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Association of Vitamin D Receptor Gene Bsm1 (A>G) and Fok1 (C>T) Polymorphism in the Pathogenesis of Impaired Glucose Tolerance in Bangladeshi Subjects

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Abstract

The pathophysiology of prediabetes (impaired fasting glucose and impaired glucose tolerance) still to be clearly understood as .

The present study was undertaken to determine genotype of Vitamin D receptor (VDR) gene Bsm1 (A>G) and Fok1 (C>T) polymorphic allele in a group of impaired glucose tolerance (IGT) subjects of Bangladeshi origin and investigate its association with insulin sensitivity and cell secretory capacity.

i) VDR gene Bsm1 (A>G) and Fok1 (C>T) polymorphic alleles are not associated with IGT of Bangladeshi origin. ii) The polymorphic marker alleles did not have any effect on fasting and two hour blood glucose and insulinemic status of the IGT and Controls iii) The study reconfirmed that insulin sensitivity is predominantly present in the IGT subjects of Bangladeshi origin.

Key words: Diabetes, IGT, polymorphism, Vitamin D receptor gene, insulin sensitivity, insulin secretory capacity

Introduction:

Diabetes mellitus, or simply diabetes, is a group of metabolic diseases in which a person has high blood glucose, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced[1]. Diabetes mellitus is suggested to pass through an intermediate stage of impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) which is together termed as impaired glucose regulation (IGR) by WHO or prediabetes by ADA. This early stage of diabetes may persist for a variable period of time[2] and has recently been adopted as a part of the natural history of diabetes and not a type of diabetes by ADA[3] and WHO[4]. Moreover, a prospective study has shown that passage from normal to impaired glucose tolerance (IGT) and finally to T2DM was accompanied by a progressive decline in β cell secretory capacity[5]. This is supported by the findings of UKIPDS study which has shown that the loss of β cell function begins some 10-12 years before diabetes is diagnosed[6]. Vitamin D deficiency was linked to glucose abnormality for some years[7,8]. Several reports have ascribed of critical role to vitamin D in the functional regulation of the endocrine pancreas. Not only are receptors for 1,25(OH)2D3 found in β cells[9], but the effectors part of the vitamin D pathway is also present in the form of vitamin D dependent calcium binding protein, also known as calbindin-D28k[10]. The expression of calbindin D28K has been shown to protect β cell from cytokine mediated cell death[11].

Vitamin D and its receptor complex may play a regulatory role in β cell insulin secretion

1. Vitamin D deficiency enhances the prevalence of T2DM
2. The replacement of vitamin D may increase the secretion of insulin
3. The vitamin D receptor (VDR) is a member of the steroid/thyroid hormone receptor family
4. The VDR is expressed in pancreatic beta-cells, the BsmI restriction enzyme polymorphism of the gene influences susceptibility to T1DM and
5. Another polymorphic site of the VDR gene (ApaI) influences the insulin secretory capacity of the β cells in healthy Asians[12].

The general objective of the present study was to determine VDR gene Bsm1 (A>G) and Fok1 (C>T) polymorphism in the Impaired Glucose Tolerance subjects in a Bangladeshi population to explore its association with the phenotype.

Materials and Methods

Subjects

This study was conducted in the Research Division, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM). A total number of 84 (Eighty-four) non-related subjects (age range 35 to 55 yrs.) were consecutively recruited in the study, irrespectively of race, religion and socioeconomic status, purposively. Written consent was taken from all the volunteers.

Methodology:

Anthropometric measurements were taken using standard methods. Fasting and postprandial serum glucose was measured using glucose–oxidase method, and the fasting serum lipid profile

(cholesterol, triglyceride and HDL) was determined by enzymatic colorimetric method, fasting serum creatinine by alkaline picrate method, and serum SGPT by UV-spectrophotometric method using commercial kits (Randox Laboratories Ltd., UK). Serum LDL was calculated using the formula of Friedewald[13], the method was not applied in case of triglyceride level exceeds 400 mg/dL. . Fasting serum insulin levels were determined through the enzyme-linked immunosorbent assay (ELISA) method (Linco Research Inc., USA). Insulin secretory capacity (HOMA B %) and insulin sensitivity (HOMA S %) were calculated from fasting glucose and fasting insulin using HOMA-CIGMA software[14]. Extraction of DNA was performed using GenElute DNA extraction kit (QIAGEN, USA). The kit uses the principal of silica gel DNA isolation from whole blood adapted in spin column.

Check for DNA extraction

DNA yield for each sample was checked by agarose gel (1%) electrophoresis.

Vitamin D receptor (VDR) gene polymorphic markers analyses

Vitamin D receptor (VDR) gene polymorphic markers (A>G and C>T) were analyzed by PCR and RFLP. The two polymorphic markers were determined by different PCR amplifications and restriction enzyme digestion. The DNA segment containing A>G (Bsm1 restriction) polymorphic marker was amplified using the following primer set: Forward primer: 5′-CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA-3′Reverse primer: 5′-AAC CAG CGG GAA GAG GTC AAG GG -3′. PCR was carried out in 10 µl reaction volume. Product size for the above mentioned primer set is 825 bp. 3 µl of PCR product was checked for amplification in 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 (Figure 2).

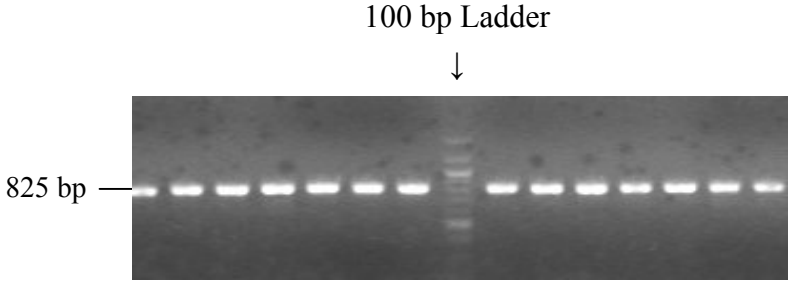

Figure 2: Gel image of VDR PCR for A>G markers containing DNA fragment in 1.5% agarose gel

RFLP analysis of VDR gene candidate markers

A>G polymorphism restricts Bsm1 site. hence the polymorphism was determined by Bsm1 restriction enzyme digestion. Restriction enzyme digestion was performed using standard digestion protocol.

The DNA segment containing C>T (Fok1 restriction) polymorphic marker was amplified using the following primer set: Forward primer: 5′-AGC TGG CCC TGG CAC TGA CTC TGC TCT-3′Reverse primer: 5′-ATG GAA ACA CCT TGC TTC TTC TCC CTC -3′. PCR was carried out

in 10 µl reaction volume. Product size for the above mentioned primer set is 265 bp (Figure 4).

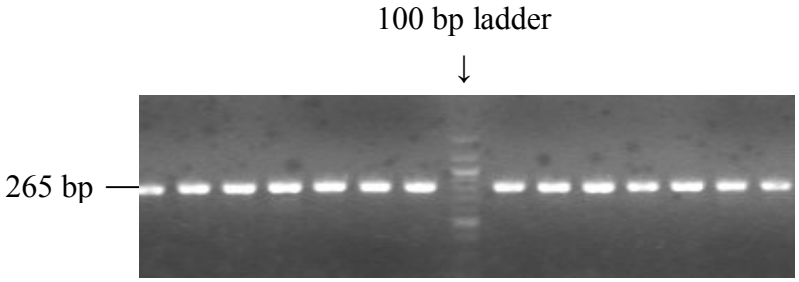

Figure 4: Gel image of VDR PCR for Fok1C>T markers containing DNA fragment in agarose gel

The polymorphism was determined by Fok1 restriction enzyme digestion as C>T polymorphism restricts Fok1 site. Restriction enzyme digestion was performed using standard digestion protocol.

Statistical analysis

Data were expressed as mean (±SD) and number (percentage) as appropriate. Difference between two groups was determined by unpaired Student's't' test and Chi-square test where applicable. Data were managed using statistical package for social science (SPSS) for Windows Version 17.

Results

A total number of 51 IGT subjects were recruited in the study and 33 healthy subjects served as control. Male and female distribution of IGT and healthy control was 28 (54.90%) and 23 (45.10%) and 15 (45.45%) and 18 (54.54%) respectively.

Anthropometric measurement of the study subjects

Age, systolic blood pressure, diastolic blood pressure, body mass index (BMI), waist to hip ratio (WHR) did not show statistically significant difference between the IGT subjects and the control group (Table 1).

Glycemic and Lipidemic status of the study subjects

Triglyceride (TG, mg/dl) value in the IGT group was significantly (p=0.013) higher and high-density lipoprotein cholesterol (HDL-c, mg/dl) value was significantly (p=0.042) lower in the IGT group compared to the control. Whereas serum total cholesterol (mg/dl) and low-density lipoprotein cholesterol (LDL-c, mg/dl) showed, no difference between the two groups (Table 1).

Insulinemic status of the study subjects

Fasting serum insulin (µU/ml) of the IGT group was significantly higher compared to the Controls (p=0.035). Mean HOMA%B of the IGT group (p=0.566) did not show any statistical difference but mean HOMA%S of the IGT group was significantly higher compared to the

Controls (p=0.035) (Table 1).

VDR gene Bsm1 A>G genotype of the study subjects
Genotype frequencies of the VDR gene Bsm1 A>G variants were 0.333, 0.455, and 0.212 for wild type, heterozygous (Ht) variant and homozygous (Hz) variant respectively. In the IGT group, the frequencies were 0.275, 0.471 and 0.255 respectively and the frequencies in the two groups did not show statistical significant association (χ^2=0.398. p=0.820) (Table 2).

When heterozygous and homozygous variant genotypes were grouped together, this distribution also did not show significant association (χ^2=0.332. p=0.565) for the genotype frequencies (Table 3). The Odd Ratio was calculated Bsm1 (A>G) polymorphic allele which was 1.3214 [95% CI- 0.5112 to 3.4161, p=0.5652].

VDR gene Fok1 C>T genotype of the study subjects
This frequency distribution of VDR Fok1 gene C>T variants did not show statistical significant association (χ^2=1.048. p=0.592) (Table 4). Hardy-Weinberg distribution in the Control (χ2= 0.229. p= 0.632) as well as IGT group (χ2= 0.907. p= 0.341) and total (χ2= 0.210. p= 0.647) were found to be in equilibrium. When heterozygous and homozygous variant genotypes were grouped together, the distribution did not showed significant association (χ^2=0.449. p=0.503) (Table 5). The Odd Ratio was calculated for Fok1 (C>T) polymorphic allele which was 1.350 [95% CI- 0.5608 to 3.250, p=0.5032].

Glycemic and insulinemic status of study subjects on the basis of Bsm1 A>G genotype
Fasting glucose levels in subjects with wild and variant (heterozygous and homozygous together) genotype was found to be almost similar in the control (p=0.178) as well as in IGT subjects (p=0.820). Postprandial glucose levels in subjects with wild and variant (heterozygous and homozygous together) genotype was found to be almost similar in the control (p=0.933) as well as in IGT subjects (p=0.129). Insulin levels in subjects with wild and variant (heterozygous and homozygous together) genotype was found to be almost similar in the control (p=0.990) as well as in IGT subjects (p=0.873). HOMA%B in subjects with wild and variant (heterozygous and homozygous together) genotype was found to be almost similar in the control (p=0.189) as well as in IGT subjects (p=0.890). HOMA%S in subjects with wild and variant (heterozygous and homozygous together) genotype was found to be almost similar in the control (p=0.269) as well as in IGT subjects (p=0.880) (Table 6).

Glycemic and insulinemic status of study subjects on the basis of Fok1 C>T genotype
Fasting glucose levels in subjects with wild and variant (heterozygous and homozygous together) genotype was found to be almost similar in the control (p=0.891) as well as in IGT subjects (p=0.957). Postprandial glucose levels in subjects with wild and variant (heterozygous and homozygous together) genotype was found to be almost similar in the control (p=0.905) as well as in IGT subjects (p=0.887) (Table 7). Insulin levels in subjects with wild and variant (heterozygous and homozygous together) genotype was found to be almost similar in the control

(p=0.738) as well as in IGT subjects (p=0.789). HOMA%B in subjects with wild and variant (heterozygous and homozygous together) genotype was found to be almost similar in the control (p=0.385) as well as in IGT subjects (p=0.791). HOMA%S in subjects with wild and variant (heterozygous and homozygous together) genotype was found to be almost similar in the control (p=0.363) as well as in IGT subjects (p=0.986) (Table 7).

Table 1: Clinical and biochemical characteristics of the study subjects

Variables	Control (n=33)	IGT (n=51)	t/p values
Age (yrs)	41.5±5.7	42.8±6.3	0.962/0.339
BMI (kg/m^2)	25.8±4.3	24.9±4.3	0.376/0.376
SBP (mmHg)	114.6±12.8	116.1±145.9	0.432/0.667
DBP (mmHg)	77.3±9.6	75.2±10.3	0.891/0.376
WHR	0.9±0.6	0.9±0.01	0.339/0.736
FSG (mmol/l)	5.1±0.4	6.4±1.7	2.658/0.009*
PPG (mmol/l)	5.8±1.1	9.3±0.9	16.045/0.001*
F Insulin (µU/ml)	11.0±3.8	13.2±5.1	2.141/0.035*
HOMA%B	115.3±30.1	120.4±43.9	0.576/0.566
HOMA%S	85.6±58.4	65.7±24.1	2.150/0.035*
TG (mg/dl)	130.8±52.2(32)	169.7±76.2(50)	2.534/0.013*
T Chol (mg/dl)	208.7±35.6(33)	200.8±47.9(49)	0.807/0.422
HDL-c (mg/dl)	35.9±9.6(29)	30.9±10.9(50)	2.667/0.042*
LDL-c (mg/dl)	132.9±41.7 (29)	146.6±39.2 (47)	1.425/0.048*

Results were expressed as mean±SD. Statistical comparison between groups was performed using unpaired Student's't' test. BMI, Body Mass Index. SBP, Systolic Blood Pressure. DBP, Diastolic Blood Pressure. IGT, Impaired Glucose Tolerance. WHR, Waist Hip Ratio. IGT, Impaired Glucose Tolerance. FSG, Fasting Serum Glucose. PPG, Postprandial Glucose. F insulin, Fasting Insulin. HOMA%B, Homeostatic model assessment β cell function in percent. HOMA%S, Homeostatic Model Assessment of insulin sensitivity in percent. TG, Triglycerides. T chol, total cholesterol. HDL-c, High-density lipoprotein cholesterol. LDL-c, Low density lipoprotein cholesterol. IGT, Impaired Glucose Tolerance

Discussion

Diabetes mellitus has become a major public health problem all over the world. About 90-95% of the total diabetic patients are of type 2 variety[4]. Both insulin resistance and / or secretory defect implicated in its pathogenesis. Some of the established polymorphic genetic markers, implicated in the pathogenesis of T2DM, have been studied at BIRDEM in Bangladeshi population. These include INS-VNTR, CAPN10, NAT2 and VDR gene common polymorphism both in T2DM and young onset diabetes mellitus. Among the Bangladeshi subjects genotype

Table 2: VDR gene Bsm1 A>G genotype of the study subjects

VDR Bsm1 A>G genotype	Control (n=33)	IGT (n=51)	Total (n=84)
Wild (AA)	0.333 (11)	0.275 (14)	0.298 (25)
Ht variant (AG)	0.455 (15)	0.471 (24)	0.464 (39)
Hz variant (GG)	0.212 (7)	0.255 (13)	0.238 (20)
	χ^2=0.398	*p=0.820*	
Allele Frequency			
Allele A	0.561	0.511	
Allele G	0.440	0.491	

Results were expressed as frequency (number). Chi-square test was performed to calculate statistical association. Hz Wild, homozygous wild. Ht variant, heterozygous variant. Hz variant, homozygous variant. VDR, vitamin D receptor. IGT, Impaired Glucose tolerance

Table 3: VDR gene Bsm1 A>G genotype (Ht and Hz variant together) of the study subjects

VDR Bsm1 A>G genotype	Control (n= 33)	IGT (n= 51)	Total (n= 84)
Wild (AA)	0.333 (11)	0.275(14)	0.298 (25)
Variant (AG and GG)	0.667(22)	0.725(37)	0.702 (59)
	χ^2= 0.332	p=0.565	

Results were expressed as frequency (number). Chi-square test was performed to calculate statistical association.

Table 4: VDR gene Fok1 C>T genotype of the study subjects

VDR Fok1 C>T genotype	Control (n= 33)	IGT (n= 51)	Total (n= 84)
Wild (CC)	0.545 (18)	0.471 (24)	0.500 (42)
Ht variant (CT)	0.364 (12)	0.471 (24)	0.429 (36)
Hz variant (TT)	0.091(13)	0.059 (3)	0.071 (6)
	χ^2= 5.339	*p=0.069*	
Allele Frequency			
Allele C	0.727	0.707	
Allele G	0.273	0.295	

Results were expressed as frequency (number). Chi-square test was performed to calculate statistical association. Hz Wild, homozygous wild. Ht variant, heterozygous variant. Hz variant, homozygous variant. VDR, vitamin D receptor. IGT, Impaired Glucose tolerance

Table 5: VDR gene Fok1 C>T genotype (Ht and Hz variant together) of the study subjects

VDR Fok1 C>T genotype	Control (n=33)	IGT(n= 51)	Total (n= 84)
Wild CC)	0.545 (18)	0.471 (24)	0.500 (42)
Variant (CT and TT)	0.455 (15)	0.529 (27)	0.500 (42)
	χ^2= 0.449	p= 0.503	

Results were expressed as frequency (number). Chi-square test was performed to calculate statistical association.

Table 6: Fasting and 2hr glucose, and insulinemic status of the study subjects on the basis of Bsm1 A>G genotype

	FSG (mmol/l)	PPG (mmol/l)	F Insulin (pmol/l)*	HOMA%B	HOMA%S
Control subjects					
Wild (n=11)	5.2±0.4	5.8±1.4	11.0±4.7	105±28.9	101.8±91.6
Variant (n=22)	5.0±0.4	5.8±1.0	11.0±3.3	120.3±30.1	77.6±31.7
t/p values	*1.379/0.178*	*0.085/0.933*	*0.012/0.990*	*1.342/0.189*	*1.124/0.269*
IGT subjects					
Wild (n=14)	5.4±0.7	9.6±0.8	13.0±5.6	121.8±51.9	66.6±23.2
Variant (n=37)	5.5±0.7	9.2±0.9	13.3±4.9	119.8±41.1	65.4±24.8
t/p values	*0.228/0.820*	*1.543/0.129*	*0.161/0.873*	*0.139/0.890*	*0.152/0.880*

Results were expressed as mean ± SD. Statistical comparison between groups was performed using unpaired Student's't' test. Variant, heterozygous and homozygous variant together. FSG, fasting serum glucose. IGT, Impaired Glucose Tolerance. PPG, postprandial glucose. F insulin, Fasting Insulin. HOMA%B, Homeostatic model assessment β cell function in percent. HOMA%S, Homeostatic Model Assessment of insulin sensitivity in percent.

frequencies of the INS VNTR A>T polymorphic alleles were 0.690, 0.254 and 0.056 (for wild heterozygous and homozygous respectively) which however, did not show association with T2DM of Bangladeshi origin[15]. In one study done in BIRDEM lab by Shapla (Dept of Biotechnology and Genetic Engineering, Kushtia Islamic University) found that, CAPN10 gene single nucleotide polymorphism (SNP) 19 and SNP 63 were in vest gated in T2DM subjects of Bangladeshi origin which, however, was found not to be associated with T2DM in other reported studies. NAT2 gene polymorphic markers (481C>T, 590G>A and 857 G>A) were also investigated for its possible role in the pathogenesis of T2DM of Bangladeshi origin. The investigator did not find any association for NAT2 polymorphic markers and T2DM (Paul 2010). VDR gene Bsm1 (A>G) and Taq1 (T>C) polymorphic markers did not but Apa1 (G>T) showed significant association with Bangladeshi T2DM subjects. VDR gene polymorphic markers were not found to be associated with VDR gene polymorphic markers with young onset diabetes mellitus of Bangladeshi origin. Vitamin D has suggested to be strongly related to both pancreatic

Table 7: Fasting and 2hr glucose and insulinemic status of the study subjects on the basis of Fok1 C>T genotype

	FSG (mmol/l)	**PPG** (mmol/l)	**F_Insulin** (pmol/l)	**HOMA%B**	**HOMA%S**
	Control subjects				
Wild (n=18)	5.1±0.5	5.8±1.2	10.8±4.3	111.1±33.5	94.2±72.8
Variant (n=15)	5.1±0.3	5.7±1.1	11.2±3.1	120.4±25.7	75.3±33.9
t/p values	*0.138/0.891*	*0.121/0.905*	*0.337/0.738*	*0.882/0.385*	*0.923/0.363*
	IGT subjects				
Wild (n=24)	5.4±0.8	9.3±0.8	13.4±5.7	122.1±47.5	65.8±27.1
Variant (n=27)	5.4±0.5	9.3±0.9	13.0±4.5	118.8±41.1	65.7±21.6
t/p values	*0.053/0.957*	*0.142/0.887*	*0.269/0.789*	*0.267/0.791*	*0.017/0.986*

Results were expressed as mean±SD. Statistical comparison between groups was performed using unpaired Student's't' test. Variant, heterozygous and homozygous variant together. FSG, fasting serum glucose. IGT, Impaired glucose tolerance. PPG, postprandial glucose. F insulin, Fasting Insulin. HOMA%B, Homeostatic model assessment β-cell function in percent. HOMA%S, Homeostatic Model Assessment of insulin sensitivity in percent.

β-cell function and insulin sensitivity[7,16]. VDR gene polymorphisms, however, found to be associated with insulin secretion[17], insulin sensitivity[7], fasting glucose and T2DM[18]. In the natural history of diabetes, in particular T2DM, variety, prediabetes (IFG and IGT) has been recognized as the intermediate state[3,4]. Like that of T2DM the prediabetes is found to be increasing fast all over the world. The world has been sharp rise in prevalence of T2DM. There has been rise in prevalence of T2DM in Bangladesh from 4% to 8% over the last couple of decades[19]. A recent study involving normal to overweight healthy adults living in Dhaka city demonstrated that 16% had T2DM and 14% IGT. These findings have reconfirmed the increasing trends of T2DM and IGT in the Bangladeshi population (personal communication). T2DM in the Bangladeshi population found to show both insulin resistance and secretory defect[20]. However, the exact pathophisiological mechanism in the development of IGT is still to be clearly understood. Since VDR gene polymorphisms have been linked to the pancreatic β-cell insulin secretory status of normal and abnormal glucose tolerance state, in the present study VDR gene Bsm1 (A>G) and Fok1 (C>T) polymorphic markers were analyzed in the IGT subjects of Bangladeshi origin to explore its association with the phenotype and their glycemic and insulinemic status. A total number of 51 IGT subjects were included in the study and 33 healthy subjects screened as control. Male and female distribution at IGT and Healthy control was 28 (54.90%) and 23 (45.10%) and 15 (45.45%) and 18 (54.54%) respectively. The IGT subjects had significantly higher serum insulin level compared to the controls (p=0.035) which has reflected by the significantly lower HOMA% S value (p=0.035). Two candidate polymorphic markers in VDR gene, Bsm1 (A>G) and Fok1 (C>T) were analyzed. The genotypes frequencies of Bsm1 (A>G) polymorphic allele in the Controls were 0.333, 0.455, 0.212 (for wild, heterozygous and homozygous variant respectively) and in IGT 0.275, 0.471, 0.255 respectively which did not show any significant association (χ^2=0.398, p=0.820) (Table 4.4). Among the 569

North Indian subjects genotype frequencies were 0.070, 0.230 and 0.763. It appeared that Bsm1 (A>G) genotype frequencies in the Bangladeshi subjects were relatively different from the North Indian subjects but almost similar to those of 0.33, 0.48 and 0.19, European origin[12]. Data in the present study, however, needs to be reconfirmed since smaller number of subjects were analyzed. The genotypes frequencies of Fok1 (C>T) polymorphic allele in the Controls were 0.545, 0.364, 0.091 (for wild, heterozygous and homozygous variant respectively) and in IGT 0.471, 0.471, 0.059 respectively which also did not show any significant association (χ^2=5.339, p=0.069) (Table 4.6). This is for first time VDR gene Fok1 (C>T) polymorphic allele was analyzed in a Bangladeshi subjects. Hence the data in the controls cannot be compared with any previous report. Fok1 (C>T) genotype frequencies among the North Indian subjects were 0.437, 0.491 and 0.072[21] and French subject 0.370, 0.454 and 0.316. There seems to be a relative deviation of Fok1 genotype frequencies in the Bangladeshi subjects which, however, needs to be confirmed by increasing number involving a large scale study. Genotype frequencies were tested for its association fasting and 2-hour and insulinemic healthy controls and IGT groups. For this purpose the Ht and Hz variants were pooled together. In case of Bsm1 polymorphism, fasting and 2-hour glucose insulinemic status did not show significant different between those with wild 'AA' and variant 'AG' and 'GG' allele both among Control and IGT subjects (Table 6). These findings, however, in contradiction with the results of Ongunkolade et al[17 (pp 2294-2300)] who demonstrated that Bsm1 polymorphism was associated with reduce secretoy capacity and the finding of Ortlepp et al[18 (pp 451-454)] who found variant allele to be associated with elevated fasting glucose. In case of Fok1 polymorphism as well both in the controls and IGT groups subjects with wild 'CC' and variant CT and TT' allele did not show any statistical difference regarding fasting and 2-hour glucose and insulinemic status (Table 7).

Conclusions:

VDR gene Bsm1 (A>G) and Fok1 (C>T) polymorphism did not show association with Bangladeshi IGT subjects. The polymorphic marker alleles did not have effect on fasting and two hour blood glucose and insulinemic status of the IGT and Controls. Data reconfirmed that insulin sensitivity is significantly lowered in the IGT subjects of Bangladeshi origin.

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