
Research article

Vitamin D receptor gene polymorphisms in Sudanese children with type 1 diabetes

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Abstract: Type 1 diabetes mellitus (T1DM) is a T cell mediated autoimmune disease. Vitamin D was found to suppress the incidence of diabetes when bind to its receptor (VDR), probably by suppressing T cell activations. Thus the VDR gene polymorphism may have an impact on pathophysiology of this disease. Since there was no consistent association between VDR polymorphisms and the risk of T1DM, this study aimed to investigate a VDR gene polymorphism in Sudanese children with T1DM. We examined the VDR gene *Bsm1* (rs1544410), *Apa1* (rs7975232), and *Taq1* (rs731236) single nucleotide polymorphisms in 174 children with T1DM, and 56 children as control, and the association of these polymorphisms with the diabetic control. Among study patients, the majority (85.63%) of diabetic patients reported metabolically poor controlled (HbA1c > 8%). As compared with the control, patients with T1DM presented more commonly with *Bsm1* B allele ($p = 0.001$; OR 0.283; 95% CI 0.131–0.609) and *Taq1* T allele ($p = 0.05$; OR 2.429; 95% CI 1.073–5.496). *Apa1* A allele was less common in patients with T1DM without statistical difference ($p = 0.862$; OR 1.085; 95% CI 0.546–2.156). Our study suggests that, *Bsm1* and *Taq1* polymorphisms of the VDR gene associated with the prevalence of T1DM.

Keywords: Type 1 diabetes mellitus; Vitamin D receptor; Gene polymorphisms; Sudanese children

1. Introduction

Type 1 diabetes mellitus (T1DM) is a multi-factorial autoimmune disorder, characterized by the destruction of pancreatic beta cells resulting in the deficiency of insulin secretion [1], caused by complex interaction between genetic and environmental factors, accounting for 5–10% of diabetes

cases worldwide [2], and it is common among children and young adults and usually developed early under age 30 years.

It is known that the active vitamin D (1,25-dihydroxy vitamin D₃), play an important role in the regulation of immune cells proliferation and differentiations, lymphocytes activation, cytokines production [3], and insulin secretion [4]. Vitamin D bioactivity is through the vitamin D receptor (VDR), which is belonging to the nuclear hormone receptors superfamily [5].

The VDR gene is located on the long arm of chromosome 12 (12q12) and 14 (q14), and four common single nucleotide polymorphisms (SNPs) have been identified [6-9] namely *Bsm1*, *Fok1*, *Apa1* and *Taq1* polymorphisms (designated as rs1544410, rs10735810, rs7975232, and rs731236 SNP, respectively). VDR polymorphism was found to be associated with many autoimmune disorders in human, such as celiac disease [10], multiple sclerosis [11,12], systemic lupus erythematosus [13-15], rheumatoid arthritis [16,17], and hashimoto,s throiditis [18]. The role of VDR polymorphisms in the pathogenesis of T1DM was not clear, large number of studies [5,7,19-25] has been investigated the association between the aforementioned SNPs and the risk of T1DM but the results were inconsistent.

This study was conducted to investigate the association between VDR polymorphisms (at position *Bsm1*, *Taq1* and *Apa1*) and susceptibility to T1DM in Sudanese population.

2. Materials and Methods

2.1. Patients and controls

The study encompasses 174 T1DM patients [mean onset age 7.68 ± 3.59 (range, 1–16) years old; 90 female and 84 male] refer to the diabetic clinic at Wad Medani Pediatric hospital which was founded in 1987 to serve children at Gezira state and the out skirt provinces. The patients group was diagnosed according the American Diabetes Association for type 1 diabetes [2]. Fifty six unrelated healthy children [9.50 ± 4.23 (range 4–16) years old; 32 female and 24 male]. The patients were classified into metabolically poor glycemic control with $HbA_{1c} > 8\%$ and well glycemic control with $HbA_{1c} \leq 8\%$. HbA_{1c} was assessed by chromatographic-spectrophotometer ion exchange (BioSystems). The demographic and the clinical information were obtained from each subject through well structured questionnaire. Informed consent was obtained from the children guardians or relatives, and the Faculty of Medicine Institutional committee approval was obtained.

2.2. VDR gene analysis

DNA was extracted from the whole blood by using the QIAamp DNA Blood Mini Kit protocol (Qiagen, USA). The target gene of the VDR was extracted from whole gene sequence available at the Pubmed (NCBI Reference Sequence: NG_008731.1), and amplified by the PCR based on primers specific for *Bsm1*, *Apa1* and *Taq1* polymorphisms (designated as rs1544410, rs7975232, and rs731236 SNP, respectively) (Table 1). The PCR condition and digestion used for amplification of *Bsm1* as follows; 94 °C for 5 min, and 30 cycles using the following temperature profile: 94 °C for 1 min, 56 °C for 1 min, 72 °C for 1 min, and final elongation for 5 min. The PCR products were 825-bp long (B allele), and were digested with Mva12691 (*BsmI*) at 37 °C for 3 hrs, and then subjected to electrophoresis in 2% agarose gel stained with 5 µL ethidium bromide. The lengths of the restriction fragments were 649 and 176 bp (mutant b allele). The PCR condition and digestion

used for amplification of *Apa1* and *Taq1* as follows; 94 °C for 10 min, and 30 cycles using the following temperature profile: 94 °C for 1 min, 62 °C for 1 min, 72 °C for 1 min, and final elongation for 5 min. The PCR products were 716-bp long (A or T alleles) and were digested with *ApaI* and *TaqI* at 37 °C and 65 °C for 2 hrs respectively, and then subjected to electrophoresis in 2% agarose gel stained with 5 µL ethidium bromide. The lengths of the *ApaI* restriction fragments were 481 and 235 bp (mutant “a” allele), and for *TaqI* were 419 and 297 bp. Five percent of the samples were checked twice for results accuracy.

Table 1. Primers design and DNA fragment length of VDR gene polymorphism.

SNP		PCR primer sequences (5'-3')	Fragment size
<i>Bsm1</i>	Forward	CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA	B: 825 bp
	Reverse	AAC CAG CGG AAG AGG TCA AGG G	b: 649 bp, 176 bp
<i>Apa1</i>	Forward	GGG ACG CTG AGG GAT GGC AGA GC	716 bp
	Reverse	GGA AAG GGG TTA GGT TGG ACA GGA	481 bp, 235 bp
<i>Taq1</i>	Forward	GGG ACG CTG AGG GAT GGC AGA GC	716
	Reverse	GGA AAG GGG TTA GGT TGG ACA GGA	481 bp, 235 bp

2.3. Statistical analysis

A chi-squared test (χ^2 test) was used to evaluate the associations between different genotypes of *Bsm1*, *Taq1*, and *Apa1* variants and the disease (T1DM vs controls). The comparisons of the genotype frequencies between groups was analysed using the t-test. Odd ratios (ORs) and 95% confidence intervals (CIs) were calculated for each allele and genotype using logistic regression. The differences were considered significant if the *P*-value less than 0.05.

3. Results

The base line characteristics of the study subjects are shown in (Table 2). The male to female ratio in diabetic and control groups was 1:1.1 and 4.3 respectively. Among diabetic patients 59.2% were having family history of diabetes, the mean HbA_{1c} among diabetic group was significantly high ($P < 0.001$) as compared with the control group, on the contrary, BMI which was significantly low among diabetic group, 85.6% of T1DM patients characterized by metabolically poor diabetic control.

Table 2. Baseline characteristics of diabetic and control subjects.

Characteristics		Diabetics (N = 174)	Non diabetics (N = 56)
Gender (years)	Male	84	24
	Female	90	32
Age at diagnosis (years)		11.48 ± 3.39	9.50 ± 4.23
Age at onset of disease		7.68 ± 3.59	-
Duration of diabetes (years)		3.82 ± 2.82	-
Family history of diabetes	Negative	71	40
	Positive	103	16
BMI (kg/m ²)		16.22 ± 2.37	19.10 ± 4.61*
HbA _{1c} (%) (normal rang = 5.4–14.5)		10.72 ± 2.18	6.05 ± 1.34*
Diabetic control	Well (≤8.0%)	25 (14.4%)	-
	Poor (>8.0%)	149 (85.6%)	-

* $p < 0.001$

3.1. Genetic set

There was a difference in the VDR genotype and allele frequencies between T1DM patients and the control group. *Bsm1* BB and B allele were significantly ($P = 0.004$ and $p = 0.001$ respectively) higher frequency in patients with T1DM (Table 3). The complete cleavage of *Bsm1* to the target gene into homozygous (wild type *BB* = 825 bp or mutant *bb* = 649 bp) (Figure 1). On the other hand, *Taq1* *TT* genotype ($p = 0.012$) and *T* allele ($p = 0.05$) were more frequent among study patients (Table 4), where as its complete cleavage of the target gene into homozygous (wild type *TT* = 712 bp or mutant *tt* = 419 bp) (Figure 2). *Apa1* AA genotype ($p = 0.932$) and A allele ($p = 0.862$) were less frequent in T1DM patients without significant difference (Table 5), its complete cleavage to the target gene generates homozygous (wild type *AA* = 716 bp or mutant *aa* = 481 bp) (Figure 3). *Bms1* BB genotype frequency was significantly ($p < 0.02$) high among patients with poor metabolic control group of T1DM as compared with well metabolic control group (Table 6).

Table 3. The allele frequencies of *Bsm1* in diabetic patients and controls.

<i>Bsm1</i> Group	Genotypes		Allele	
	Bb	BB	Bb	B
Diabetic patients	17 (17%)	51 (51%)	32 (32%)	0.61
Control	21 (42%)	19 (38%)	10 (20%)	0.42
<i>P</i> -value	= 0.004 ^a		= 0.001	
	$\chi^2 = 11.145$		$\chi^2 = 11.014$	

^a statistical analysis was performed between BB + Bb and bb; Odd Ratio = 0.283; 95% CI 0.131–0.609.

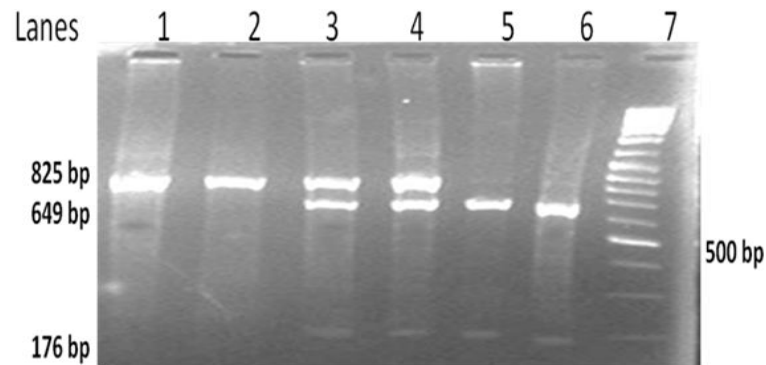


Figure 1. Detection of *Bsm1* polymorphism by PCR-RFLP method. Lane 1 and 2: wild-type homozygote (BB), Lane 3 and 4: heterozygote (Bb), Lane 5 and 6: mutant homozygote (bb), and Lane 7: 100-bp DNA marker.

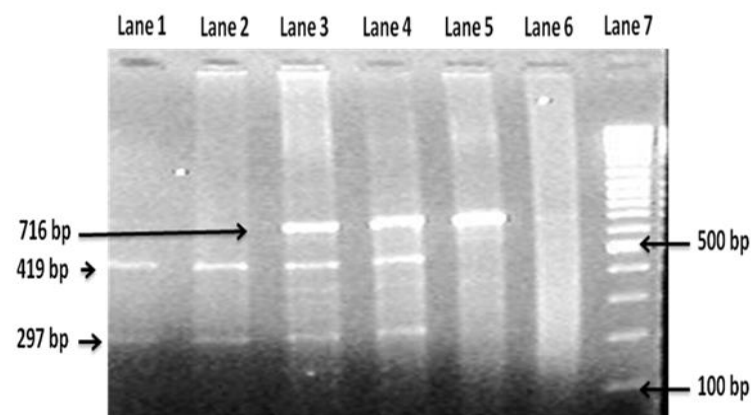


Figure 2. Detection of *Taq1* polymorphism by PCR-RFLP method. Lane 1 and 2: mutant homozygote (tt), Lane 3 and 4: heterozygote (Tt), Lane 5: wild-type homozygote (TT), and Lane 7: 100-bp DNA marker.

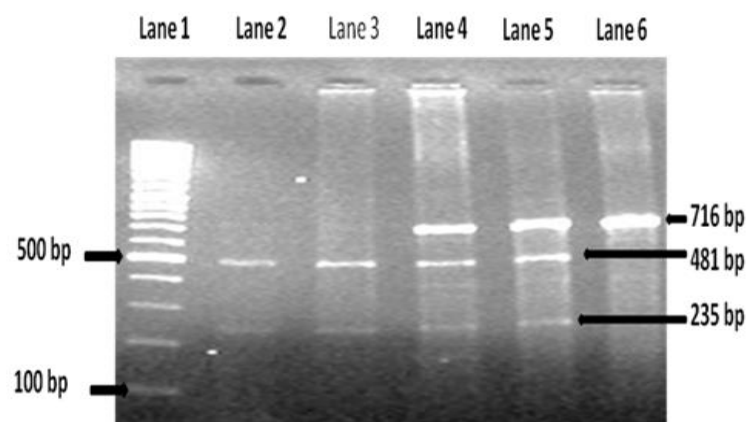


Figure 3. Detection of *Apa1* polymorphism using PCR-RFLP method. Lane 1: 100-bp DNA marker, Lane 2 and 3: mutant homozygote (aa), Lane 4 and 5: heterozygote (Aa), and Lane 6: wild-type homozygote (AA).

Table 4. The allele frequencies of *Taq1* in diabetic patients and controls.

<i>Taq1</i>	Genotypes			Allele	
Group	Tt	TT	Tt	T	T
Diabetic patients	22 (22%)	63 (63%)	15 (15%)	0.54	0.46
Control	16 (32%)	19 (38%)	15 (30%)	0.41	0.49
<i>P</i> -value	= 0.012 ^a $\chi^2 = 8.877$			= 0.05 $\chi^2 = 4.688$	

^a statistical analysis was performed between TT + Tt and tt; Odd Ratio = 2.429; 95% CI 1.073–5.496.

Table 5. The allele frequencies of *Apa1* in diabetic patients and controls.

<i>Apa1</i>	Genotypes			Allele	
Group	Aa	AA	Aa	A	A
Diabetic patients	44 (44%)	49 (49%)	7 (7%)	0.68	0.32
Control	21 (42%)	26 (52%)	3 (6%)	0.68	0.32
<i>P</i> -value	= 0.932 ^a $\chi^2 = 0.141$			= 0.862 $\chi^2 = 0.054$	

^a statistical analysis was performed between AA + Aa and aa; Odd Ratio = 1.085; 95% CI 0.546–2.156.

Table 6. Frequency of VDR polymorphisms among diabetic control groups.

Genotype frequency		HbA _{1c} (>8%) (n = 87)	HbA _{1c} (≤8%) group (n = 13)	<i>P</i> value
<i>Bsm1</i>	BB	55 (63.2 %)	6 (46.2 %)	0.02 ^a
	Bb	9 (12.3 %)	3 (23.0 %)	
	Bb	23 (26.4 %)	4 (30.8 %)	
<i>Taq1</i>	TT	45 (41.4 %)	7 (53.8%)	0.13 ^b
	Tt	29 (33.3 %)	4 (30.8 %)	
	Tt	13 (14.9 %)	2 (15.4 %)	
<i>Apa1</i>	AA	47 (54.0 %)	7 (53.8 %)	0.46 ^c
	Aa	33 (37.9 %)	5 (38.5 %)	
	Aa	7 (8.0 %)	1 (7.7 %)	

^{a,b,c} statistical analysis was performed between BB + Bb and bb; TT + Tt and tt; AA + Aa and aa.

4. Discussion

Vitamin D could play a role in the pathogenesis of autoimmune diseases particularly T1DM, with its metabolites could inhibit T cell proliferation and suppress the production of certain pro-inflammatory cytokine such as TNF- α and IL-1 [26-29]. Our study showed high frequency of patients with family history of diabetes. An early family study has supported the association between first degree relatives with T1DM and the risk of developing T1DM [30], while other study indicated that, this association depends on which parent has diabetes [31]. Several studies investigated the association between VDR gene polymorphisms and T1DM in different populations like Greece [5], Taiwanese [9], Germany [1;], Finnish [20], Portuguese [21], Japanese [22], Iranian [23], Dalmatian [24], and Egyptians [25]. Most of the aforementioned studies reported controversial

findings, which were most likely due to ethnic variations in addition to the role of the environmental and genetic factors. Thus far, no study indicated the association between VDR gene polymorphisms and susceptibility to T1DM in Sudanese population. Sudanese regions, particularly central Sudan, is inhabited by ethnically, linguistically and culturally diverse populations which makes the comparison of our results with previous studies from different regions is comparably useful, previous studies combines the genotype data of Sudanese populations with other subordinate countries such as Somalia, Egypt and Uganda [32], in addition to Ethiopia and Fulani [33]. Our study analysed three well characterized VDR polymorphisms (*Bsm1*, *Taq1*, and *Apa1*) among Sudanese T1DM children. We were not being able to investigate the fourth *Fok1* polymorphism owing to difficulties associated with the enzyme itself.

Bsm1 and *Apa1* are located in the intron between exon 8–9, which seems not affecting VDR protein structure, however, *Bsm1* may influence VDR mRNA stability because it was found strongly linked with the 3 polyA microsatellite repeat in 3' untranslated region [3,38]. Our results showed both *Bsm1* *BB* genotype and *B* allele were significantly high in patients than controls and is associated with increased risk of T1DM. This result was consistent with findings from other populations [13,16,35,36]. On the contrary, population study in Iran [14], showed no significant association between *Bsm1* and susceptibility to T1DM, and was found to have protective role in Greece population [5].

Taq1 is a silent SNP within the 3' noncoding sequences in exon 9 [37], its exact role in the pathogenesis of T1DM is not well defined. In this study and similar to *Bsm1*, *Taq1* *TT* genotype and *T* allele exerted susceptibility action to T1DM, which is agreed with other study populations from Greece and Uruguayan [5,29]. This data was not in agreement with other populations such as Portuguese, Iranian, and Korean, where there was no association found [21,23,39], or there was low frequency in Egyptian population [25]. Our data showed no significant difference in *Apa1* between patients and control. In agree with our result, *Apa1* genotype and alleles frequencies showed no significant differences between T1DM and the control among Portuguese [21]. In contrary, *Apa1* gene polymorphism was found associated with T1DM in Greece and Taiwanese populations [5,7].

5. Conclusion

In conclusion, the VDR *Bsm1* and *Taq1* were associated with susceptibility to T1DM among Sudanese population. Our sample size was not large enough to represent the whole population, although it enrolls the majority of the patients from central Sudan, nevertheless, the strength of an association between SNPS and T1DM in this study is measured by the odds ratio (OR). Hardy-Weinberg equilibrium for the genotypes was not used in this study because of small sample size and to override violation. Since Sudan is known as small continent and characterized by different ethnic groups, further studies from different regions is necessary to correlate between the genetic and environmental factors, VDR polymorphisms, and T1DM.

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Conflict of interest

The author declares no conflict of interest.

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