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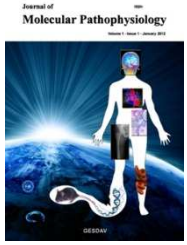

Original Research

Association of vitamin D receptor gene G>T and T>C polymorphisms in young onset diabetes mellitus in a Bangladeshi population

Debashish Paul[1,2], Imran Khan[1], Omar Faruque[3], Naiyuum Choudhury[2], Zahid Hassan[2], Liaquat Ali[4]

[1]Dept of Physiology and Molecular Biology, [2]Dept of Biotechnology, BRAC University
[3]Dept of Biochemistry and Cell Biology, [4]Coordinator, Biomedical Research Group, BIRDEM

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Corresponding Author:
Debashish Paul,
Dept of Physiology and Molecular Biology, BRAC University,66 Mohakhali Dhaka 1212, Bangladesh.

bapi_ads@yahoo.com

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Abstract

Background: Vitamin D has an important immunomodulatory property and vitamin D receptor (VDR) gene polymorphism has also been found to be associated with insulin secretory capacity and glucose intolerance. The present study was undertaken to determine genotype of VDR gene common variants in young onset diabetic subjects of Bangladesh to explore its association with diabetes and B cell secretory capacity.

Methods: A total number of 94 young diabetic subjects and 92 healthy controls were recruited. Blood parameters were estimated by standard methods and DNA was extracted using QIAGEN Kit. VDR gene variants [G>T and T>C] were determined by PCR-RFLP using restriction endonuclease Apa1 and Taq1 respectively. Data were managed using Statistical Program for Social Science (SPSS).

Results: C-peptide level was found to be significantly lower 3.297 ($p<0.001$) and HOMA-%B found lower 3.102 ($p=0.003$). The G>T and T>C genome frequencies (wild, heterozygous and homozygous variants) were in the control (0.183, 0.516, 0.316 and 0.370, 0.522, 0.109 respectively) and (0.198, 0.484, 0.319 and 0.484, 0.418, 0.090 respectively) in young diabetes mellitus (YDM) which did not show significant association with YDM ($p=0.906$ and 0.288 respectively). Genotype frequency of the marker allele did not show significant association with glucose, C-peptide, HOMA-%S and IR either in controls or YDM subjects except the HOMA-%B shown to have relatively lower.

Conclusions: VDR gene G>T and T>C polymorphic marker are not associated with diabetes mellitus in the young Bangladeshi diabetic patients, Insulin secretory dysfunction is main feature of pathogenesis of diabetes in the young. VDR gene G>T genotype is associated with higher secretary capacity.

INTRODUCTION

Diabetes mellitus is a complex heterogeneous group of metabolic condition characterized by elevated levels of blood glucose, caused mainly by impairment of insulin action and or insulin secretion [1]. Diabetes is probably caused by a complex interaction of environmental factors and genetic predisposition. Environmental risk factors are thought to act as either damage or dysfunction among the genetically predisposed subjects.

Vitamin D has been suggested to play an important role in the immunomodulation [2] and the pathogenesis of type 1 diabetes mellitus (T1DM) although the

mechanism is yet to be clearly understood. Vitamin D deficiency to reduce insulin secretion, and $1\alpha,25(OH)_2D_3$ supplementation improve in B cell secretory function and consequently in glucose tolerance [3] which is studies on rats and humans. The vitamin D receptor (VDR) mediates the majority of the effects of vitamin D (as the active form 1α,25-dihydroxyvitamin D (calcitriol)) on gene expression via formation of a heterodimer with the retinoid X receptor which binds to promoter regions of many target genes. There are 6 known polymorphisms in the VDR locus with a range of possible effects). Studies have demonstrated a link between VDR polymorphism and T2DM, although the findings differ from one population to another. A study involving Bangladeshi population demonstrated that the Apa1 polymorphism influences insulin secretion in response to glucose [4] while association between the VDR Apa1 and higher fasting plasma glucose levels and glucose intolerance were observed in a community based study of older adults without known diabetes [5]. Vitamin D itself and VDR gene polymorphism have links to the pathogenesis of both type 1 and type 2 diabetes since young diabetes mellitus (YDM) seems to be representing unique groups of diabetic patients.

The prevalence of diabetes mellitus in Bangladesh is about 7% [6]. Study on newly diagnosed normal to overweight T2DM subjects reporting that they have both insulin secretory dysfunction and insulin resistance but a substantial number of patients diagnosed and registered at BIRDEM are of younger age i.e. less than 30 years who are lean, normal to moderate over weight, low body mass index (BMI) (BMI<19) and usually present with moderate to severe hyperglycemia.

METHODS

Subjects

In the present study 92 patients with YDM, where male and female were 37 (40.2%) and 55 (59.8%) respectively and 94 were healthy controls in control group male and female distribution was 55 (58.5%) and 39 (41.5%) respectively. In YDM subjects age limit up to 30 years and duration of the diabetes 3 months. These subjects were recruited from the Outpatient department, BIRDEM, the central institute of Bangladesh Diabetic Association (BADAS). Healthy control subjects were recruited through personal communication from the community. Diagnosis of diabetes was made according to WHO criteria (WHO, 1999). Informed consent was obtained from all the volunteers.

Collection of blood samples and biochemical analysis

Overnight fasting (8-10 hours) blood was collected between 8.00-9.00 am. Venous blood (10 ml) was obtained by venepuncture following standard procedure. A portion of blood (5 ml) sample was taken into a tube containing EDTA (1 mg/ml), mixed thoroughly and preserved at -30^{o}C for future DNA extraction. The other portion of blood sample was taken into plain tube and allowed to clot for 30 minutes and serum was separated by centrifugation for 10 min at 3000 rpm using refrigerated centrifuge and preserved at -30^{o}C for further biochemical analyses.

Serum glucose was measured by glucose oxidase and insulin by ELISA methods. Insulin secretory capacity (HOMA-%B) and insulin sensitivity (HOMA-%S) were also determined using HOMA-SIGMA software (Levy et al. 1998).

Genetic analysis

Vitamin D receptor (VDR) gene polymorphic markers (G>T and T>C) were analyzed by PCR and RFLP. Genomic DNA was isolated using GenElute DNA extraction kit (QIAGEN, USA). The DNA segment containing G>T (Apa1 restriction) and T>C (Taq1 restriction) polymorphic marker was amplified using the following primer set:

Forward primer: 5′-CAG AGC ATG GAC AGG GAG CAA G-3′ and Reverse primer: 3′-GCA ACT CCT CAT GGC TGA GGT CTC A-5′

PCR was carried out in 15 µl reaction volume. Product size for the above mentioned primer set is 740 bp.

PCR condition: Conditions for the amplification of the above mention product include initial incubation at 94^{o}C for 15 min followed by 35 cycles of denaturation at 94^{o}C for 30 seconds, annealing at 64^{o}C for 45 seconds and elongation at 72^{o}C for 45 seconds and after 35th cycle was followed by a step of final elongation 72^{o}C for 10 minutes.

Evaluation of PCR: 3 µl of PCR product was checked for amplification in a 1.5 % agarose gel. The optimum size of the product was ascertained comparing it with 100 bp DNA ladder. The amplified DNA was visualized using under UV light and gel image captured and documented.

RFLP analysis of VDR gene candidate markers

G>T polymorphism restricts Apa1 site. Hence the polymorphism was determined by Apa1 restriction endonuclease digestion. The digestion was carried out in a reaction volume of 15µl. The enzyme digestion protocol is as follows:

Apa1 restriction enzyme digestion was carried out at 25°C for 2 hours in a water bath and the digest was resolved in 3% agarose gel and visualized using gel documentation system following ethidium bromide straining.

T>C polymorphism creates a restriction site for Taq1 endonuclease. Hence the polymorphism was determined by Taq1 restriction enzyme digestion. Restriction enzyme digestion was performed using standard digestion protocol. The digestion was carried out in a reaction volume of 15µl.

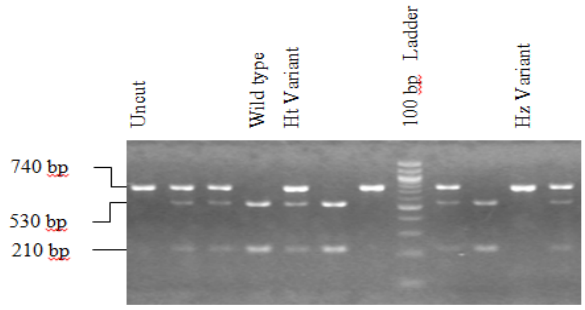

Figure 1. Agarose gel image of VDR gene Apa1 G>T candidate marker analysis by Apa1 restriction enzyme digestion

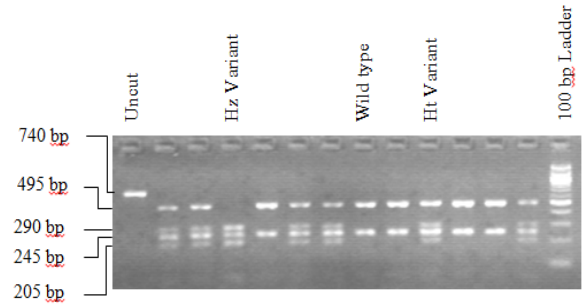

Figure 2. Agarose gel image of VDR gene Taq1 T>C candidate marker analysis by Taq1 restriction enzyme digestion

Taq1 restriction enzyme digestion protocol

Taq1 restriction enzyme digestion was carried at 65°C for 2 hours in water bath. Enzyme digestion product was resolved in 3% agarose gel and digested product was visualized using gel documentation system following ethidium bromide straining.

Statistical Methods

Data were expressed as mean (±SD) and number (percentage) as appropriate. Difference between two groups was determined by unpaired Student's 't' test, Chi-square test where applicable. Data were managed using statistical package for social science (SPSS) for Windows Version 10. P< 0.05 was taken as level at significance.

RESULTS

A total number of 186 unrelated subjects were included in the study. Of them 94 were healthy controls and 92 were YDM. In control group male and female distribution was 55 (58.5%) and 39 (41.5%) respectively. In YDM group the distribution was 37 (40.2%) and 55 (59.8%) respectively.

Anthropometric and biochemical measurements of the study subjects

In between two groups age, BMI, pressure and HOMA-B% of Mean (±SD) was statistically significantly lower (p=0.001) in the YDM group compared to the controls.

Table 1. Age, BMI and blood pressure and biochemical measurement of the study subjects

Variables	Control (n=94)	YDM (n=92)	*t/p values*
Age (yrs)	23±4	19±6	4.780/<0.001
BMI (kg/m^2)	20.4±3.7	18.2±5.1	3.355/0.001
SBP (mmHg)	112.4±7.4	103.7±12.4	5.775/<0.001
DBP (mmHg)	71.6±5.9	68.8±9.1	2.433/0.16
FSG (mmol/l)	4.9±0.5	15.4±5.2	
C-peptide (ng/ml)	1.6 ±0.6	1.2±0.9	3.291/<0.001
HOMA-%S	94.1±39.8	102.6±83.3	0.873/<0.384
HOMA-%B	112.6 ±38.5	19.4±21.3	20.452/0.001

Results were expressed as mean±SD.

VDR gene G>T (Apa1 restriction) and T>C (Taq1 restriction) variant

The genotype frequency of G>T and T>C distribution did not show any statistical significant association between the two groups (χ^2=0.198, 2.492; p=0.906, 0.288) (Table 2). For all the study subjects the Hardy-Weinberg distribution was the found to be in equilibrium (χ^2=0.661; p=0.416). The T>C genotype frequency found to be in compliance with Hardy-Weinberg equilibrium. Odd-Ratio for the variant genotype to the condition was 0.626 (range 0.347-1.230), which did not show any significant risk association (p=0.136).

Table 2. VDR gene Apa1 G>T and Taq1 T>C genotype of the study subjects

VDR Apa1 G>T genotype	**Control** (n= 94)	**YDM** (n= 92)
Wild (GG)	0.183 (17)	0.198 (18)
Ht variant (GT)	0.516 (48)	0.484 (44)
Hz variant (TT)	0.301 (25)	0.319 (29)
	χ^2= 0.198	*p*=0.906
VDR Taq1 T>C genotype		
Wild (TT)	*0.370 (34)*	*0.484 (44)*
Ht variant (TC)	*0.522 (48)*	*0.418 (38)*
Hz variant (CC)	*0.109 (10)*	*0.090 (9)*
	χ2= 2.492	*p*=0.288

Results were expressed as frequency (number).

Table 3. Fasting glucose insulinemic status of the study subjects on the basis of Apa1 G>T genotype

	FSG (mmol/l)	**C-Peptide** (ng/ml)	**HOMA%B**	**HOMA%S**
Control subjects				
Wild (n=17)	5.1±0.6	1.6±0.7	101.0±31.6	95.0±30.9
Variant (n=76)	4.9±0.5	1.6± 0.6	115.0±39.85	94.1±41.8
t/p values	1.243 / 0.227	0.219 / 0.829	1.496 / 0.146	0.090 / 0.929
YDM subjects				
Wild (n=30)	16.2± 4.5	0.9± 0.8	11.6±11.6	106.5±46.2
Variant (n=71)	15.3± 5.5	1.2±.9	20.9±22.7	102.4±90.7
t/p values	0.637 / 0.463	1.177 / 0.013	2.424 / 0.019	0.183 / 0.855

DISCUSSION

Diabetes Mellitus has emerged as a major health problem all over the world and is posing additional burden to the already exhausted health care budget like many other developing countries including Bangladesh.

Vitamin D has been suggested to play an important role in the pathogenesis of diabetes mellitus, both in type 1 and type 2 varieties [7]. Moreover, VDR gene [8] polymorphisms have been found to be associated with T1DM in different population. However, studies also revealed lack of association of VDR gene polymorphism with either T1DM or T2DM [9]. The lack or presence of association of VDR gene polymorphism with diabetes mellitus points to the importance of gene environment and gene to gene interaction in its pathogenesis. In this respect ethnic variation appeared to be an important factor. The YDM subjects in the present study were found to be having low insulin secretory capacity as revealed by HOMA-%B (19.4±21.3) and HOMA-IR (0.74±0.54) values compared to the control group. The present finding is in agreement with the observation of Khan et al. [9].

VDR gene polymorphic (G>T and T>C) markers genotype in the present study determined were in compliance with the Hardy-Weinberg equilibrium. VDR gene G>T polymorphism did not show any

association with YDM ($\chi 2$=0.198; p=0.906). This is in agreement with Ishizaka et al. [8] who did not finding any association between VDR gene G>T variation and juvenile onset diabetes in the Japanese population. The finding is also substantiated by the lack of association in the study involving in Finnish type 1 diabetic patient. They have concluded that single nucleotide polymorphism is unlikely to be associated with type 1 diabetes in Finnish population [10]. The lack of association of the VDR gene G>T polymorphism in the present study is , however, in contrast to that observed in the type 2 diabetic patients [11].

In the present study VDR gene T>C (Taq 1 restriction) polymorphism did not show any association with YDM ($\chi 2$=2.49; p=0.288). This is again agreement with findings of Ichiro et al [8] in the Japanese population. VDR gene T>C polymorphism also lacked association with type 1 diabetes in Caucasoid population [5] Presence of VDR gene T>C variant allele also did not show any relationship regarding insulin secretory status of pancreatic B cell YDM subjects. The lack of any relationship of VDR gene G>T and T>C polymorphism with YDM as a group and there insulinemic status does not exclude possible role of other polymorphic marker in the pathogenesis of diabetes in the young which needs to evaluated.

REFERENCE

1. American Diabetes Association (ADA). Diagnosis and classification of diabetes mellitus. Diabetes Care 2005; 28:37-42. doi:10.2337/diacare.28.suppl_1.S37
2. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004; 80(6 suppl):1689-96.
3. Kumar S, Davies M, Zakaria Y, Mawer EB, Gordon C, Olukoga AO, Boulton AJ. Improvement in glucose tolerance and *β*-cell function in a patient with vitamin D deficiency during treatment with vitamin D. Postgrad Med J 1994; 70:440-3.
4. Hitman GA, Mannan N, McDermott MF, Aganna E, Ogunkolade BW, Hales CN, Boucher BJ. Vitamin D receptor gene polymorphism influences insulin secretion in Bangladeshi Asians. Diabetes 1998; 47(4)**:**688-90.
5. Oh JY, Barrett-Connor E. Association between vitamin D receptor polymorphism and type 2 diabetes or metabolic syndrome in community-dwelling older adults: the Rancho Bernardo Study. Metabolism 2002; 51(3): 356-9.
6. Sayeed MA, Mahtab H, Khanam PA, Latif ZA, Banu A, Khan AK (2007). Prevalence of diabetes and impaired fasting glucose in urban population of Bangladesh. Bangladesh Med Res Counc Bull 2007; 33(1):1-12.
7. Norman AW, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency inhibits pancreatic secretion of insulin. Science 1980; 209(4458):823-5.
8. Ishizaka N, Ishizaka Y, Takahashi E, Unuma T, Tooda E, Nagai R, Togo M, Tsukamoto K, Hashimoto H, Yamakado M. Association between insulin resistance and carotid arteriosclerosis in subjects with normal fasting glucose and normal glucose tolerance. Arterioscler Thromb Vasc Biol 2003; 23(2):295-301.
9. Khan AA, Ali L. Tropical calcific pancreatitis and fibrocalculus pancretitic diabetes in Bangladesh. J Gastroenterol Hepatol. 1997; 12(6):S48-52.
10. Turpeinen H, Hermann R, Vaara S, Laine AP, Simell O, Knip M, Veijola R, Ilonen J. Vitamin D receptor polymorphisms: no association with type 1 diabetes in the Finnish population. Eur J Endocrinol 2003; 149(6):591-6.
11. Hitman GA, Mannan N, McDermott MF, Aganna E, Ogunkolade BW, Hales CN, Boucher BJ. Vitamin D receptor gene polymorphisms influence insulin secretion in Bangladeshi Asians. Diabetes 1998; 47:688-90.