VITAMIN D AND VITAMIN D RECEPTOR GENE VARIANT IN EGYPTIAN MULTIPLE SCLEROSIS PATIENTS: A CASE-CONTROL STUDY

Doaa Mohammed Sultan[1], Iman A. Mandour[2], Khaled Mohamed Geba[3], Asmaa Galal-Khallaf[3] and Noha A. Radwan[2]

[1]Department of Zoology Chemistry, Faculty of Science, Ain Shams University, Egypt

[2]Clinical and Chemical Pathology, Kasr Alainy Medical School, Cairo University, Egypt

[3]Molecular Biology Department, Faculty of Science, Monifeya University, Egypt

Corresponding Author: Noha A. Radwan, Kasr Alainy Medical School, 1057 2nd district, 6 October City, Giza

E-mail: nohakhaled11@cu.edu.eg

ORCiD Number: https://orcid.org/0000-0003-4048-6378

ABSTRACT:

Aim: Multiple sclerosis (MS) is an autoimmune disease with a controversial etiology. Both genetic and environmental factors are thought to be involved in the risk of developing the disease. The purpose of this study was to assess the association of the Vitamin D receptor (VDR) BsmI variant with MS and to investigate the interaction of this variant with vitamin D levels. **Method:** 100 subjects were recruited for this study. Fifty patients were diagnosed with MS and 50 were healthy individuals. BsmI was genotyped by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analyses in both groups and serum 25-hydroxyvitamin D [25(OH)D] levels were determined in MS patients by high-performance liquid chromatography (HPLC). **Results:** The distribution of the genotype of VDR polymorphism BsmI did not differ significantly between MS patients and healthy controls**.** The G allele of BsmI was a statistically significant higher percentage in MS patients (*p*-value 0.045). There is no statistically significant difference in the level of 25(OH)D between MS patients and the control group. **Conclusions:** The study findings suggest that the VDR gene variant BsmI G allele may increase the risk of the development of MS.

Keywords: VDR gene – RFLP – Vitamin D - HPLC

INTRODUCTION:

Multiple sclerosis (MS) is an autoimmune chronic inflammatory disease of the central nervous system (CNS) causing inflammatory demyelination and axonal transection with irreversible neurologic damage. Diagnosis of MS generally is between the first and second decades of life (1). In 2016, the prevalence of MS in Egypt was 13.7/100,000 for the age ≥ 17 years. MS is a multifactorial disorder, in which environmental factors interact with genetically susceptible individuals. The McDonald's criteria are the most commonly used criteria for MS diagnosis (2).

Vitamin D is a secosteroid mainly produced in the skin by the action of Ultra-Violet B (UVB) rays. Vitamin D is crucial for bone and calcium homeostasis, adequate neuromuscular functions, brain neurotransmission, and immune system regulation (3). Vitamin D initiates its biological responses by binding to the nuclear vitamin D receptor (VDR), a phosphoprotein that regulates gene expression via zinc finger-mediated DNA binding and protein–protein interactions (4). The *VDR* gene is located on chromosome 12 (12q13.11) and contains nine exons. Several common allelic variants have been identified, and some variants may have consequences on VDR function and activity. The most widely studied are Apa-I (rs7975232), Bsm-I (rs1544410), Fok-I (rs10735810), and Taq-I (rs731236) (5).

Our research aimed to study the genotype and the frequency of *VDR* gene BsmI A/G in the intron 8 (rs1544410) variant and serum level of Vitamin D in patients with Multiple Sclerosis at the diagnosis.

MATERIALS and METHODS:

Subjects

One hundred patients were recruited in this study which was performed in the Multiple Sclerosis Unit at Kasr Elainy Medical School. The subjects were divided into 2 groups: group I, 50 MS patients that were diagnosed according to McDonald criteria, and group II, 50 healthy subjects of matching age and sex were included as a control group. All patients were subjected to thorough history-taking clinical, and neurological examinations. Informed consent to participate in the study was obtained from all participants.

VDR gene polymorphisms genotyping by PCR-RFLP

Genomic DNA was extracted from EDTA-peripheral blood samples obtained from both groups using Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit (USA #K0781) following the manufacturer's recommendations. The extracted DNA was stored at − 20 °C till used. The targeted SNP was identified by PCR-RFLP where the targeted SNP was amplified by conventional PCR followed by restriction digestion. SNV primer sequences, PCR thermal profiles, and expected amplicon size following restriction digestion are summarized in **Table 1**. PCR-RFLP reactions were performed in a 50-μl final volume; 25 μl Thermo Scientific Dream Taq green PCR master mix, 2 μl of forward and reverse SNP primers (Sigma Aldrich, USA), 10 μl genomic DNA, and 11 μl DNase-free water and PCR protocols designed to the corresponding SNP were applied as in **Table 1**. The amplicons were examined by 3% agarose gel electrophoresis to ensure appropriate amplification. The amplified PCR products were digested using the corresponding restriction enzymes, and the resulting RFLP products were analyzed by 3% agarose gel electrophoresis and visualized by the Dolphin-Doc gel documentation system (Wealtec, USA). The restriction enzyme mixture reaction was 30 μl final volume and contained 17 μl nuclease-free water, 2 μl 10X FastDigest green buffer, 10 μl PCR product, and 1 μl FastDigest enzyme. Restriction enzymes were used following the manufacturer's instructions: BsmI code number: #FD0964 (Thermo Scientific FastDigest, USA).

Table (1): Primers sequences and PCR thermal profile of VDR gene BsmI (rs1544410) variant.

SNV	Primers	PCR thermal profiles	Amplicon (bp)
BsmI	F 5′-GGG AGA CGT AGC AAA AGG-3′ R 5′-AGA GGT CAA GGG TCA CTG-3′	Initial denaturation 94°C for 3 min; 32 cycles:94°C for 45s, 60°C for 45s, and 72°C for 45s; and final extension: 72°C for 5 min	358

Serum Vitamin D Assay by HPLC

The assay was performed on Agilent 1260 HPLC instrument (5301 Stevens Creek Blvd Santa Clara, California 95051, USA) with a photodiode array detector set at 265 nm and reversed-phase Agilent column (Microsorb-MV 100-5 C18, 250 × 4.6 mm, 5 μm). Vitamin D Extraction was done according to Hanna et al. The mobile phase ratios were changed to acetonitrile: methanol: water of 45:45:10, v/v. The flow rate was slowed down to 1.0 ml/min for 18 min (6).

Statistical Analysis

Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) vs. 28. Numerical data were summarized using means and standard deviations or medians and/or ranges, as appropriate. Categorical data were summarized as numbers and percentages. Estimates of the frequency were done using numbers and percentages. Numerical data were explored for normality using the Kolmogrov-Smirnov test and Shapiro-Wilk test. Chi-square or Fisher's tests were used to compare the independent groups to categorical data, as appropriate.

Comparisons between two groups for normally distributed numeric variables were done using the Student's t-test while for non-normally distributed numeric variables, comparisons were done by the Mann-Whitney test. Comparisons between more than 2 groups were performed by Kruskal-Wallis for non-normally distributed variables, then followed by post hoc if needed (Post Hoc comparison was done and the P value was adjusted using Bonferroni adjustment). All tests were two-tailed & Probability (p-value) ≤ 0.05 is considered significant.

Results

Demographic data

The studied cases were divided into group I (MS patients; n = 50, 16 males (32%) and 34 females (68%), age mean 28 ± 6 years) and group II (healthy controls; n = 50, 23 males (46%) and females 27 (54%), age mean 31 ± 6 years). Demographically among MS patients, Females were presented more than males with a percentage of 68%.

VDR gene polymorphism genotype and allelic distribution (Table 2)

VDR gene polymorphisms were genotyped for all studied subjects, and the resulting RFLP products were visualized by 3% agarose gel electrophoresis **(Fig. 1)**

Table (2): VDR gene polymorphism genotype and allele frequencies in the two study groups.

		Cases		Control		p-value	OR	95%CI for OR
		Number	%	Number	%			
BsmI SNV	AA	10	20	16	32	0.124*		
	AG	20	40	23	46		1.4	0.5-3.9
	GG	20	40	11	22		3	0.99-9
BsmI Allele	A (Major Allele)	40	40	52	55.3	0.045*	1.8	1.1-3.2
	G (Minor Allele)	60	60	42	44.7			

*p value <0.05 is considered significant, OR: Odds ratio, CI: Confidence interval
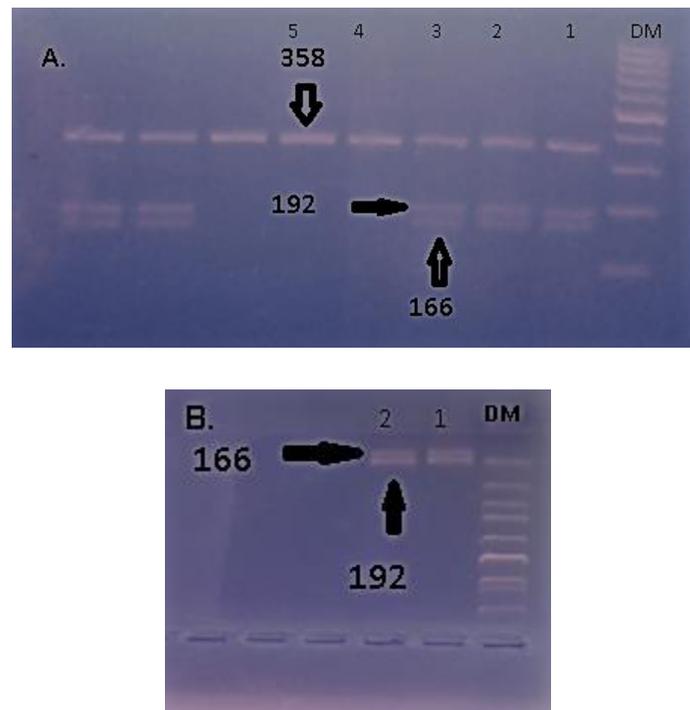
Figure (1): PCR-RFLP-based genotyping of VDR gene variant. The RFLP bands were visualized by 3% agarose gel electrophoresis. A. BsmI, lane 5: AA (Homozygous), lane 3: AG (Heterozygous). B. Lane 2: GG (Homozygous). DM, 100 bps DNA marker, size of the band in bps is written above the corresponding band.

Serum level of 25 (OH) Vitamin D and relation with VDR gene Variants

The serum level of 25 (OH) Vitamin D median for MS patients was 16.2 ng/mL with a range from 5 ng/mL to 51 ng/mL. In the control healthy group, the median for 25 (OH) Vitamin D was 15.7 ng/mL with a range from 5.3 ng/mL to 37.3 ng/mL. The relation between the serum level of 25 (OH) Vitamin D and BsmI variants is represented in **Table (3).**

Table (3): Relation between Serum level of 25 (OH) Vitamin D and BsmI Genotype

BsmI							
	AA		AG		GG		*p*-value
	Median	Range	Median	Range	Median	Range	
25 (OH) Vitamin D (ng/mL)	19.3	7.8 – 37.9	13.4	6.7 – 40.8	20.1	5 – 51	0.955*

p-value <0.05 is considered significant

Discussion

Studying VDR gene variants in association with MS has provoked a large body of research with a special interest in the studied race and population. In various studies, female patients with MS outnumbered male patients with M. This was stated before in El-Tallawy et al. An age-specific prevalence ≥17 years was stated to be 13.7/100,000 (2).

In the present study, we analyzed the VDR gene variant BsmI (rs1544410) and its relation to MS in Egyptian patients for a better understanding of MS susceptibility. There is no statistically significant difference between Genotypes AA, AG, and GG in MS and the control group (*p*-value, 0.124). Our result is following other studies that demonstrated that the BsmI variant (rs1544410) had no statistically significant difference between the MS group and the control group (7,8).

The BsmI variant (rs1544410) is located in the 3′UTR. 3′UTR regulatory region affects mRNA stability and translational activity, which is important in understanding the functionality of sequence variations in the 3′UTR. In the MS group, there was a statistically significant higher percentage of the G allele more than the A allele (*p*-value 0.045) which is in accordance with the study done by (9). This is not in accordance with Cakina et al. who stated that there is no statistical difference between the A allele and G allele in the two groups (8).

A meta-analysis done by Imani et al. stated that 16 case-control studies with 1793 cases and 1815 control subjects were included to examine the association between BsmI polymorphism and MS risk. The pooled results demonstrated no significant association between BsmI polymorphism and MS risk under all genetic models, but subgroup analysis

revealed that BsmI polymorphism across the recessive model

Increased the risk of MS in the Asian population (OR = 1.78, 95%CI = 1.01–2.93, random effect) compared to the European population (OR = 0.84, 95%CI = 0.66–1.06, random effect) (10). In our study, there is an increase in the risk of MS with GG Genotype (OR = 3, 95%CI = 0.99–9).

Vitamin D is a fat-soluble prohormone supplied in the diet and/or formed in the skin upon exposure to ultraviolet radiation (UVR). In the liver, Vitamin D is metabolized to 25(OH)D, which represents the major circulating vitamin D metabolite and a reliable indicator of vitamin D status. Vitamin D controls a wide variety of biological responses, including calcium homeostasis, bone formation, apoptosis, and immune homeostasis (4).

In the current study, the serum level of vitamin D between the MS group and the control group had no statistically significant difference with a p-value of 0.0.491. These results are following Cakina et al. who stated that there was no statistical difference between serum level of vitamin D in MS and control groups (8). Also, there is no difference between Vitamin D and the different genotypes of the BsmI variant (*p*-value 0.955). This result was the same as the study done by Agnello et al. (4).

Conclusion

Thus far, VDR has been investigated in many studies to determine the relationship with MS, but there are contradictory conclusions. Data from this study suggested that the G allele of BsmI is associated more with Egyptian MS patients. Serum level of 25 (OH) vitamin D has no statistical difference between Egyptian MS patients and Control group. Small sample sizes, differences in ethnicities, extensive geographic variation, interactions with other genetic or environmental factors, and/or clinical heterogeneity may be the reasons for this disparity.

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