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Research Article

Vitamin D Receptor Gene Polymorphisms and Vitamin D Level Among Multiple Myeloma Sudanese Patients: A Cross-Sectional Study

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Abstract:
Background and objective: Multiple myeloma (MM) is associated with vitamin D deficiency, which may impact disease progression. Vitamin D acts through the vitamin D receptor (VDR), and single nucleotide polymorphisms (SNPs) in the VDR gene could influence MM susceptibility.
This study aimed to ascertain the genotypes, and allelic distribution of the VDR gene polymorphisms (rs7975232, and rs731236) and its relationship to MM in Sudanese individuals.
Methods: in this comparative cross-sectional study; 50 patients and 50 apparently healthy individuals age matched (as controls) were enrolled. Serum samples were analyzed for vitamin D level using Cobas e 601 (Roch Germany). Genomic DNA was extracted from whole blood by using specific extraction kit Qiagen. By using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP); then all samples were genotyped for the VDR polymorphisms (rs7975232), and (rs731236).
Results: Significant association was found between VDR gene polymorphisms and MM. There was a significant difference in genotypic frequencies of the rs7975232, rs731236 SNPs and alleles frequencies between MM patients and control group. MM patients had a significantly decreased serum vitamin D when compared with control group, results also showed that decreased serum vitamin D, VDR Taq1 (CC allele), and ADIPOQ VDR Apa1 (TT allele) had independently predicted the incidence of multiple myeloma in the Sudanese population.
Conclusion: Along with deficiency of Vitamin D level, the VDR genetic variants (rs 7975232and rs731236) are connected to MM risk among the Sudanese population.

Keywords: Vitamin D Receptor, Genotypes, polymorphisms, Serum Vitamin D, Multiple Myeloma,

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Introduction

Multiple myeloma (MM) is a form of blood cancer marked by the irregular proliferation of clonal plasma cells within the bone marrow. This unchecked growth results in considerable complications, such as serious bone deterioration, anemia, kidney failure, and increased calcium concentrations in the bloodstream.(**1**) MM represents around 1% of all cancers and 10% of blood-related malignancis.(**2**) In certain cases of monoclonal gammopathy of undetermined significance (MGUS); more advanced and asymptomatic pre-malignant phase known as smoldering multiple myeloma (SMM) can be identified through clinical evaluation, which occurs in about 0.5% of the population aged 40 years or older and has a progression rate to multiple myeloma of approximately 10% annually during the first five years post-diagnosis, 3% annually in the subsequent five years, and 1.5% annually thereafter.(**3-4**) A large proportion of patients with hematological malignancies have low vitamin D (VitD) levels ranging from 30% to 80%, regardless of the type of malignancy (myeloid or lymphoid tumors) or the treatment received.(**5**) VitD insufficiency has been shown to have a detrimental influence on plasma cell neoplasms, with a direct association between low blood VitD levels and a late illness stage according to the International Staging System (ISS).(**6**)

The vitamin D receptor (VDR), a member of the nuclear receptor superfamily, plays a crucial role as a transcriptional regulator in the signaling pathway of calcitriol,als known as 1-alpha,25-dihydroxycholecalciferol (1α,25(OH)2D). Upon binding with 1α,25(OH)2D, VDR activates and forms a heterodimer with the retinoid X receptor (RXR), this complex of 1α,25(OH)2D-VDR-RXR then translocate to the nucleus, where it regulates the transcription of genes associated with the effects of vitamin D, which include the metabolism of phosphorus and calcium, cell proliferation, and the regulation of both innate and adaptive immunity.(**7**) Although serum VitD levels and VDR gene polymorphisms are related to the development of MM in several populations.(**8-9**) no data exist for MM among Sudanese. This is the first study aiming to determine the relationship between VDR gene polymorphisms and the MM. The results founded from this study, will certainly have an impact in clinical practice.

Materials and Methods

This comparative cross-sectional study was carried out in Khartoum state in Sudan during the period from 2021-2022. The study was approved by the Research Ethics Committee of the Ministry of Health (ID No 44), after obtaining an informed consent (from participants or parents of minors); a total of one hundred Sudanese participants were enrolled in this study. Participants were divided into two groups; 50 MM patients (23 males and 27 females with age ranged from 17- 69 years who admitted to radio and isotope center in Khartoum state) and 50 apparently healthy individuals as control (28 males and 22 females with age ranged from 16-69 years). Patients who had any disorders that may affect the levels of VitD or treated with it; were excluded from the study.

Demographic data were collected through structured questionnaire, and samples were collected from patients before starting chemotherapy and radiotherapy. Five milliliters of venous blood were drawn from each participant (patients or control group) and divided into two containers: a whole blood EDTA container for Complete Blood Count (CBC was done by Sysmex KX21 autoanalyzer) and DNA extraction; and a plain container for serum Vitamin D measurements which was done by Cobas e 601 (Roch Germany).

DNA Extraction

Genomic DNA was extracted from whole blood using a Qiagen extraction protocol.(**10**) The quality of DNA was assessed using a Nano-Drop spectrophotometer (Thermo Fisher Scientific**)** to measure the A260/A280 ratio and confirm DNA purity.

Screening of VDR SNPs

PCR-RFLP was used to amplify and genotype ADIPOQ variants Apa1 (rs7975232) and Taq1 (rs731236). The amount of the PCR reaction mixture was 25 µL, and the annealing temperatures varied depending on the primers (specific temperatures optimized for each primer pair), which was performed using an Exicycler Real-Time PCR system from Bioneer (Korea).(**11**) A standard horizontal gel electrophoresis system was used to test the amplified products for electrophoresis, ApaI and TaqI restriction enzymes were used in this study, following the method described by Gunes.(**12**) Following the manufacturer's recommendations, 1U of the relevant restriction enzyme was used to digest 5 µL of the amplified products in a total reaction volume of 20 µL. The 100 bp DNA ladder and the digestion products were resolved on 2.0% agarose gels stained with ethidium bromide and visualized under a UV transilluminator (e.g., Bio-Rad Gel Doc system or equivalent) (**Figure 1).**

Statistical Analysis:

Statistical Package for Social Sciences (SPSS; Chicago, USA, version 20.0) was used for all statistical analyses. One-Sample Kolmogorov-Smirnov test was used to detect the normal distribution of data for baseline parameters. The Hardy-Weinberg equilibrium (HWE) was evaluated for all the SNPs in patients and controls by comparing the observed and expected frequencies of the genotypes using the Chi-square test. An independent T test was used to analyze baseline parameters. The distribution of the genotypes and allele frequencies of VDR SNPs for patients and control were compared using the Chi-square test with two × two contingency

tables using GraphPad Prism 6.07 software. The Association of SNPs with Vitamin D level was performed using the One-Way ANOVA test. The association of the VDR SNPs with the risk of MM was assessed by odds ratio (OR) with 95% confidence intervals (CI) and logistic regression to predict risk factors. Results were considered statistically significant for values of $P \leq 0.05$.

Results

Table (1) summarizes the anthropometric measurements, hematological parameters and biochemical parameters of the participants. The means of red blood cells (RBCs), hemoglobin (HB), packed cell volume (PCV), and platelet counts (PLTs) showed a significant decrease in MM patients, while mean of WBCs showed no significant difference. There was a significant decrease in Vitamin D level in MM patients when compared with control. Genetic analysis showed that the VDR gene polymorphisms Apa1 (rs7975232), and Taq1 (rs731236) were detected in all the participants. PCR products of T/t SNP (rs7975232), and C/c SNP (rs731236), were 300bp, and 250bp, respectively (**Figure 1).** Restriction digestion was carried out following amplification. For the rs7975232 genotype, the wild genotype (TT) produced a single band of 300bp; the heterozygous genotype (Tt) had three fragments of 300bp, 200bp, and 100bp; and the homozygous mutant genotype (tt) produced two bands of 200bp and 100bp. For rs731236, the wild genotype (CC) had a single band 250bp; the heterozygous genotype (Cc) produced three fragments 250bp, 150bp, and 100bp; and the homozygous mutant genotype (cc) produced two bands 150bp and 100 bp (**Figure 2).** The genotypes and alleles frequencies of VDR - polymorphisms (ApaI &TaqI) are summarized in (**Tables 2-3).**

Table (2) shows a significant association in ApaI in genotype TT (*P*=0.000, OR 19.00 CI=3.109-116.1), dominant model (TT+Tt) (*P*=0.014 OR=6.000 CI=1.241-2900) and allele T (*P*=0.001, OR =2.481 CI=1.398-4.04). In **table (3)** TaqI is also show significant association in genotype CC (*P*=0.000, OR=18.957 CI=3.185-110.4), dominant model (CC +cc) (*P*=0.025, OR=5.268 CI=1.076-25.79) and allele C (*P*=0.000, OR=3.049 CI=1.688-5.506).

Furthermore, there were no significant differences found in the serum vitamin D levels and VDR-SNPs (Apa1 and Taq1) in patients (**Figure 3).**

Significant risk variables for multiple myeloma were found by the logistic regression analysis as shown in (**Figure 4).** These risk factors include vitamin D deficiency, the VDR Apa1 (rs7975232) TT genotype, and the VDR Taq1 (rs731236) CC genotype, which are predictive of susceptibility to the disease.

Table 1: Comparison between anthropometric measurements, hematological parameters and biochemical parameters in MM patient with control group

Variables	MM (N=50)	Control (N=50)	*P*-value
Age (Year)	47.66±16.2	46.02±16.3	0.616
Gender count (M/F)	23/27	28/22	-
WBCs (10^3/UL)	6.694±2.335	7.142±1.824	0.288
RBCs (10^6/UL)	3.796±1.165	5.035±0.479	0.000*
Hb (g/dl)	10.79±3.19	13.96±1.26	0.000*
PCV (%)	32.78±8.97	41.81±3.38	0.000*
Plt (10^3/UL)	173.88±65.6	268.20±67.2	0.000*
Vit D (ng/ml)	16.42±7.04	20.15±6.00	0.005*

Data were presented as mean value ± SD or counts. WBC: white blood cell, RBCs: Red blood cell, Hb: Hemoglobin, PCV: Packet cell volume, PLTs: platelet, VitD: Vitamin D. An independent t.test was used. Two-tailed P-value ≤ 0.05 was considered as statistically significant.

Table 2: Comparison between genotypes and allele frequency of VDR (ApaI) gene polymorphisms in MM patient and control group:

Gene Polymorphisms	Group		*P*-value	OR	CI Lower-Upper
	MM	Control			
VDR ApaI					
Genotype					
TT	19 (38.0%)	5 (10.0%)	0.000*	19.00	(3.109-116.1)
Tt	29 (58.0%)	35 (70.0%)	0.064	4.143	(0.840-29.00)
tt	2 (4.0%)	10 (20.0%)	reference		
dominant model					
TT+ Tt	48 (96%)	40 (80%)	0.014*	6.000	(1.241-29.00)
Allele					
T	67 (67.0%)	45(45.0%)	0.001*	2.481	(1.398-4.404)
t	33 (33.0%)	55 (55.0%)	reference		

Data are reported as numbers and percentages (%) for genotype counting across all participants (multiple myeloma group and control group). Analyses were conducted using the equation of allelic frequencies. A Chi-square test was performed. A two-tailed P-value ≤ 0.05 is considered significant. OR: odd ratio, CI: confidence interval.

Table 3: Comparison between genotypes and allele frequency of VDR (TaqI) gene polymorphisms in MM patient and control group:

Gene Polymorphisms	Group MM	Control	*P*-value	OR	CI Lower-Upper
VDR TaqI Genotype					
CC	25 (50.0%)	6 (12.0%)	0.000*	18.75	(3.185-110.4)
Cc	23 (46.6%)	35 (70.0%)	0.174	0.158	(0.585-14.95)
Cc	2 (4.0%)	9 (18.0%)	reference		
dominant model					
Cc + cc	48 (96%)	41 (82%)	0.025*	5.268	(1.076-25.79)
Allele					
C	73 (73.0%)	47 (47.0%)	0.000*	3.049	(1.688-5.506)
c	27 (27.0%)	53 (53.0%)	reference		

Data are reported as numbers and percentages (%) for genotype counting across all participants (multiple myeloma group and control group). Analyses were conducted using the equation of allelic frequencies. A Chi-square test was performed. A two-tailed P-value ≤ 0.05 is considered significant. OR: odd ratio, CI: confidence interval.

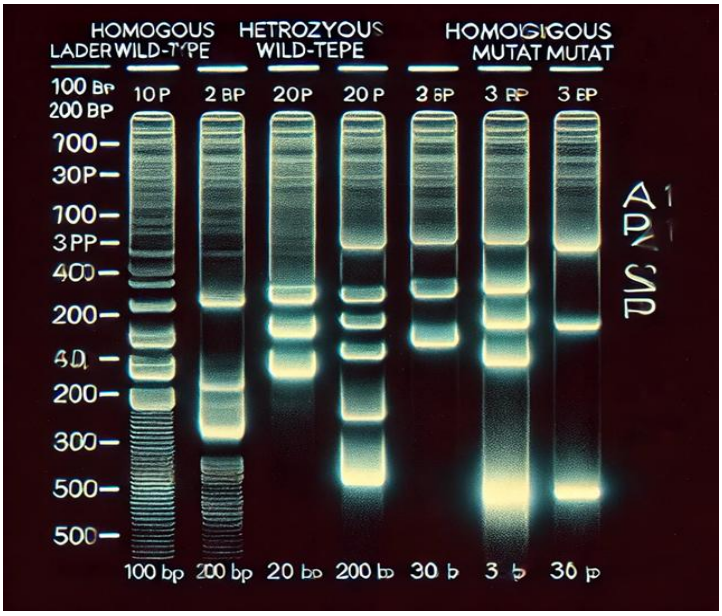

Figure 1: generated image representing an electrophoresis gel for Apa1 SNP analysis, with labeled lanes and distinct band patterns for different genotypes

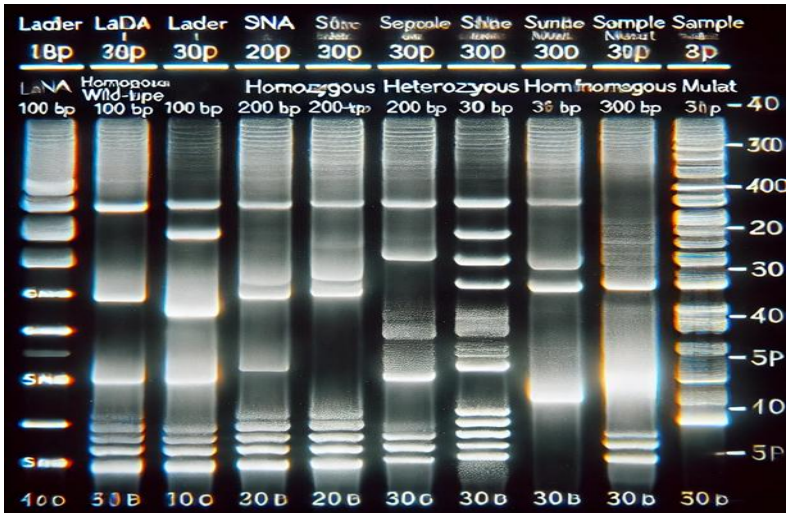

Figure 2: Gel electrophoresis image representing SNP analysis using the TaqI enzyme, with distinct band patterns for different genotypes.

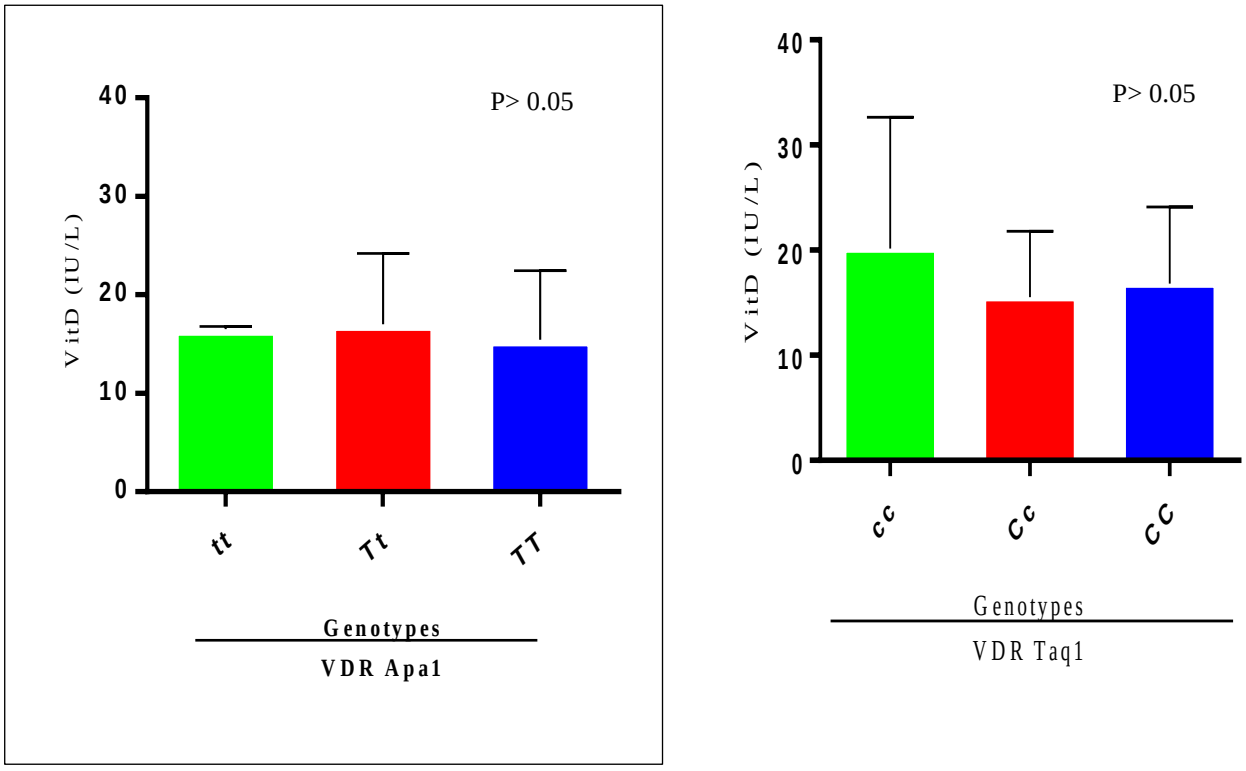

Figure 3: Comparison between VDR (Apa1 and Taq1) gene polymorphisms and VitD level in MM patients.

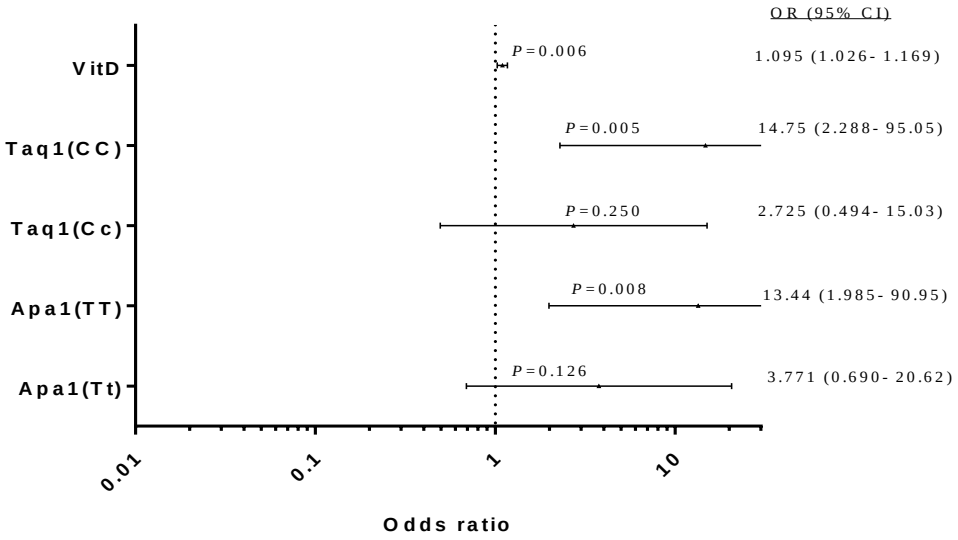

Figure 4: Logistic regression analysis of risk factors affecting MM patients after adjusts age and sex
OR: odd ratio, CI: confidence interval. *P*-value ≤ 0.05 was considered statistically significant

Discussion

Multiple myeloma patients have lower levels of vitamin D, which is mediated by vitamin D receptor (VDR). Single nucleotide polymorphisms in the VDR can modify its function, impacting vitamin D status. (**13**) This study results showed that VDR gene polymorphisms (rs795232, and rs731236) were significantly associated with MM in the Sudanese individuals. In the analysis of the genotype distribution of TaqI (rs731236) conducted in this study, the MM Group showed a significantly greater proportion of individuals with the CC genotype (carriers) (50.0%) compared to control group (12.0%). Results also revealed that the rs731236 C allele raises the probability of developing multiple myeloma among participants by 3.049 times (P =0.000). This comes in line with Li Y et al. study that noted, C Allele is important genetic factor in MM in the Chinese Han population, another study done by Lyu et al. report that, C was found TaqI is associated with MM in the dominant model and heterozygous model.(**14-15**)

Additionally, this study demonstrated a statistically significant difference in the genotype distribution of Apa1 (rs795232) between MM cases and controls. There were significantly more carriers of the TT genotype in the MM group (38.0%) than in the control group (10.0%). It is also discovered that the rs795323 T allele increases the risk of developing multiple myeloma in the study participants by 2.481 folds (P =0.000). Our results support research results done by Lyu C et al., that indicates the genetic polymorphisms at the VDR Apa1 (rs795232) loci enhanced the risk factor for the onset of MM risk in Asian populations. (**13**) This finding was counteracted by Shaifa et al., that found no association between ApaI and MM. (**15**)

The study results also revealed significant deficiency of serum Vitamin D level in MM group compared to control group. Our results come on line with results reported by Lauter et al., that indicates the relatively high prevalence of vitamin D deficiency in patients with myeloma.(**16**) This finding was counteracted by Clement Z, that found a relatively high prevalence of vitamin D deficiency in patients with myeloma. (**17**)

Furthermore, this study found no significant differences in serum vitamin D levels and VDR-SNPs (TaqI (rs731236), and Apa1 (rs7975232) among patients. However, those with the TaqI CC and Cc genotypes exhibited lower serum vitamin D levels.

In evaluation of the polymorphisms of VDR gene (TaqI, ApaI) and serum Vit D level with MM risk; the results showed that TaqI, ApaI, polymorphisms along with low serum Vit D levels, were associated with MM risk. However, due to geographical and ethnic differences in SNPs and serum Vit D levels and limited studies were found regarding the association between VDR gene polymorphisms, low serum Vit D level and MM. the results concerning SNPs (TaqI & ApaI) and low serum Vit D levels may be difficult to extrapolate to non-Sudanese populations as the studies was confined to Sudanese participants.

In conclusion, the results of this study give strong support for the hypothesis that the multiple myeloma risk in the Sudanese population was strongly influenced by VDR gene polymorphisms (ApaI, and TaqI), and Vit D deficiency. Considering the importance of gene-gene interactions, more studies are needed to determine the substantial contributions of VDR gene variants that serve as genetic markers for identifying individuals at heightened risk for MM.

Conflict of Interest

All authors declare no conflict of interest regarding this study

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