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Evaluation of VDR Gene Polymorphisms with Nephropathy Stages in Men with Type 2 Diabetes Mellitus

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ABSTRACT

To appraise the association of VDR FokI and TaqI polymorphisms with diabetic nephropathy patients, a total of 60 men with type 2 diabetes mellitus (T2DM) were enrolled in the recent paper, as well as 17 healthy men as a control. The patients have been divided according to the albumin/creatinine ratio (Normo albuminuria, micro-albuminuria, and macro-albuminuria,). ARMS PCR was used to diagnose the genotypes and allele frequencies of FokI "rs10735810*" and TaqI "rs731236*". The results indicated that FF, Ff and TT, Tt genotype were significantly higher in patients with T2DM than the control (OD=3.375, p=0.001), (OD=4.333, p=0.001), (OD=4.222, p=0.001), and (OD=2.833, p=0.025) respectively. Moreover, the results showed a notable increase of FF, Ff, and TT, Tt genotypes in the patients with micro-albuminuria and macro-albuminuria when compared with patients of normo albuminuria, (p=0.002), (p=0.005), (p=0.001), (p=0.010) respectively. The current outcomes propose that the F allele of FokI and T allele of TaqI polymorphisms in the VDR gene are associated with a higher risk of T2DM and nephropathy stages. Futhermore, the FokI and TaqI can be used as vital markers to predict the risk of complications of diabetes mellitus especially nephropathy.

Keywords: ARMS-PCR, Diabetes Type 2, Diabetes Nephropathy, VDR Gene Polymorphisms.

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INTRODUCTION

Diabetic nephropathy (DN) is one of the most common complications of microvascular disease for diabetics. Epidemiological studies showed that 30-40% of diabetics have been developed diabetes-related kidney disease (1). DN is defined as a gradual increase in the rate of secretion of albumin accompanied by a rise in blood pressure and a sharp decline in the glomerular filtration rate, which leads to end-stage renal failure (ESRD)(2). DN is a chronic disorder, and it is not detectable until it develops significant renal damage (3,4). Vitamin D is one of those fat-soluble vitamins are produced after the skin is exposed to sunlight from ultraviolet rays. The kidneys play an important role in converting the 25(OH)D3 to 1,25(OH)2D3 by 1-alpha hydroxylase or CYP27B1 (5), it has been found in diverse types of the cells involving epidermis cells, bone cells, macrophages, placental cells, parathyroid cells, and cells of prostate and colon cancer (6). Thus, vitamin D3 can be produced locally in various tissues, about one billion people worldwide have vitamin D shortages or insufficiencies (7). Vitamin D exerts an obvious role in glucose homeostasis and normal insulin release mechanisms (8). Many studies indicated that vitamin D deficiency may play a significant role in type 2 diabetes mellitus pathogenesis (T2DM) (9), as a transcription factor that regulates insulin release from pancreatic beta cells through vitamin D receptor (VDR) (10). VDR gene is a member of a family of steroid hormone receptors and located on chromosomes 12 with about four identified single nucleotide polymorphisms (SNPs) (11,12) are FokI (rs10735810), TaqI (rs731236), ApaI (rs7975232) and BsmI (rs1544410)(13). The present study focused on investigating possible associated SNPs of FokI and TaqI with stages of nephropathy in patients with T2DM.

MATERIAL &METHODS

Blood and urine samples were taken from 60 men with T2DM who had been attending diabetes and endocrinology center in Al Sadr Medical city in Najaf Governorate, in addition to 17 healthy men as control group during the period of March 2019 to June 2019. The patients have been divided according to the albumin/creatinine ratio measured by (Cobas e411) device. The genomic DNA was extracted via an extraction kit from peripheral leukocytes from Intron Co. ARMS PCR assay used to determine VDR gene polymorphisms in FokI and TaqI regions through used 3 primers which involved: one common primer and 2 allele-specific primers. The primers and PCR product sizes were approved according to(14) FokI: rs10735810* FokI/F5'TGGCCGCCATTGCCTCCG3' FokI /f5'TGGCCGCCATTGCCTCCA3' FokI/C5'AGCTGGCCCTGGCACTGA3' product size 77bp and TaqI:rs731236* TaqI/T5'CAGGACGCCGCGCTGATT3 TaqI/t 5'CAGGACGCCGCGCTGATC 3' TaqI/C 5'CCTCATTGAGGCTGCGCAG 3' product size 148bp and supplied by Macrogen CO. Two tubes were used for each reaction; one of them contained the wild type (F, T), and the other mutant type (f,t), the volume of the PCR reaction was 20 µl. In addition, the sequences have been amplifying by thermo-cycler (Bio-Rad Technology, USA), and ARMS PCR reaction was done at 95 C for 5 minutes as initiation denaturation, followed by 30cycles (denaturation at 95C for 30 seconds, annealing at 62 C for 60 seconds and extension at 72 C for 45seconds, and then final extension at 72 C for 5 minutes). PCR products electrophoresed in 2% agarose gel treated by Red safe dye. Concern to the statistical analysis of the findings, it was based on multiple regression tests and Odd ratio by using SPSS version 23 software.

RESULTS AND DISCUSSION

The results of the current study revealed that the assessment of polymorphisms within the FokI region in the VDR gene was more in T2DM patients than control. FF and Ff genotypes reached (46.7%)(43.4%) in the patients when compared to (47.1%) (35.3%) in control respectively. Whereas ff genotype frequencies in the patients with T2DM were (10%) in compared with the control (17.6%). The findings also showed that the F allele differences were higher in the patients than control (p=0.001), (table 1). The reason may be attributed to the allele F activity is about 1.7 times more than the allele f, and the allele f could has a protective role to prevent type 2 diabetes mellitus (15,16). Moreover, the FokI polymorphisms within VDR gene may increase insulin-sensitive, and thus be a cause of diabetes (17).

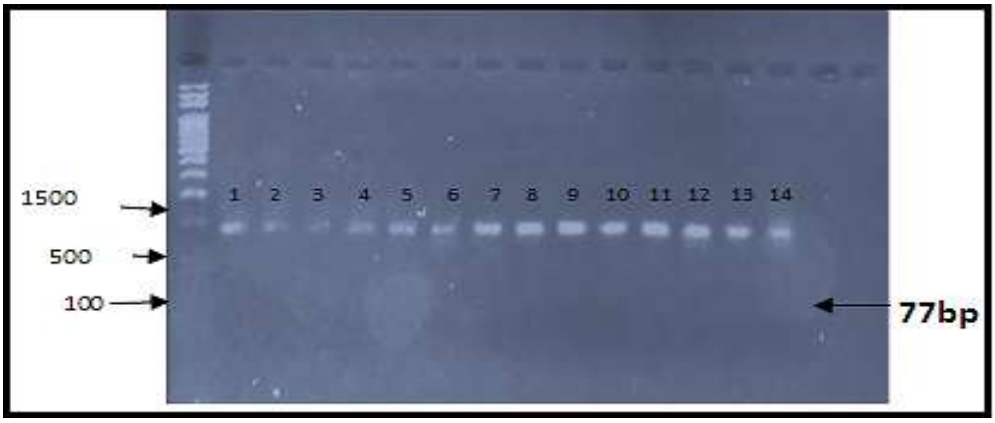
Furthermore, there is a marked elevation in frequencies TT and Tt genotypes within TaqI region in VDR gene; it reached (65 %) and (28.4%) in the patients compared with (52.9%) (35.3%) in control respectively, while tt genotype frequencies in the patients were (6.6%), and in the control were (11.6%). The data of the present study also exhibited that the T allele significantly raised in the patients compared to the control (p=0.001), as in (table 1). Several studies have shown that individuals with TT and Tt genotypes have higher insulin resistance than those who have tt genotype (18,19). One the other hand, the results of study were incompatible with(20), perhaps due to the regions of the VDR gene vary according to race, environment, and lifestyle. Some studies have reported that there is a critical role for F allele in renal impairment (21), and it has been found that there is a relation between active vitamin D and FF genotype in the related stages of renal damage(22). The findings also noticed an observable increment (p<0.05) of the FOKI polymorphism region in FF, Ff genotype in the patients with microalbuminuria, and macroalbuminuria stages when compared with normoalbuminuria patients, while ff genotype did not appear in microalbuminuria and macroalbuminuria stages, as shown in (table 2), the results were agreed with another study proposed that the F allele had a critical role in nephropathy patients(23).In addition to, the VDR FokI polymorphisms have a limited parathyroid gland response in patients with chronic renal failure as confirmed by some studies (24), also there is a negative correlation between $1.25(OH)_2D_3$ levels and nephropathy stages for ff genotypes compared with FF genotypes (22). Besides, there was a remarkable increase (p<0.05) of the TaqI polymorphism region in TT, Tt genotype in the patients with microalbuminuria, and macroalbuminuria stages comparable to normoalbuminuria patients, while no significant tt genotype in macroalbuminuria when compared to microalbuminuria and normoalbuminuria patients (table 2). This is due to the role of the T allele in the progress of the renal impairment stages (25,26,27).

Table 1: Number of samples, percentage, odds ratio of genotype and alleles at FokI and Taq position in T2DM patients and control

Genotype or alleles	Patient N=60	%	Control N=17	%	Odds Ratio	CI	Sig
FF	28		8	47.1	3.375	-10.52	0.001*
Ff	26	46.7	6	35.3	4.333	-7.42	0.003*
ff	6		3	17.6	2.000	-1.56	0.327
F	82	43.4	22	64.70		0.533-7.997	
f	38	10	12	35.3	0.231		0.001*
		68.3				0.372.0.143-	
		31.3					
TT	39	65	9	52.9	4.222	2.03-8.732	0.001*
Tt	17		6	5.3 3	2.83	1.117-7.186	0.025*
tt	4	28.4	2	11.6	2.000	10.919 -0.36	0.423
T	95	6.6	24	70.58			
t	25	78.2	10	29.4	0.087	0.051-0.147	0.001*
		21.8					

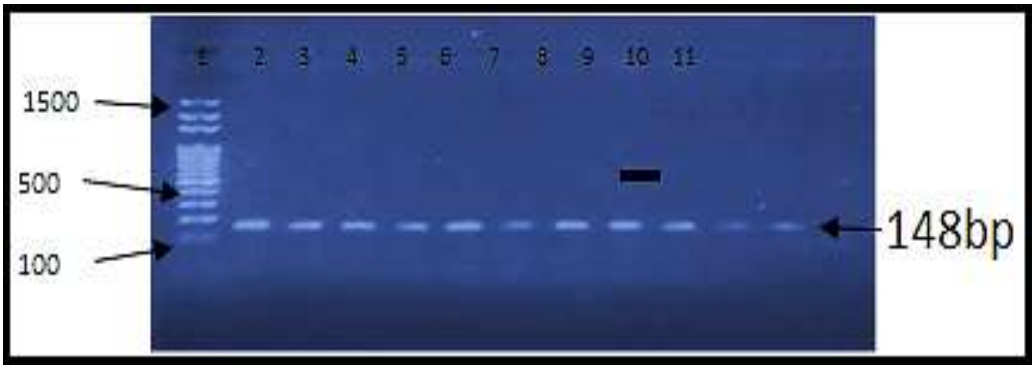
Table 2: Association of VDR FokI& TaqI gene polymorphisms with the DN stage

Genotype	Normoalbuminuria	Microalbuminuria Odds Ratio	CI	Sig	Macroalbuminuria Odds Ratio	CI	Sig
FF	Reference category	0.143	0.479-	0.002*	0.143	0.479-	0.002*
Ff		0.263	0.043	0.008	0.211	0.043	* 0.005
ff			0.705-	*		0.619-0.072	
			0.098				
TT	Reference category	0.219	0.496-	0.001*	0.125	0.353-0.044	0.001*
Tt		0.143	0.097	0.010 *	0.143	0.629-0.032	* 0.010
tt			0.629-		0.333	3.205-0.035	0.341
			0.032				

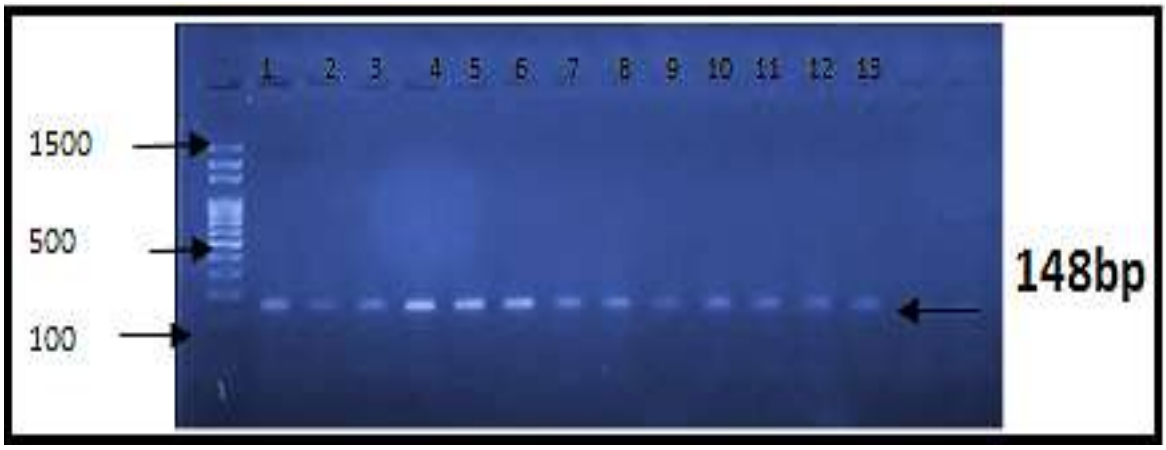


Agarose gel electrophoresis (2% at 90 volts for 2-hour) of ARMS-PCR reaction for VDR gene FokI polymorphisms in healthy men. These bands showed 77bp from (1-5) FF genotype, (6-11) Ff genotype, and (12-14)ff genotype which stained with Red safe, Ladder (100-1500), visualized by U.V light.

Agarose gel electrophoresis (2% at 90 volts for 2-hour) of ARMS-PCR reaction for VDR gene FokI polymorphisms in type 2 diabetes mellitus patients. These bands showed 77bp from (1-7)FF genotype, (8-15) Ff genotype, and (16-19)ff genotype which stained with Red safe, Ladder (100-1500), visualized by U.V light.



Agarose gel electrophoresis (2% at 90 volts for 2-hour) of ARMS-PCR reaction for VDR gene TaqI polymorphisms in healthy men. These bands showed 148bp from (1-5) TT genotype,(6-8)Tt genotype, and(9-11) tt genotype which stained with Red safe, Ladder (100-1500), visualized by U.V light.



Agarose gel electrophoresis (2% at 90 volts for 2-hour) of ARMS-PCR reaction for VDR gene TaqI polymorphisms in type 2 diabetes mellitus patients. These bands showed148bp from (1-4)TT genotype,(5-9)Tt genotype, and (10-13) tt genotype which stained with Red safe, Ladder (100-1500), visualized by U.V light.

CONCLUSION

The current study indicated an association between VDR FokI (rs10735810*) and TaqI (rs731236*) polymorphisms and earlier progression of nephropathy in patients with T2DM. The current study is the first of its kind in Iraq; therefore, require more research on this topic which may open new horizons in preventing the development of complications are related with type 2 diabetes mellitus in the future.

CONFLICT OF INTEREST

None

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