Association of Single Nucleotide Polymorphisms of Adiponectin Gene with Type 2 Diabetes Mellitus, and Their Influence on Cardiovascular Risk Markers

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Abstract Type 2 diabetes mellitus is a genetically heterogeneous condition, characterized by insulin deficiency and/or insulin resistance. The etiology of type 2 diabetes is complex, with involvement of genetic and environmental factors. The adipose tissue protein ‘adiponectin’ is known to increase insulin sensitivity with decreased risk of type 2 diabetes mellitus. The gene for adiponectin is present on chromosome 3q27, the association of number of single nucleotide polymorphisms of adiponectin gene with type 2 diabetes and its complications have been reported. In the present study the two most common SNPs +45T/G & +276G/T, and their association with type 2 diabetes mellitus and cardiovascular markers were studied. The significant difference in genotype frequencies of +45T/G & +276G/T was found in type 2 diabetic patients and controls, with odds ratio of 1.13 & 1.26 respectively. BMI, Fasting blood glucose, fasting insulin, HOMA IR, triglyceride and VLDL cholesterol levels were increased, and HDL cholesterol level was decreased in patients carrier for +45T/G SNP than the wild type. While only decrease in the HDL cholesterol was reported in carriers for SNP +276G/T than the wild type. The logistic regression analysis revealed the positive association of SNP +45T/G with total cholesterol & LDL cholesterol. And negative association of HDL cholesterol

was found with SNPs +45T/G and +276G/T. The haplotype analysis shows the alterations in means of biochemical markers in the patients having haplotype (GG) for mutant allele of SNP +45T/G and wild allele for SNP +276G/T.

Keywords Type 2 diabetes mellitus · Adiponectin · Adiponectin gene · SNP+45T/G · SNP+276G/T

Introduction

We are in the midst of a worldwide epidemic of type 2 diabetes, obesity, and metabolic syndrome, each created by complex interactions between genes and environment [1]. Despite advances in treatment, diabetes is the leading cause of chronic renal failure, adult blindness, and limb amputation; and a major risk factor for heart disease, stroke, and birth defects [2]. The largest proportion of public health problems related to kidneys, eyes, brain etc. is derived from type 2 diabetes which accounts for more than 90 % of diabetes worldwide [3].

Type 2 diabetes is a genetically heterogeneous disease, with several relatively rare monogenic forms and a number of more common forms resulting from a complex interaction of genetic and environmental factors. Previous studies using a candidate gene approach, family linkage studies, and gene expression profiling uncovered a number of genes related to type 2 diabetes, but the genetic basis of common type 2 diabetes remained unknown [1].

Adipose tissue, in addition to its function as the major energy reservoir, produces and secretes proteins such as adipon, tumor necrosis factor- α , and leptin [4]. One of its secretory products adiponectin, has been shown to increase insulin sensitivity via promotion of lipid β -oxidation in skeletal muscles and reducing hepatic gluconeogenesis. It

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has direct vasoprotective actions such as stimulation of prostaglandin I₂ synthase and endothelial nitric oxide synthase activity [5].

Adiponectin (AdipoQ) is an adipose tissue-specific protein of 247 amino acids that shares significant similarity with collagens VIII and X and complement protein C1q. It is encoded by AdipoQ gene in adipose tissue and is expressed exclusively in differentiated adipocytes [6, 7]. The adiponectin gene consists of three exons and two introns located on chromosome 3q27, where a diabetes susceptibility locus has been mapped. Single nucleotide polymorphisms (SNPs) of the adiponectin gene have shown to be associated with BMI, insulin sensitivity, and type 2 diabetes in some cross-sectional studies, but these associations have not been seen in all studies [6, 8].

Several studies have been performed aiming to investigate the association of single nucleotide polymorphisms in the adiponectin gene with obesity, insulin resistance, type 2 diabetes and polycystic ovary syndrome [4, 5, 7, 9–13]. The most common reported variants are the T/G polymorphism of SNP +45 (+45T/G) in exon 2 and the G/T polymorphism of SNP +276 (+276G/T) in intron 2 in AdipoQ gene, which have been found to be related to obesity, insulin resistance syndrome and type 2 diabetes.

Recently, El-Menyar et al. [14] studied the association of these adiponectin gene variants with acute coronary syndrome, it is shown that +45T/G is associated with high risk for acute coronary syndrome among Arab populations.

In this study, we investigated genetic variants +45T/G & +276G/T in the adiponectin gene. The aim of the present study was to examine the association of SNPs +45T/G and +276G/T in the adiponectin gene with markers of cardiovascular diseases and insulin resistance parameters in a group of patients with type 2 diabetes mellitus. This will help to understand the pathophysiology of type 2 diabetes mellitus and risk of cardiovascular diseases in type 2 diabetics. However, there are very few studies reported on the association of +45T/G & +276G/T single nucleotide polymorphisms of the adiponectin gene with the risk of type 2 diabetes from India. We therefore hypothesized that presence of common single nucleotide polymorphisms in the adiponectin gene may take part in progression of insulin resistance and type 2 diabetes.

Material & Methods

Subjects

A case control study was conducted at department of biochemistry, B. J. Government Medical College, Pune. The sample size was calculated from the previous study of SNP +45T/G done in Indian population by Biswas D et al. [15].

150 patients of diabetes mellitus (diagnosed as per ADA guidelines) in the period of 2011–2013 were selected by simple random sampling method from out patients Department of Medicine, B. J. Government Medical College, Pune, between the age group 40–60 years of either sex. Patients, who were pregnant, had angina or heart failure, renal failure, hypertension and severe concurrent illness were excluded from the study. Age & sex matched healthy volunteers without any clinical & biochemical evidence of diabetes or hyperlipidemia, chronic kidney or liver diseases or thyroid disorders were selected as controls, the control subjects were selected from community those who were coming for routine checkup. Moreover, all the control subjects had normal fasting (<110 mg/dl) and post prandial (<140 mg/dl) plasma glucose level. An informed consent was taken from every patient after complete explanation of procedure. Every patient was advised for at least 12–14 h overnight fasting and venous blood samples were collected in fluoride and plain vacutainers, and plasma & serum were separated. Serum samples were analyzed for total cholesterol, triglycerides, HDL cholesterol and fluoride plasma was used for the estimation of fasting blood glucose. 3 ml of blood was collected in EDTA vacutainer to extract DNA for analysis of SNPs +45T/G & +276G/T of adiponectin.

Lipid Analysis

To study the effect of the SNPs +45T/G & +276G/T on lipid levels in T2DM, plasma triglycerides, total cholesterol and HDL cholesterol and other lipid levels were measured in T2DM patients and controls by using Biosystem A15 fully automated biochemistry analyzer. Blood glucose was estimated by GOD–POD method [16]. Serum total cholesterol [17] and triglyceride [18] concentrations in serum were measured by enzymatic methods. HDL cholesterol [19] was determined with a turbidimetric assay. LDL & VLDL cholesterol were calculated according to the equation of Friedwald's et al. [20] insulin concentrations were detected using chemiluminescence immunoassay after the serum samples were thawed at room temperature. Insulin resistance was calculated by means of HOMA-IR [21].

Genetic Analysis

Venous blood samples in EDTA were subsequently used for DNA extractions by salting out method [22]. Single nucleotide polymorphisms +45T/G & +276G/T of adiponectin for patients and controls were determined by Polymerase chain reaction followed by restriction digestion [12, 23]. For the +45T/G locus, we used the primer pair (Sigma Aldrich) as follows: forward primer, 5'-

AGGTGGGCTGCAATATTCAG-3'; reverse primer, 5'-CCTGGATCTCCTTTCTCACC-3'. The +276G/T polymorphic locus was amplified by the use of forward primer 5'-TCTCTCCATGGCTGACAGTG-3' and reverse primer 5'-AGATGCAGCAAAGCCAAAGT-3'. PCR reactions were carried out in a 24-well programmable thermal cycler. The PCR was carried out in a total volume of 25 µl of reaction mixture, containing 50 ng of genomic DNA; 12.5 µl 2X master mix containing 200 µmol/L of deoxynucleotides triphosphates; 10 mmol/L Tris-HCL, pH 8.8; 1.5 mmol/L MgCl₂; 50 mmol/L KCl, and 1 mL/L Triton X-100; 2.5 U of Taq DNA Polymerase; and 8 nmol/L each primer. The PCR amplification conditions used for +45T/G were as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation for 35 s at 94 °C, annealing for 30 s at 58.8 °C, and extension for 30 s at 72 °C followed by final extension for 5 min at 72 °C. PCR conditions for +276G/T were as follows: Initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 62 °C, and extension for 30 s at 72 °C followed by final extension of 5 min at 72 °C.

Restriction Digestion

The PCR products were digested with *Sma*I (Sigma Aldrich) for 6 h at 25 °C for SNPs +45T/G, and *Bsm*I (Sigma Aldrich) for 14 h at 65 °C for +276G/T. The digested DNA fragments were electrophoresed on a 2 % agarose gels containing ethidium bromide and visualized by an ultraviolet transilluminator. In regard to the +45T/G, a single 390-bp band indicates homozygosity for the T allele; the two bands, 217- and 173-bp, indicate homozygosity for the G allele; the presence of three fragments, 390-, 217- and 173-bp bands, indicate heterozygosity for the T & G allele.

As for the +276G/T, presence of a single 468-bp band indicates homozygosity for the G allele; presence of two fragments, 320- and 148-bp, indicates homozygosity for the T allele; and three fragments, 468-, 320- and 148-bp indicate heterozygosity for the G & T allele.

Statistical Analysis

Deviations of the genotype distribution from expected for a population were tested by means of Hardy–Weinberg equilibrium (HWE). The frequencies of SNPs among the T2DM patients & controls were compared using Chi square test within codominant & dominant genetic models. The association of SNPs was calculated by means of Odds ratio. The demographic and biochemical variables viz. BMI, fasting blood glucose, fasting insulin, HOMA IR index, triglyceride, total cholesterol, HDL cholesterol, LDL

cholesterol and VLDL cholesterol, among dominant genetic model subgroups of +45T/G & +276G/T (wild Vs Carriers) were compared, and the *p* value of <0.05 is considered significant. The demographic and biochemical parameters were compared between the groups of SNPs based on dominant genetic model. The models were adjusted for sex, smoking and alcoholic status. Data were log transformed when necessary to approximate a normal distribution. The association between SNPs and biochemical markers was studied by logistic regression analysis. And the pairwise association between the SNPs was calculated by means of linkage disequilibrium (LD) & the frequencies of possible haplotypes were calculated. Statistical analysis was done using SNPStat software. The linkage disequilibrium and haplotype analysis was done by statistical software THIASIS version 3.1.

Results

Sample Characteristics and Frequencies of +45T/G and +276G/T SNPs

The mean age was 56.1 ± 10.73 years and 54.5 ± 11.65 years for type 2 diabetics and controls respectively. The diabetic group out of 150 patients consisted of 74 males and 76 females, while the control group out of 150 volunteers consisted of 81 males and 69 females, when compared no significant difference was found in age & gender between type 2 diabetics and controls. The genotype frequencies were distributed according to Hardy–Weinberg equilibrium for +45T > G and +276G > T SNPs in the adiponectin gene in type 2 diabetics as well as controls.

The genotype frequencies were compared between type 2 diabetics and controls for +45T > G and +276G > T SNPs with codominant & dominant genetic models. For +45T > G, the dominant and codominant models show a significant difference in genotype frequencies between the study and control groups with associated *p* values of <0.0001 & = 0.0018 respectively, while for +276G > T variant genotype frequencies were also significant for dominant and codominant genetic models with associated *p* value of <0.0001 for both (Table 1). The odds ratio calculated from the above values for +45T > G variant was found to be 1.13 with 95 % confidence interval of 0.8161 to 1.5754, and for +276G > T it was 1.26 with 95 % confidence interval of 0.897–1.7677. We also studied Pairwise linkage disequilibrium for SNPs +45T/G and +276 G/T, no statistical (*p* = 0.058) association of these two SNPs was found, associated linkage disequilibrium statistics values were $D' = 0.3411$, $R^2 = 0.2304$.

Table 1 Allele and genotype frequencies of +45T/G and +276G/T SNPs of Adiponectin gene in Type 2 diabetics and controls

		Total (n = 300)	T2DM patients (n = 150)	Controls (n = 150)	<i>p</i> value
+45T/G (+45T > G) Genotype frequencies: expressed as 'n' & (%)					
Codominant	T/T	271 (90.33)	127 (84.67)	144 (96.0)	<0.01*
	T/G	27 (9.0)	21 (14.0)	06 (4.0)	
	G/G	02 (0.67)	02 (1.33)	0 (0)	
Dominant	T/T	271 (90.33)	127 (84.7 %)	144 (96 %)	<0.0001*
	T/G–G/G	29 (9.67)	23 (15.3 %)	06 (4 %)	
Allele frequencies	T	0.95	0.92	0.98	
	G	0.05	0.08	0.02	
+276G/T (+276G > T) Genotype frequencies: expressed as 'n' & (%)					
Codominant	G/G	244 (81.33)	108 (72.0)	136 (90.67)	<0.0001*
	G/T	48 (16.0)	35 (23.33)	13 (8.67)	
	T/T	08 (2.67)	07 (4.67)	01 (0.67)	
Dominant	C/C	244 (81.33)	108 (72 %)	136 (90.7 %)	<0.0001*
	C/T–T/T	56 (18.67)	42 (28 %)	14 (9.3 %)	
Allele frequencies	G	0.89	0.84	0.95	
	T	0.11	0.16	0.05	

* highly significant

Effect of +45T/G and +276G/T SNPs on Biochemical Parameters

The results of the demographic and biochemical parameters were expressed as Mean $\pm$ Standard error (SE). When results of demographic and biochemical parameters were compared between subgroups of SNPs on the basis of dominant genetic model, adjusted for sex, smoking and alcoholic status, BMI, fasting glucose, fasting insulin, HOMA IR index, TG & VLDL-C were elevated in carriers for +45 SNP than wild type. And HDL cholesterol was decreased in subjects carriers for variant at position +45 than wild type. Plasma concentration of HDL cholesterol was decreased significantly among subjects wild type for variant at positions +276 than carriers (Table 2).

Logistic regression analysis was evaluated to see the association of +45T/G and +276G/T variants in adiponectin gene with biochemical parameters. The positive association of total cholesterol (<0.0001), and LDL cholesterol (<0.0001) and negative association of HDL cholesterol (<0.0001) was found with +45T/G variant of adiponectin gene. The regression model was also evaluated by ROC curve analysis with the area under the ROC curve of 0.901 and standard error of 0.0375 showing overall strong association. While +276G > T variant was only associated negatively with HDL-C (0.0452). The regression model was also evaluated by ROC curve analysis with the area under the ROC curve of 0.604 and standard error

of 0.0500. The results of logistic regression analysis are shown in Table 3.

+45T/G/+276G/T Haplotype Analysis

The frequencies of all possible haplotypes were calculated for SNPs +45T/G and +276G/T. The possible haplotypes were TG, TT, GG and GT with frequencies of 0.773960, 0.142707, 0.062707 and 0.020627 respectively in type 2 diabetics. The phenotypes were compared among all haplotypes. The phenotypic means of all parameters did not differ between wild type for both variants TG vs. +276G/T variant haplotype TG. +45T/G variant haplotype GG vs. TG was associated with higher fasting blood glucose, fasting insulin levels, HOMA-IR index, total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol, no significant association was found between BMI, WHR, and HDL cholesterol levels.

Whereas we found an increase only in triglyceride and VLDL cholesterol levels for the +45T/G & +276G/T variants haplotype GT vs. TG, and no association found with fasting glucose, fasting insulin, HOMA IR index, total cholesterol, HDL cholesterol and LDL cholesterol. The haplotype frequencies for +45T/G and +276G/T and the phenotypic means for BMI, fasting blood glucose, fasting insulin, HOMA IR index, total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol are shown in Table 4.

Table 2 Association of adiponectin +45T/G & +276G/T variants with biochemical parameters (mean $\pm$ SE) adjusted for sex, smoking and alcoholic status in type 2 diabetics

	SNP +45T/G (+45 T > G) genotype			SNP +276G/T (+276G > T) genotype		
	TT n = 127	TG + GG n = 23	p value	GG n = 109	GT + TT n = 41	p value
Age (years)	56.35 (0.95)	54.7 (2.23)	>0.05	56.59 (1.06)	54.78 (1.51)	>0.05
BMI (Kg/m ²)	26.97 (0.23)	28.42 (0.51)	<0.05*	27.21 (0.25)	27.15 (0.38)	>0.05
WHR	0.96 (0.01)	0.96 (0.02)	>0.05	0.97 (0.01)	0.95 (0.01)	>0.05
Fasting glucose (mg/dl)	130.92 (1.41)	138.12 (3.37)	<0.05*	132.65 (1.58)	130.36 (2.35)	>0.05
Fasting insulin (μ IU/ml)	9.91 (0.32)	12.17 (0.8)	<0.01**	10.36 (0.36)	10.0 (0.53)	>0.05
HOMA IR	3.24 (0.12)	4.2 (0.32)	<0.01**	3.43 (0.14)	3.26 (0.2)	>0.05
Total cholesterol (mg/dl)	203.06 (2.71)	214.36 (6.9)	>0.05	204.97 (2.99)	204.31 (4.9)	>0.05
Triglyceride (mg/dl)	158.5 (1.89)	192.22 (3.69)	<0.0001**	162.83 (2.21)	165.9 (4.15)	>0.05
HDL cholesterol (mg/dl)	44.24 (0.51)	41.04 (0.92)	<0.05*	44.33 (0.56)	42.21 (0.77)	<0.05*
LDL cholesterol (mg/dl)	127.13 (2.6)	134.87 (6.65)	>0.05	128.09 (2.8)	128.92 (4.9)	>0.05
VLDL cholesterol (mg/dl)	31.7 (0.38)	38.44 (0.74)	<0.0001**	32.56 (0.44)	33.18 (0.83)	>0.05

* significant ** highly significant

Table 3 Logistic regression analysis of adiponectin +45T > G & +276 G > T variants with biochemical markers in T2DM patients

Variables	Coefficient	Std. error	'p' values
+45T > G			
Cholesterol	0.49321	0.11323	<0.0001*
HDL-C	−0.54786	0.11784	<0.0001*
LDL cholesterol	0.49437	0.11386	<0.0001*
+276G > T			
HDL	−0.070943	0.035425	0.0452*

* statistically significant

Discussion

Adiponectin is an adipose tissue-specific protein with insulin-sensitizing and anti-atherogenic properties. Therefore, the adiponectin gene is a promising candidate gene for type 2 diabetes [7]. In this study we have studied association of two common SNPs +45T/G & +276G/T of AdipoQ gene, with type 2 diabetes mellitus and the cardiovascular diseases risk factors. Of these +45T/G is situated in the 2nd exon while +276G/T is in 1st intron of AdipoQ gene, on chromosome 3q27, where a diabetes susceptibility locus has been mapped [4].

The genotype frequencies for both the variants were within Hardy–Weinberg equilibrium in study group as well as in controls. The genotype frequencies differ significantly among type 2 diabetics and controls, which indicate the role of these variants in the development of type 2 diabetes mellitus. The subjects carrying +45T/G

variant were at 1.13 times higher risk of having type 2 diabetes mellitus, while the presence of +276G/T variant increases the risk of having type 2 diabetes mellitus by 1.26 times. Also insulin resistance is found to be strongly associated with SNP +45T/G, and play an important role in the pathogenesis of type 2 diabetes. While +276G/T was not statistically associated with insulin resistance. Therefore, it is evident that the variant +45T/G of adiponectin is having role in the development of insulin resistance as well as progression of type 2 diabetes. While +276G/T variant may take part in the progression of type 2 diabetes mellitus.

Kazuo Hara et al. [4] presented an association between SNPs at positions 45 and 276 in the adiponectin gene and type 2 diabetes with odds ratios of 1.70 and 2.16 respectively. Jelena Zacharova et al. [7] found G allele of SNP +45 had 1.8-fold and the T allele of SNP +276 had a 4.5-fold higher risk of developing type 2 diabetes, also concluded that the combined effect of SNP +45 and SNP +276 on the development of type 2 diabetes was stronger than that of each SNP alone.

In South Indian population Biswas et al. [15] have shown a positive association between SNP +45T/G and type 2 diabetes mellitus with the odds ratio of 3.797. A study in Romanian patients to assess possible link to prevalence of type 2 diabetes mellitus, demonstrated no genotype difference between type 2 diabetics and controls. Further regression analysis disclosed age (OR 1.086), waist circumference (OR 1.084), but not genotype as predictors for the presence of diabetes in this population [23]. The reduced risk of obesity (OR 1.32), metabolic syndrome (OR 1.33) and diabetes mellitus (OR 1.28) was found in

Table 4 Expected phenotypic means according to possible haplotypes for SNPs +45 and +276 of the adiponectin gene in type 2 diabetics

Haplotypes Haplotype frequencies	TG 0.773960	TT 0.142707	GG 0.062707	GT 0.020627
BMI	13.61 [13.18–14.03]	13.72 [12.95–14.5]	14.76 [13.46–16.06]	15.22 [12.64–17.8]
WHR	0.48 [0.46–0.49]	0.48 [0.45–0.51]	0.50 [0.46–0.53]	0.40 [0.29–0.51]
Fasting blood glucose [¶]	66.28 [63.44–69.13]	63.12 [57.88–68.36]	73.75* [66.46–81.03]	64.69 [46.23–83.15]
Fasting insulin [§]	4.5 [4.43–5.57]	4.85 [3.65–6.06]	7.04* [5.78–8.31]	4.78 [0.47–9.1]
HOMA IR index [⊖]	1.66 [1.45–1.86]	1.52 [0.1–2.04]	2.57* [2.10–3.03]	1.50 [0.01–2.99]
Total cholesterol [¥]	100.33 [95.57–105.09]	107.32 [99.38–115.26]	122.97* [107.56–138.38]	80.34 [51.11–109.58]
Triglycerides [±]	78.53 [74.85–82.22]	76.7 [69.79–83.61]	107.37* [96.47–118.26]	106.3* [87.41–125.19]
HDL cholesterol	22.06 [21.19–22.94]	20.89 [19.15–22.63]	19.77 [16.47–23.06]	17.3 [1.36–33.24]
LDL cholesterol [‡]	62.60 [57.90–67.30]	70.96 [63.62–78.29]	81.39* [67.04–95.74]	42.36 [13.57–71.14]
VLDL C [†]	15.70 [14.96–16.45]	15.34 [13.94–16.74]	21.47* [19.26–23.68]	21.28* [17.44–25.12]

[¶] GG versus TG, $p = 0.043266$

[§] GG versus TG, $p = 0.002538$

[⊖] GG versus TG, $p = 0.000230$

[¥] GG versus TG, $p = 0.007090$

[±] GG versus TG, $p = 0.000001$; GT versus TG, $p = 0.003099$

[‡] GG versus TG, $p = 0.016944$

[†] GG versus TG, $p = 0.000001$; GT versus TG, $p = 0.003461$

* statistically significant

presence of G allele of SNP +276G/T in intron 2 in one of the study [24]. In Arabs acute coronary syndrome patients and healthy controls from Qatar, had high risk of acute coronary syndrome due to presence of G allele of SNP (+45T/G) in the adiponectin gene [14].

In French Caucasians Nabila Bouatia-Naji et al. [25] found an association between severe forms of obesity (OR 1.19) and +276T allele. Shirin Hasani Ranjbar et al. [26] examined association between +45T/G SNP and type2 diabetes in an Iranian population, no significant association was found between adiponectin gene polymorphism +45T/G and diabetes, also no association found between +45 polymorphism and complications of type 2 diabetes mellitus.

In an association study by Labros Melistas et al. [10] showed higher fasting insulin levels ($9.65 \pm 5.91 \mu\text{IU/ml}$) and higher HOMA index (2.38 ± 1.63) for insulin resistance were associated with +276G/T variant, and SNP +45T/G was associated with lower insulin levels ($6.30 \pm 2.13 \mu\text{IU/ml}$) and HOMA-IR (1.50 ± 0.48). No association with plasma lipid levels was observed. Antonio Petrone et al. [27] showed the +45G carriers had higher fasting and 2-hour glucose levels ($p = 0.01$ for both) and lower adiponectin levels ($p = 0.02$). Many studies stated the reduced circulating adiponectin levels when +45T/G and/or +276G/T are present in AdipoQ gene [28, 29] and higher levels of circulating adiponectin are associated with a lower risk of type 2 diabetes, independently of intra-

abdominal fat deposition, and that adiponectin may confer a benefit in both persons with and without insulin resistance [30].

Analysis of +45T/G & +276G/T SNPs association with phenotypic traits was done considering dominant genetic model for both variants. In type 2 diabetics, carriers (T/G + G/G) for +45T/G variant demonstrated higher BMI, fasting blood glucose, fasting insulin, HOMA IR index, triglycerides & VLDL cholesterol levels when compared with the wild type (TT), while lower level of HDL cholesterol was found in carriers than the wild type. The heterozygous and homozygous (G/T + T/T) for the +276G/T variant shows no difference in all phenotypic traits except for HDL cholesterol, which was decreased in compared with wild type (GG). Since, total cholesterol, triglycerides, LDL, HDL & VLDL cholesterol are the independent risk factors for cardiovascular diseases, results of present study indicate the association of these variants with increased risk of cardiovascular diseases via the increased levels of lipids and lipoproteins, which may be due to the presence of +45 and/or +276 variant in adiponectin gene lowering the activity of the hormone.

In the present study the logistic regression analysis of +45T/G with the biochemical parameters, indicated the presence of G allele at position 45 of adiponectin gene is associated with increased total cholesterol, & LDL cholesterol and decrease in HDL cholesterol. While only decrease in HDL cholesterol was associated with presence of T allele at position +276 of adiponectin gene. In another

study [24], the association of GT and TT genotypes with fasting plasma glucose concentrations was shown than those of the GG genotype of +276G/T variant. Maurizio Cesari et al. [31] in a series of 60 pairs of healthy twins, found significant genetic variance and heritability, independent of BMI and partly accounted for by the +45 T/G.

Haplotype analysis revealed that subjects having mutant haplotype for both SNPs i.e. +45T/+276G (GT) had significant role in development of insulin resistance than did non-carriers of this haplotype [10]. In a previous study haplotype analysis for these common variants, indicated homozygotes for the haplotype had higher body weight, waist circumference, systolic and diastolic blood pressure, fasting glucose and insulin levels, HOMA IR index, and total to HDL cholesterol ratio. They concluded that the variability at the adiponectin locus at these positions is associated with obesity and other features of the insulin resistance syndrome [6].

Conclusion

The G allele of variant +45T/G of the adiponectin gene is responsible to increase the risk of insulin resistance and type 2 diabetes mellitus and may take part in development and progression of cardiovascular diseases. While T allele of +276 of adiponectin gene may take part in the progression of type 2 diabetes mellitus but not insulin resistance. The haplotype analysis suggests the increased risk of cardiovascular diseases, with the presence of G allele of +45T/G along with +276 variant alleles.

Compliance with Ethical Standards

This study was not funded by any funding agency.

Conflict of Interest Author A. A. Momin declares that he has no conflict of interest. Author M. P. Bankar declares that he has no conflict of interest. Author G. M. Bhoite declares that she has no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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