BsmI*: line 1: BB, lines 2, 4: bb, and line 3: Bb.

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polymorphism in healthy and RRMS individuals and compare the values. Prism version 5 software was used to draw diagrams.

Findings

The mean and standard deviation of the age of the participants in this study in the test and control groups were 32.16 ± 5.87 years and 31.58 ± 8.14 years, respectively. The independent t-test did not demonstrate a statistically significant difference in the mean age of the two groups, and both groups were similar in age ($p=0.829$). 72% of the test

group and 41.6% of the control group were women ($p=0.364$). The mean and standard deviation of the duration of disease in the patients participating in this study in the test group was 45.44 ± 41.74 months. The mean and standard deviation of the age of disease onset in the patients participating in this study in the test group was 28.52 ± 5.07 years. 28% of newly admitted patients were in the relapse phase while taking no medication, and 72% of the patients were only treated with intron beta and with a gap of at least 3 months from the relapse phase. The average and deviation of the time since the last attack in the patients participating in this study in the test group was 3.60 ± 2.44 months.

In the test group, 88% of the participants had a deficiency of 25-hydroxyvitamin D level, and in the control group, 66.7% of the participants had insufficient vitamin D. According to the results of the Spearman test, the two groups had a statistically significant difference in terms of the level of 25 hydroxy vitamin D ($p<0.001$). According to Table 2,

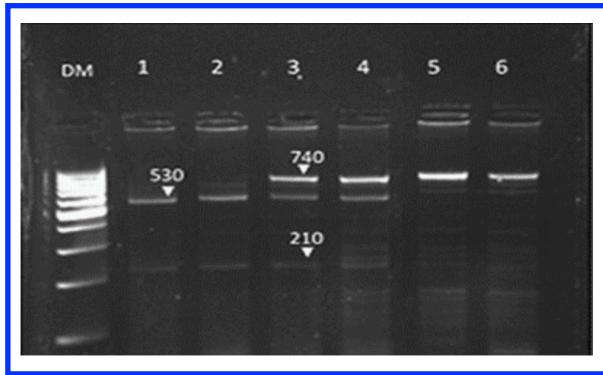


Figure 3. *ApaI*: lane 1, 2: aa, lane 3, 4: Aa, and lane 5, 6: AA.

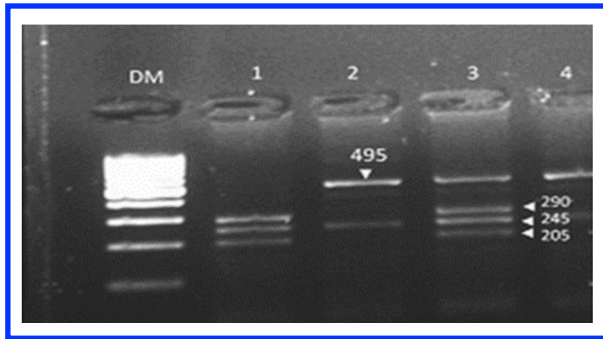


Figure 4. *TaqI*: lane 1: tt, lane 2: TT, and lane 3: Tt.

Table 1. RFLP bands with 3% agarose gel electrophoresis.

SNP type	Size (bp)	The number of bands	Genotype
FokI	265	1	FF
	265, 196, 69	3	Ff
	196, 69	2	ff
BsmI	358	1	BB
	358, 192, 166	3	Bb
	192, 166	2	bb
ApaI	740	1	AA
	740, 530, 210	3	Aa
	530, 210	2	aa
TaqI	495, 245	2	TT
	495, 290, 245, 205	4	Tt
	290, 245, 205	3	tt

Table 2. Polymorphisms and haplotypes of the VDR gene.

SNP	Primers sequences	Amplicon (bp)
FokI	F 5'-AGC TGG CCC TGG CAC TGA CTC TGC TCT-3' R 5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3'	265
BsmI	F 5'-GGG AGA CGT AGC AAA AGG-3' R 5'-AGA GGT CAA GGG TCA CTG-3'	358
ApaI	F 5'-CAG AGC ATG GAC AGG GAG CAA-3' R 5'-GCA ACT CCT CAT GGC TGA GGT CTC-3'	740
TaqI	F 5'-CAG AGC ATG GAC AGG GAG CAA-3' R 5'-GCA ACT CCT CAT GGC TGA GGT CTC-3'	495, 245

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the mean and standard deviation of 25 hydroxy vitamin D levels of the patients participating in this study in the control and control groups were 16.20 ± 4.01 ng/mL and 25.50 ± 4.87 ng/ml, respectively. According to the Mann-Whitney statistical test, there was a statistically significant difference in terms of the level of 25 hydroxy vitamin D between the test and control groups ($p < 0.001$), so in the test group, vitamin D deficiency was observed, and in the control group, insufficient vitamin D in the company. There was the mean serum level of 25-hydroxyvitamin D in patients with the disease was significantly lower than that of healthy subjects ($p < 0.001$) (Table 3).

According to Table 4, in the present study, the value of Spearman's correlation coefficient was -0.435 . Because the p-value in this study was calculated as 0.030 and this value is smaller than 0.05 , then this correlation coefficient is significant at the 0.05 level. So, it can be concluded that there is a statistically significant relationship between the EDSS scale scores and the level of 25 hydroxy vitamin D in MS patients participating in the study. The negative sign of the

correlation coefficient indicates that the relationship is not aligned. This means that MS patients with lower levels of 25-hydroxyvitamin D had higher scores and patients with higher levels of 25-hydroxyvitamin D had lower scores. As seen in Table 4, there was a significant negative trend between serum levels of 25 hydroxy vitamin D and EDSS score ($p < 0.001$).

According to Table 5, in univariate conditional logistic regression analysis, the odds ratio (OR) was 1.55 (95% CI, $1.187-2.040$; $p < 0.001$), indicating that low levels of 25-hydroxyvitamin D can 1.55 to increase the odds of MS disease, this relationship was statistically significant, and a strong relationship was observed ($p < 0.001$).

According to Table 6, the mean and standard deviation of 25 hydroxy vitamin D levels in patients participating in this study in the mild and severe groups were 18.42 ± 3.64 ng/mL and 15.33 ± 3.89 ng/mL, respectively. According to the Mann-Whitney statistical test, a statistically significant difference was observed in terms of the level of 25-hydroxyvitamin D between the two mild and severe groups

Table 3. Mean values of 25 hydroxy vitamin D levels in patients with relapsing-remitting MS in both experimental and control groups.

Groups	Test (n=25) Number (percent)	Witness (n=12) Number (percent)	P-value
25 hydroxy vitamin D level (ng/mL)		1 (3/8)	Spearman correlation > 0.001
Shortage	22 (88) 3 (12)	8 (7/66) 3 (25%)	
Insufficient vitamin D	0	$87/4 \pm 50/25$	Mann-Whitney test > 0.001

Table 4. Correlation between serum levels of 25 hydroxy vitamin D and Extended Disability Status Scale (EDSS) score.

		Vitamin D serum level		EDSS
Spearman's statistical test	Vitamin D serum level	Correlation	1/100	-0/435
		p-value		0/030
		Number	25	25
Spearman's statistical test	EDSS	Correlation	-0/435	1/000
		p-value		0/030
		Number	25	25

Table 5. Odds ratio of MS disease with 25 hydroxy vitamin D levels.

	B	S.E.	Wald	df	Sig.	Exp (B)	95% C.I. for EXP(B)	
							Lower	Upper
Vitamin D	442.0	0.138	10.23	1	0.001	1.55	1.187	2.040
Constant	-9.76	2.93	11.07	1	0.001	0		

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($p < 0.001$), so the level of vitamin D in the severe group was significantly lower than the mild group.

According to Table 7, the FokI genotype distribution in RRMS patients was as follows: FF (wild) =72%, Ff (heterozygous) =28% and ff (mutant) =0%; in the control group: FF=25%, Ff=41.7%, and ff=33.3%.

The statistically significant difference was observed between the percentage of genotypes in both test and control groups ($p=0.003$).

Statistical analysis of FokI polymorphism showed a significant increase in risk in patients with FF genotype compared to the control group (OR=7.28: 95% CI; 1.86, 28.41).

The odds ratio was 7.28.

The distribution of ApaI genotype in RRMS patients was as follows: AA (wild) =40%, Aa (heterozygous) =56% and aa (mutant) =4%; in the control group: AA=50%, Aa=25%, and aa=25%.

No statistically significant difference was observed between the percentage of genotypes in the two test and control groups ($p=0.075$).

Statistical analysis of ApaI polymorphism showed no significant risk increase in patients with AA genotype compared to the control group (OR=1.28: 95% CI; 0.45, 3.62).

The odds ratio was 1.28.

The distribution of TaqI genotype in RRMS patients was as follows: TT (wild) =48%, Tt (heterozygous) =40% and tt (mutant) =12%; in the control group: TT=33.3%, Tt=33.3% and tt=33.3%.

No statistically significant difference was observed between the percentage of genotypes in the two test and control groups ($p=0.294$).

Statistical analysis of TaqI polymorphism showed no significant increased risk in patients with TT genotype compared to controls (OR=1.89: 95% CI; 0.74, 4.84).

The odds ratio was 1.89.

The distribution of BsmI genotype in RRMS patients was as follows: BB (wild)=40%, Bb (heterozygous)=24% and bb (mutant)=36%; in the control group: BB=41.7%, Bb=25%, and bb=33.3%.

No statistically significant difference was observed between the percentage of genotypes in the two test and control groups ($p=0.987$).

Statistical analysis of BsmI polymorphism showed no significant increased risk in patients with TT genotype compared to the control group (OR=0.94: 95% CI; 0.42, 2.08).

The odds ratio was 0.94.

Table 6. Determining the relationship between 25-hydroxyvitamin D levels and MS disease progression.

Groups	Mild (n=7)	Intense (n=18)
25 hydroxy vitamin D level (ng/mL)		
Standard deviation ± mean	18.42 ± 3,64	15.33 ± 3.89
Z		-2.56
P-value		0.010

Table 7. Relationship between polymorphisms and haplotypes of the VDR gene and the odds ratio of polymorphisms.

SNPs	Genotype	Genotypic frequency (%)		Significance	Odds ratio (95% CI)
		RRMS (n=25)	Control (n=12)		
FokI	rs2228570	FF	18 (72)	3 (25)	$\chi^2= 11.96$ P= 0.003
		Ff	7 (28)	5 (41.7)	
		ff	0	4(33.3)	
ApaI	rs7975232	AA	10 (40)	6 (50)	$\chi^2=5.19$ P=0.075
		Aa	14 (56)	3 (25)	
		aa	1 (4)	3 (25)	
TaqI	rs731236	TT	12 (48)	4 (33.3)	$\chi^2=2.44$ P=0.294
		Tt	10 (40)	4 (33.3)	
		tt	3 (12)	4 (33.3)	
BsmI	rs1544410	BB	10 (40)	5 (41.7)	$\chi^2=0.025$ P=0.987
		Bb	6 (24)	3 (25)	
		bb	9 (36)	4 (33.3)	

χ^2 chi-square test; p, p-value; Significant at $P \leq 0.05$.

Discussion

In the present study, a statistically significant difference was observed between the percentage of FokI genotypes in RRMS patients and healthy individuals. FokI polymorphism showed a significantly increased risk with an odds ratio of 7.28 in patients with the FF genotype compared to healthy individuals. ApaI, TaqI, and BsmI were not significantly different between the two groups. In previous studies, the association between VDR gene polymorphisms and MS was investigated in several populations.¹⁵⁻¹⁷ The results of the present study are consistent with some studies conducted on different populations. A study in Iran found a big difference in the types of genes found in MS patients and healthy people when it comes to polymorphism and allelic frequency for ApaI, BsmI, and TaqI.¹⁸ A Japanese group found an increased frequency of the ApaI AA genotype and A allele in MS.¹⁸

Sioka *et al.* showed no association between ApaI, BsmI, and TaqI polymorphisms of the VDR gene.¹⁹ The results of our study are consistent with those found by Sioka *et al.*¹⁹ Previous epidemiological and biological evidence has supported the protective effect of vitamin D on the underlying disease course of MS.^{20,21} The present study's findings revealed low serum levels of 25-hydroxyvitamin D in MS patients. Patients with MS had a significantly lower mean serum level of 25-hydroxyvitamin D than healthy individuals, which was considered statistically significant. The present study observed a statistically significant relationship between low serum levels of 25-hydroxyvitamin D and the progression of MS.

Fitzgerald *et al.* also linked low levels of 25-hydroxyvitamin D in the blood to a higher risk of MS, higher disease activity, and a faster rate of progression in early MS and clinically isolated syndromes, in line with the findings of this study.²² Similarly, the present study observed a significant association between low levels of 25-hydroxyvitamin D and increased disease activity, as measured by the EDSS score. Although Mowry *et al.* reported that vitamin D levels were inversely associated with MS activity on brain MRI.²³ Another study reported that there is no relationship between 25-hydroxyvitamin D levels and disability scores in MS patients in Iran.²⁴ However, the study by Zhang *et al.* in China reported the same findings as the present study.²⁵

Sutherland *et al.* illustrated that vitamin D deficiency is common in MS patients, affecting approximately 50% of MS patients in Europe and 77% in African Americans.²⁶

Dong *et al.* reported that 76% of Kuwaiti MS patients were vitamin D deficient.²⁷ Similarly, Braidy *et al.* in their study showed that, more than half of the MS patients had vitamin D deficiency.²⁸ In addition, the serum level of 25(OH) D was lower in MS patients compared to the control group. According to Beecham *et al.* study serum vitamin D levels were low in Japanese MS patients.²⁹

Al-Temaimi *et al.* found that there was no significant difference in vitamin D levels between MS and healthy subjects. In this study, the small sample (N=50) and the high rate of vitamin D deficiency (82%) in the control group should be considered.³⁰

Niino *et al.* showed that the serum level of 25(OH) D was higher in MS than in the control group (healthy subjects).³¹

Vickaryous *et al.* illustrated that population-based prospective cohort study of 145 people with RRMS living in southern Tasmania and Australia from 2002 to 2005 found that higher 25(OH) D levels were associated with a reduced risk of relapse.³²

Wasnik *et al.* showed that Every 10 nmol/L increase in 25(OH) D results in a 12% reduction in the risk of recurrence. The radiological signs of MS disease activity also correlate with these clinical findings.⁴

According to Simpson Jr. *et al.* Results from a prospective cohort study of 1,482 MS patients in the BEYOND study who were administered interferon beta 1b found that a 50.0 nmol/L increase in blood 25(OH)D levels was associated with a 31% was associated with a lower rate of new lesions on MRI. Patients with 25(OH) D levels above 100.0 nmol/L had the lowest rate of new lesions on MRI. However, we did not observe any significant association with the recurrence rate.³³

The current study had limitations such as the small sample size and the need to select more studies with a larger sample size; It is also very important to select the sample size from different regions. Conducting a study to investigate polymorphic translocation of the VDR gene is necessary to understand the function of the VDR and increase the likelihood of detecting alleles that contribute to the risk of common diseases, as well as further research involving more cases to obtain results This can be distorted by small samples or low frequencies. Minor alleles are required.

Conclusions

Based on the findings of the present study, FokI polymorphism showed a significant risk increase with an odds ratio of 7.28 in RRMS patients with FF genotype compared to healthy individuals (OR=7.28; 95% CI; 1.86, 28.41).

List of abbreviations

VDR, Vitamin D Receptor
RRMS, Relapsing-Remitting Multiple Sclerosis
MS, Multiple Sclerosis
PCR, Polymerase Chain Reaction
MSSS, Multiple Sclerosis Severity Score
RFLP, Restriction Fragment Length Polymorphism

Conflict of interest

The authors declare no potential conflict of interest.

Ethics approval

This research was approved by the Medical Ethics Department of Iran University of Medical Sciences with no. IR.IUMS.FMD.REC.1401.429

Informed consent and patient consent for publication

All patients participating in this study signed a written informed consent form for participating in this study.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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