Association of Vitamin D receptor gene *Bsm*I polymorphism with type 2 diabetes mellitus in Pakistani population

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder with strong genetic components. The reported association of vitamin D receptor (VDR) gene polymorphisms varies among ethnic groups.

Objectives: The present study was conducted to determine association of vitamin D receptor gene *Bsm*I (rs1544410 A>G) polymorphism with type 2 diabetes mellitus in Pakistani population.

Methods: Blood samples were collected from 150 T2DM patients and 100 non-diabetic engaged by convenient sampling method. After collection of demographic data, assessment of fasting glucose (FG), vitamin D, HbA1c, renal function tests, liver function tests and lipid profile was done. Candidate gene polymorphism was analyzed by DNA amplification with polymerase chain reaction and endonuclease digestion.

Results: Biochemical parameters were significantly different among case and control groups. Associations of *Bsm*I genotype with T2DM, related complications and biochemical variables were not significant.

Conclusion: The current study did not provide evidence for the association of VDR gene *Bsm*I polymorphism with T2DM in Pakistani population.

Keywords: Vitamin D receptor gene; single nucleotide polymorphism; type 2 diabetes mellitus.

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Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder with strong genetic components. Candidate genes and variants for T2DM risk present in specific genome parts are involved in disease onset, associated pathways and functions.[1,2]

Single nucleotide polymorphisms (SNP) in vitamin D receptor (VDR) gene modulate glucose intolerance, insulin secretion and sensitivity.[3-5] Genetic polymorphism of vitamin D receptor (VDR) gene can affect insulin secretion, causing insulin resistance, affect vitamin D synthesis, transportation and action.[6] VDR gene located on chromosome 12q12-q14 mediates vitamin D action as it binds to vitamin D response elements (VDRE).[7-9]

Vitamin D binding protein (DBP) is the gene product that mediates vitamin D action. Cholcalciferol enters blood stream through binding to DBP. VDR gene genotype *Bsm*I polymorphism found in intron 8is related with onset of type 2 diabetes mellitus.[10,11] Al-Daghri et al.[12] stated that *Bsm*I SNP is significantly more common in T2DM patients. Other reports also demonstrated analogous relationship between *Bsm*I polymorphism and T2DM in different populations.[13-16] Consequently, association between *Bsm*I SNP and risk of T2DM in different ethnic groups is not conclusive.

SNP relates to disease susceptibility and response to treatment.[17] Hypovitaminosis D has been reported among Pakistani population.[18-20] However, researchers overlooked the role of VDR gene SNP in local population especially

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with reference to diabetes mellitus. Progress in identification of novel VDR gene variants predisposing to diabetes mellitus in Pakistan has been limited. Therefore, current research was conducted to assess the association of VDR gene *Bsm*I polymorphism with T2DM in Pakistani population.

Methods

Research design

Study was conducted from March, 2015 to January, 2016 at Clinico-Medical Biochemistry Lab., Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan and Molecular Labs., Department of Medical and Dentistry, Southmead Hospital, University of Bristol, Bristol, United Kingdom. Sampling was conducted within Pakistan and included consented 150 T2DM patients and 100 non-diabetic engaged by convenient sampling method. Graduates Studies and Research Board (GSRB), University of Agriculture, Faisalabad, Pakistan granted ethical approval.

Sample collection and bioassays

After collection of demographic data,fasting blood samples were taken in EDTA-coated vacutainers.Assessment of fasting glucose (FG), vitamin D, HbA1c, renal function tests,liver function tests and lipid profile was done by kits (Merck, Germany) as per manufacturer's guidelines using Dade Behring clinical chemistry system(dimension auto-analyzer, Siemens, USA) and Diastat auto-analyzer (Randox, UK).

DNA extraction and quantification

After genomic DNA extraction, the quantity and quality of DNA was assessed by Nanodrop (Nanophotometer™, Implen, Germany). Purity of the DNA was assessed by measuring optical density (OD) as OD260/ OD280.[22]

PCR-RFLP

Polymerase chain reaction (PCR) primers sequences used were: (F: CCCTGGCACTGACTCTGCTC; R: GGAAACACCTTGCTTCTTCTCC) as described earlier.[14,23] Go Taq kit (Promega Madison, Wisconsin USA) was used for about 1.0 mL of genomic DNA (100 ng/ μL). PCR conditions were optimized for annealing temperature (60 °C) and Mg^{2+} concentration. The intron 8 was amplified to study the *Bsm*I polymorphism(PCR thermocycler T100TM, BioRad). Using RFLP (restriction fragment length polymorphism) method, fragments were digested to assess the BsmI (rs1544410 A>G) genotype (New England BioLabs®, R0109S). A molecular weight marker (HyperLadder II, Bioline or VC 100 bp Plus DNA Ladder, Vivantis) and for agarose gel visualization, UV light (UVITEC system, Uvitec Cambridge) were used.[14]

All data was expressed as mean ± SD (standard deviation), % or n (number). Data analysis was performed using Statistical Package for Social Sciences (version 17; Chicago, USA). P value less than 0.05 was considered significant.

Results

Biochemical assays

Results are presented in table l. Elevated FG, HbA1c, BMI, blood pressure and decreased vitamin D ($P < 0.05$) levels were evident in case group as compared to controls with negative correlation between vitamin D and HbA1c. Liver function tests inferences were similar among both groups. Regarding RFTs and lipid profile, T2DM sample showed appreciably ($P < 0.05$) higher concentrations than control participants. Within the each group, associations of biochemical variables with vitamin D were negligible. Based upon the physician-diagnosed complications, diabetic subjects were sub-divided into CP (cardiac patients), NP (nephropathy patients), RP (retinopathy patients) and HP (hypertensive patients). Among these groups, non-significant differences in clinical and biochemical profiles were acquired, an observation that can be justified by the fact that most of the patients were either in initial complication phases or were using oral hypoglycemic medications. Therefore, data was simplified and presented as case and control participants.

Table 1: Clinical and biochemical profile

Parameters	Diabetic group (n = 150)	Control group (n = 100)	*P* value
Clinical			
Age (years)	46.4 ± 3.5	46.8 ± 4.4	>0.05
Gender (Male/Female)	80/70	53/47	-
BMI (kg/m^2)	35.3 ± 10.7	22.9 ± 5.0	<0.05
sBP (mm Hg)	149 ± 13	123 ± 12	<0.05
dBP (mm Hg)	83 ± 7	75 ± 8	<0.05
Biochemical			
FG (mg/dL)	144 ± 6.38	83 ± 5.21	<0.05
HbA1c (%)	7.13 ± 0.58	4.46 ± 0.49	<0.05
Vitamin D (mg/dL)	14.46 ± 1.29	23.87 ± 3.16	<0.05
Liver Function Tests			
Total B (mg/dL)	1.02 ± 0.52	1.09 ± 0.39	0.53
Direct B (mg/dL)	0.98 ± 0.36	0.93 ± 0.31	0.83
ALT (mg/dL)	69.86 ± 14.67	71.88 ± 13.90	0.32
AST (mg/dL)	34.54 ± 17.23	37.77 ± 18.01	0.29
ALP (mg/dL)	51.87 ± 11.27	51.24 ± 11.05	0.99
Renal Function Tests			
BUN (mg/dL)	39.57 ± 7.29	13.79 ± 5.71	<0.05
Creatinine (mg/dL)	2.18 ± 0.36	0.49 ± 0.40	<0.05
Uric acid (mg/dL)	7.01 ± 0.88	3.00 ± 0.63	<0.05
Lipid Profile			
Cholesterol (mg/dL)	276.38 ± 29.54	145.09 ± 12.87	<0.05
LDL-C (mg/dL)	169.64 ± 11.37	65.52 ± 13.73	<0.05
HDL-C (mg/dL)	37.41 ± 3.95	63.02 ± 13.26	<0.05
TG (mg/dL)	453.05 ± 147.22	175.14 ± 8.02	<0.05

Data expressed as mean ± SD (standard deviation), % or n (number) unless otherwise indicated, P<0.05: significant. BMI: body mass index, sBP: systolic blood pressure, dBP: diastolic blood pressure, FG: fasting glucose, HbA1c: glycated hemoglobin, Total B: total bilirubin, Direct B: direct bilirubin, ALT: alanine transaminase, AST: aspartate transaminase, ALP: alkaline phosphatase, BUN: blood urea nitrogen, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, TG: triglycerides

*Bsm*I polymorphisms

After amplification of intron 8 by PCR (figure 1a), heterozygosity of intron 8 was confirmed by enzymatic digestion (Figure 1b). Figure 1a-b showed typical results in current study that correlate with previously studied results. It was performed to check the action of restriction enzyme. *Bsm*I polymorphisms among T2DM complications groups were scored in figures 3-5.

Three enzymatic digested fragments of *Bsm*I (76, 115 and 191 bp) indicated heterozygosity (Bb) of *Bsm*I genotype. Differences of *Bsm*I genotypes of VDR gene were significant between T2DM and normal groups ($p < 0.01$). No substantial association was found between biochemical parameters and *Bsm*I restriction site ($p > 0.01$).

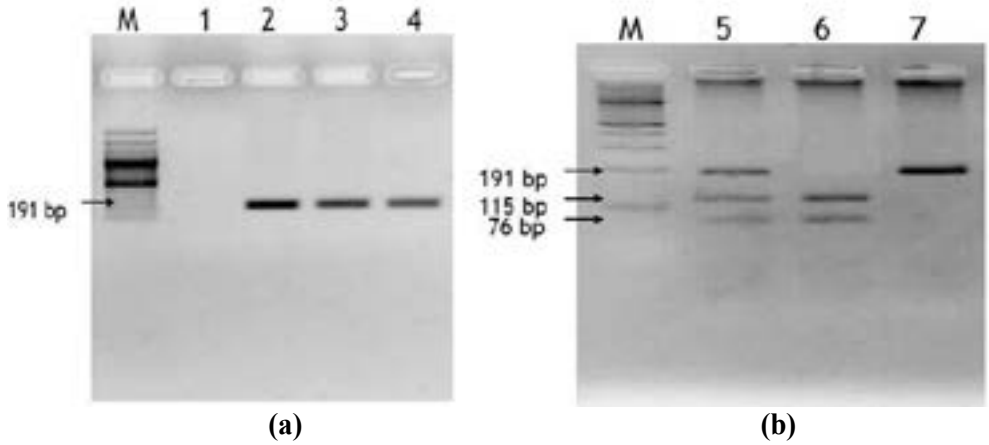

Figure 1(a-b): a: Electrophoresis of a 2% agarose gel with intron 8 PCR product loaded; 1 – negative control; 2, 3 and 4 – intron 8 fragment with 191 bp. **b:** Electrophoresis of a 3% agarose gel with *Bsm*I enzymatic digestion of *VDR* intron 8; 5 – heterozygous Bb genotype (76, 115 and 191 bp); 6 – homozygous bb genotype (76 and 115 bp); 7 – homozygous BB genotype (191 bp)

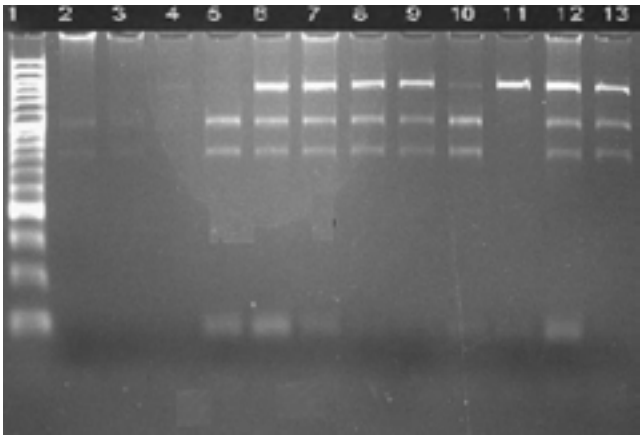

Figure 2: VDR gene *Bsm*Idigestion polymorphism products (cardiac group & hypertensive patients)
Lane 11 have BB homozygous PCR-RFLP product. Lane 5,6,7,8,9,10,11& 12 have Bb heterozygous PCR-RFLP products. Lane 5 has bb homozygous PCR-RFLP products of 175 bp. Lane 1 consist of ladder 100 bp.

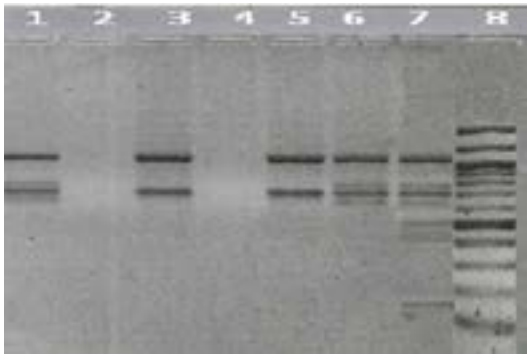

Figure 3: VDR gene *Bsm*I digestion polymorphism products (nephropathy patients group)
Lane 8 : ladder 100bp, lane; 1,3,5,6 and 7 have *Bsm* Idigestion PCR-RFLP product BB 823 bp, lane ;1,3,5,6 and 7 consists 648 bp product Bb, lane 7 has bb product of 175 bp.

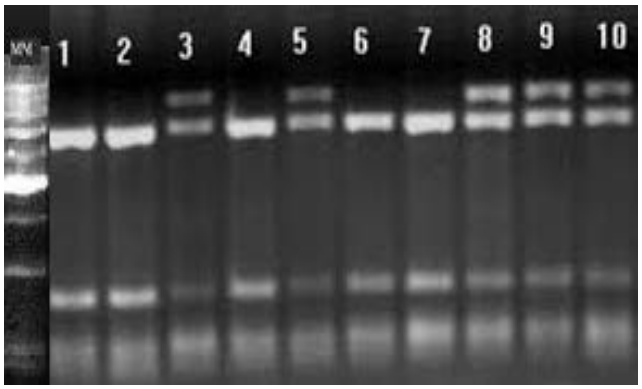

Figure 4: VDR gene *Bsm*Idigestion polymorphism products (retinopathy patients group)
Lane M; Ladder of 100bp. Lane 3,5,8,9 & 10 consist fragment BB of 823 bp. Lane 1,2,3,4,5,6,7,8,9 and 10 consist of Bb fragment of 648 bp. Lane 1,2,3,4,5,6,7,8,9 and 10 consist of fragment bb of 175 bp.

Distribution of genotype allele frequencies and carriage rate of *Bsm*I among diabetic patients, T2DM sub-groups and control group was non-significant (table 2). Effect of VDR gene polymorphisms on metabolic parameters in terms of probability to clarify their association underlying the diabetic complications are mentioned in table 3. VDR gene *Bsm*I polymorphism was related non-significantly ($p>0.05$) to the diabetic complications in the present study.

Table 2: Distribution of genotype allele frequencies and carriage rate of *Bsm*I

Groups	Genotypes			Total
	bb	Bb	BB	
Control	2	64	34	100
	2.0%	64.0%	34.0%	100.0%
Diabetic	4	119	27	150
	2.7%	79.3%	18.0%	100.0%
CP	3	59	18	80
	3.8%	73.8%	22.5%	100.0%
NP	0	18	2	20
	0.0%	90.0%	10.0%	100.0%
RP	0	19	1	20
	0.0%	95.0%	5.0%	100.0%
HP	1	23	6	30
	3.3%	76.7%	20.0%	100.0%
Total	6	183	61	250
	2.4%	73.2%	24.4%	100.0%

Data expressed as $X^2 = 15.08^{S}$, $p < 0.05$, S: significant (diabetic & control groups); $X^2 = 13.158^{NS}$, $p = 0.108$ (diabetic subgroups and control groups).
CP: cardiac patients, NP: nephropathy patients, RP: retinopathy patients, HP: hypertensive patients, NS: non-significant

Table 3: Probability values for the association of biochemical parameters and *Bsm*Igenotypes

Parameters	Control	T2DM Sub-groups			
		CP	NP	RP	HP
HbA1c	0.775	0.597	0.436	0.256	0.376
Vitamin-D	0.334	0.592	0.165	0.858	0.279
T. bilirubin	0.810	0.999	0.263	0.601	0.585
D. bilirubin	0.850	0.871	0.841	0.037	0.495
ALT	0.229	0.064	0.694	0.051	0.595
AST	0.399	0.291	0.131	0.577	0.870
ALP	0.138	0.180	0.136	0.334	0.622
BUN	0.257	0.855	0.205	0.403	0.123
Creatinine	0.536	0.137	0.620	0.868	0.658
Uric acid	0.336	0.989	0.426	0.422	0.717
Cholesterol	0.335	0.750	0.633	0.966	0.628
LDL-C	0.563	0.358	0.782	0.641	0.369
HDL-C	0.763	0.988	0.178	0.954	0.719
TG	0.206	0.629	0.562	0.914	0.969

Data expressed as p – value
CP: cardiac patients, NP: nephropathy patients, RP: retinopathy patients, HP: hypertensive patients, HbA1c: glycated hemoglobin, T. bilirubin: total bilirubin, D. bilirubin: direct bilirubin, ALT: alanine transaminase, AST: aspartate transaminase, ALP: alkaline phosphatase, BUN: blood urea nitrogen, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, TG: triglycerides

Discussion

Although numerous genes have role in T2DM,[24,25] VDR gene plays a dominant role in onset and progression of T2DM.[11,14,26] *Bsm*I polymorphism found in intron 8 of VDR gene may be involved in pathogenesis of T2DM.[27] Prevalence of vitamin D deficiency in T2DM group was higher as compared to control subjects, showing significant association of *Bsm*I with T2DM in the present study. However, Santos et al.[27] observed conflicting results in Brazilian population. Contrary to current findings, Dilmec et al.[14] found significant association of *Bsm*I to T2DM onset. While Israni et al.[28] suggested potential role of *Bsm*I polymorphisms. Wang et al.[30] studied significant association of *Bsm*I polymorphism with T2DM onset.

Review of literature indicates conflicting results regarding the impact of VDR gene polymorphisms on T2DM pathogenesis in different populations[14,26,30] either supporting or contrasting current findings. Heterogeneity in different populations and limited knowledge of underlying mechanism may be responsible for these discrepancies.[31,32]

Statistically non-significant relationship between *Bsm*I polymorphism and T2DM in current project is supported by earlier study.[33] This study suggests that the *Bsm*I may be related with susceptibility to T2DM subjects but genetic contribution of VDR gene polymorphism for the development or existing diabetic complications is not clear. In addition, vitamin D receptor gene consists of many single nucleotide polymorphisms (SNPs). To investigate whether functional changes of VDR gene may be potential risk for T2DM, future studies should focus on population or case-control studies along with family linkage with multiple SNP study[34]

Conflict of interest statement

We declare that we have no conflict of interest.

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