

# Association of ADIPOQ Gene Variant rs266729 with Circulatory Adiponectin Levels in Patients with Type 2 Diabetes in North Indian Population: A Case-Control Study

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## ABSTRACT

Adiponectin gene (ADIPOQ) has association with circulatory adiponectin levels, metabolic disorders and type 2 diabetes mellitus (T2DM) in different population. We aimed to evaluate the relationship between SNP rs266729 in promoter region of ADIPOQ gene with T2DM patients, circulatory adiponectin levels and other clinical and anthropometric parameters in North Indian adult population. This case-control study was conducted on 300 subjects (150 T2DM and 150 healthy controls), aged between 25-75 years. Biochemical investigations performed included fasting and post prandial blood sugar level, lipid profile and serum creatinine. Glycated haemoglobin (HbA1c) and circulatory adiponectin levels were assayed using commercially available kits. PCR-RFLP method was used for genotyping. Mean levels of various anthropometric and biochemical parameters were significantly higher in T2DM than healthy controls ( $p < 0.001$ ). The levels of circulatory adiponectin was found significantly lower in T2DM as compared to healthy controls ( $p = 0.014$ ). There was no significant association of CC and CG genotypes with T2DM patients ( $p = 0.81$ ). C and G allele frequencies of the rs266729 were also not significantly associated with T2DM cases as compared to healthy controls ( $p = 0.23$ ). It was observed that significant impact on circulatory adiponectin levels for rs266729 polymorphism with GG genotype having very low circulatory adiponectin level ( $p < 0.001$ ). There was no significant association of CC, CG genotype and C, G allele frequencies of rs266729 in with T2DM cases as compared to healthy controls. However, it was observed that GG genotype of rs266729 has significant impact on circulatory adiponectin levels in T2DM cases. The GG carrier females have two fold increased risk for diabetes than men.

**Keywords:** ADIPOQ gene, SNP rs266729, Type 2 Diabetes Mellitus, Adiponectin.

## INTRODUCTION

Diabetes is one of the most challenging non-communicable disease in the current century worldwide. T2DM, represents 90–95% of the total diabetes cases, ranging from predominantly insulin resistance, insulin deficiency to insulin secretory

defect<sup>1</sup>. 422 million people are affected worldwide<sup>2</sup> and its prevalence is increasing rapidly because of population and surge of obesity in many countries including India<sup>3</sup>. Prevalence of diabetes in total is 7.8%, while in males 7.9% and females 7.5% in Indian population<sup>2</sup>.

The exact pathogenesis of T2DM is unclear, it is generally accepted that T2DM is a multifactorial disorder resulting from genetic polymorphisms and several environmental factors.<sup>(4)</sup> Genome Wide Association Studies (GWAS) showed that T2DM has strong genetic link and many genes are responsible to develop T2DM and overt its complications also<sup>5</sup>. GWAS among European and Asian populations indentified ADIPOQ locus as the major gene for variation in the serum adiponectin levels<sup>6</sup>. The most widely studied adiponectin gene (ADIPOQ) has direct and indirect association with obesity, insulin resistance and metabolic traits that contributes in development of T2DM<sup>7, 8</sup>.

Adiponectin is an adipokine protein and secreted from adipocyte cells which plays a major role in regulating blood glucose levels, insulin sensitivity, and lipid metabolism<sup>9</sup>. Adiponectin levels differ according to age, sex, and body mass index with lower levels in obese individuals<sup>10</sup>. Its level is low in T2DM<sup>11</sup>. Consequently, low levels of plasma adiponectin might play a role in the etiology of insulin resistance and T2DM. There is growing evidence demonstrating the association of single nucleotide polymorphisms (SNPs) of the ADIPOQ gene with varying levels of circulating adiponectin. SNP rs266729 (-11377 C > G) and rs1501299 (+276 G > T) in the proximal promoter of ADIPOQ gene has been widely studied. This allelic variant is associated with lower adiponectin levels, has also been shown to be related with obesity<sup>12</sup>.

A functional single nucleotide polymorphism (SNP), rs266729, in the promoter region of the ADIPOQ gene causes an amino acid change leading to replacement of cytosine with guanine at nucleotide position -11377 (-11377C > G) and is associated with T2DM<sup>13</sup>. Although the G allele of the ADIPOQ rs266729 SNP may appear important in associations with T2DM risk in various populations<sup>14, 15</sup> yet genetic evidence of its effect on T2DM has been ambiguous.

Thus, these uncertain results motivated us to carry on a replication study and evaluate the relationship between SNP rs266729 in promoter region of ADIPOQ gene with T2DM in North Indian adult population. We also estimated the potential

effects of its association with circulatory adiponectin levels, other clinical and anthropometric parameters. Clinical variables of T2DM was also assessed according to adiponectin gene rs266729 genotypes distribution.

## MATERIALS AND METHODS

### Subject Selection

This is a case-control study and all subjects (T2DM and healthy controls) were enrolled from outpatients Diabetes Clinic of IIMS&R, Integral University, Lucknow (India) and King George's Medical University, Lucknow (India). Study was approved by ethical committee of the institution. Written informed consent was taken from each subject and all procedures performed in this study involving human participants were in accordance with the ethical standards of this university and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards<sup>16</sup>.

This study was conducted on 300 subjects (150 T2DM and 150 healthy controls), aged between 25-75 years. T2DM was defined according to the criteria provided by World Health Organisation (2006): fasting blood sugar  $\geq 126$ mg/dl or 2 hour post-prandial blood glucose load  $\geq 200$ mg/dl. Subjects with ischemic heart disease, angina, myocardial infarction (MI), and electrocardiogram abnormalities, those with other concurrent sickness like chronic liver disease, hypothyroidism or those on drugs like diuretics, pregnant women and women using oral contraceptives were excluded from the study.

### Laboratory investigations

5ml venous blood was taken from each subject in EDTA, fluoride and plain vials after overnight fasting for laboratory investigation. Investigations performed included fasting and post prandial blood sugar, lipid profile and serum creatinine using of Vitros 250, Johnson & Johnson fully automated analyser and HbA1c by high performance liquid chromatography of Bio-Rad D10 analyser.

### Estimation of serum adiponectin

Serum levels of adiponectin were evaluated using standard commercially available

ELISA kit (USCN, Life Science Inc. Wuhan). The test was conducted in duplicate and as per manufacturer protocol. The inter-assay variation of adiponectin was  $CV < 12\%$  and intra-assay variation was  $CV < 10\%$ . The detection limit of the assay was 0.156-10ng/ml, and its sensitivity; the minimum detectable dose of human ADP is typically less than 0.065ng/ml).

#### Anthropometric parameters

Age, gender, systolic and diastolic blood pressure (SBP and DBP respectively), body mass index (BMI), waist circumference (WC), waist hip ratio (WHR) were recorded as per standard protocol<sup>17</sup>.

#### DNA extraction and Genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using salting out method<sup>18</sup>. Isolated genomic DNA was quantified by spectrophotometer (Systronics-2205). Agarose gel electrophoresis was done to confirm the purity of isolated DNA containing 10 $\mu$ g/ml ethidium bromide and was observed under Gel Dock system (Bio-Rad, Gel Doc XR+, Universal Hood II).

PCR-RFLP method was used for genotyping. Primers were designed using Primer3 software. The ADIPOQ rs266729 (-11377C>G) polymorphic locus was amplified using the forward primer, 5'-ACTTGCCCTGCCTCTGTCTG-3' and the reverse primer, 5'-CCTGGAGAACTGGAAGCTG-3'<sup>19</sup>. Amplifications were performed using a thermal cycler (Applied Biosystems, US). Each PCR reaction consisted of a total of 15 $\mu$ l containing 100 ng template DNA, buffer (100mM Tris, pH 9.0; 500mM KCl; 0.1% gelatin), 200 $\mu$ M dNTP, 10 pmol of each primers and 1.0 unit Taq DNA polymerase. PCR reaction conditions, after an initial step of 5 min at 94°C, followed by 35 cycles of 30s at 94°C, 30s at 58°C, 30s at 72 °C, and a final extension step at 72°C for 7 min. PCR products were verified on 2% agarose gel containing 10 $\mu$ g/ml ethidium bromide and visualized by UV light. Following PCR, the products were digested with 10U of HhaI enzyme (New England Biolabs, UK), at 37°C for overnight. The restriction fragments of PCR products were separated on a 2.5% agarose gel. 50bp DNA ladder was included in each run.

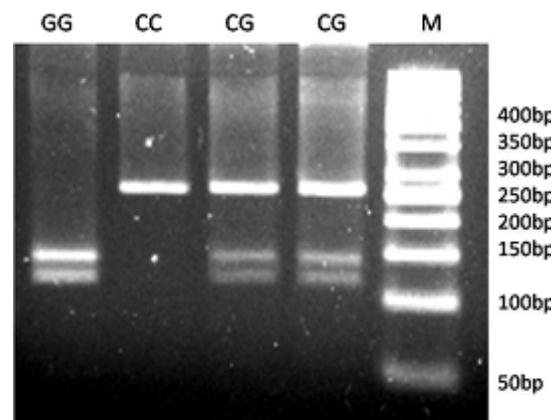
#### Statistical Analysis

All the statistical analysis was done using SPSS software version 20.0 (Armonk, NY, USA). All the phenotypic data were compared by using ANOVA or unpaired t-test. Values were given as mean  $\pm$  SD (Standard Deviation). Allelic and genotypic frequencies were presented with 95% confidence interval (CI) and were analyzed using  $\chi^2$  test. Genotypes of ADIPOQ rs266729 (-11377C>G) were tested for Hardy–Weinberg equilibrium. A p value  $< 0.05$  was considered as statistically significant for all the data analyzed.

## RESULTS

#### Anthropometric and Clinical Characteristics

A total of 300 subjects were enrolled for this case control study (150 T2DM and 150 healthy controls). Age and gender were matched between the cases and control groups ( $p > 0.05$ ). Of these, 61.3% were males and 38.7% were females. The anthropometric and biochemical profile of both the groups are given in Table 1. Mean levels of anthropometric parameters i.e. WC, WHR, SBP and DBP were significantly higher in T2DM cases whereas BMI remained indifferent between the two groups. We found gender specific differences for WC and WHR, male had significantly more WC and WHR. Similarly, biochemical parameters such as blood sugar, HbA1c, SCr were also significantly raised in T2DM cases as compared to healthy controls ( $p < 0.001$ ). There was significant elevation



**Fig. 1: Genotyping result for adiponectin gene rs266729. M: Marker, CC genotype: 250bp, CG genotype: 250/138/112bp, and GG genotype: 138/112bp.**

in triglyceride and VLDL levels in T2DM cases as compared to healthy controls ( $p=0.002$ ,  $p=0.003$ , respectively). The levels of circulatory adiponectin was found significantly lower in T2DM as compared to healthy controls ( $p=0.014$ ), (Table 1).

### Genotypes and Alleles Distribution

Fragment of 250bp was detected for the CC homozygote wild type (absence of HhaI restriction site). Fragments of 138bp and 112bp were detected for the GG mutant homozygote (presence of HhaI restriction site). The CG heterozygous contained the three fragments of 250bp, 138bp and 112bp (Fig. 1).

The genotype and allele frequencies of the rs266729 promoter region ADIPOQ gene polymorphism in T2DM patients and healthy

controls are shown in Table 2. The frequencies of the CC, CG and GG genotypes of rs266729 were 52%, 36.7%, 11.3% in T2DM cases and 56.7%, 36.7%, 6.6% in healthy controls respectively. The allele frequencies of the C and G were 70.3%, 29.7% in T2DM and 75%, 25% in healthy controls respectively. There were no significant association of homozygous CC and heterozygous CG genotype with T2DM patients (OR: 0.92; CI: 0.57-1.49;  $p=0.81$ ) and C, G allele frequencies of the rs266729 had no significant association with T2DM cases as compared to healthy controls (OR: 0.79; CI: 0.55-1.13;  $p=0.23$ ). We also analysed the dominant genotype (CC vs. CG+GG) and found no significant difference between T2DM cases and healthy controls (OR: 0.83; CI: 0.53-1.31;  $p=0.49$ ). Similarly, the recessive genotype (CG+CC vs. GG) did not show significant difference between T2DM

**Table 1: Clinical and Anthropometric parameters of Case (T2DM) and Control Groups**

Parameters		Case(n=150)	Control(n=150)	P-value
AGE (years)		48.31±10.88	48.03±11.83	0.83
Gender (M/F)		97/53	87/63	0.28
BMI (kg/m <sup>2</sup> )		24.96±4.68	24.73±4.74	0.67
Waist circumference(cm)	Male	99.46±5.49	95.18±6.16	<0.001*
	Female	95.13±7.32	96.57±8.58	0.34
Waist-Hip Ratio(WHR)	Male	0.99±0.06	0.95±0.06	<0.001*
	Female	0.95±0.07	0.97±0.09	0.19
SBP (mmHg)		140.75±26.65	114.45±7.52	<0.001*
DBP (mmHg)		82.39±15.67	70.21±8.69	<0.001*
FBS (mg/dl)		167.01±73.41	93.83±11.47	<0.001*
PPBS (mg/dl)		260.59±112.52	127.29±24.41	<0.001*
HbA1c (%)		8.01±2.09	5.30±0.72	<0.001*
Total Cholesterol (mg/dl)		167.81±53.94	157.73±46.27	0.083
Triglyceride (mg/dl)		182.27±112.76	148.35±68.36	0.002*
HDL (mg/dl)	Male	37.39±10.48	38.91±11.34	0.34
	Female	39.39±12.61	39.58±11.11	0.93
LDL (mg/dl)		92.59±47.59	91.15±47.11	0.79
VLDL (mg/dl)		36.14±22.57	29.67±13.72	0.003*
Serum Creatinine (mg/dl)		2.44±2.11	0.91±0.25	<0.001*
Adiponectin (µ74g/ml)		1.82±1.05	2.22±1.68	0.014*

Values are expressed as Mean ± Standard Deviation

\*Significant considered as  $P<0.05$ .

M: Male, F: Female, FBS: Fasting Blood Sugar, PPBS: Post-Prandial Blood Sugar, HbA1c: Glycated Haemoglobin, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Sugar, BMI: Body Mass Index, WC: Waist Circumference, TC: Total Cholesterol, TG: Triglyceride, HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein, VLDL: Very Low-Density Lipoprotein,

**Table 2: Genotypes and Allele Distribution of Adiponectin rs266729 Gene Polymorphism in Case (T2DM) and Control Groups**

rs266729 Polymorphism	Case N (%)	Control N (%)	OR (95% CI)	P-value
Co dominant				
CC	78 (52%)	85 (56.7%)	1.00	-
CG	55 (36.7%)	55 (36.7%)	0.92 (0.57-1.49)	0.81
GG	17 (11.3%)	10 (6.6%)	0.54 (0.23-1.25)	0.21
Dominant				
CC	78 (52%)	85 (56.7%)	1.00	-
CG+GG	72 (48%)	65 (43.3%)	0.83 (0.53-1.31)	0.49
Recessive				
CG+CC	133 (88.7%)	140 (93.4%)	1.00	-
GG	17 (11.3%)	10 (6.6%)	0.56 (0.25-1.26)	0.23
Alleles				
C	211 (70.3%)	225 (75%)	1.00	-
G	89 (29.7%)	75 (25%)	0.79 (0.55-1.13)	0.23

Values are expressed as Number (N) and Percentage (%)

OR: Odd Ratio, CI: Confidence Interval

Significant considered as P<0.05.

**Table 3: Clinical variables of Cases (T2DM) according to adiponectin gene rs266729 genotypes**

Genotypes→ Variables↓	CC(n=78)	CG (n=55)	GG (n=17)	P-value
FBS (mg/dl)	167.99±78.09	167.36±73.71	161.37±44.29	0.94
PPBS (mg/dl)	264.26±123.70	261.64±106.17	240.31±67.52	0.73
HbA1c (%)	8.14±2.23	7.85±1.97	7.95±1.72	0.73
TC (mg/dl)	172.23±56.84	163.61±52.33	161.11±42.35	0.57
TG (mg/dl)	177.27±105.20	192.93±130.13	170.72±77.64	0.66
HDL (mg/dl)	39.75±11.20	38.55±13.09	37.75±9.77	0.75
LDL (mg/dl)	97.32±50.90	86.91±44.40	89.24±38.71	0.44
VLDL (mg/dl)	34.89±21.05	38.54±26.02	34.09±15.51	0.61
Serum Creatinine (mg/dl)	2.22±1.55	2.53±1.63	2.57±2.18	0.49
Adiponectin (µg/ml)	1.97±1.05	1.97±0.99	0.71±0.36	<0.001*

Values are expressed as Mean ± Standard Deviation

\*Significant considered as P<0.05.

FBS: Fasting Blood Sugar, PPBS: Post-Prandial Blood Sugar, HbA1c: Glycated Haemoglobin, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Sugar, BMI: Body Mass Index, WC: Waist Circumference, TC: Total Cholesterol, TG: Triglyceride, HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein, VLDL: Very Low-Density Lipoprotein

cases and healthy controls (OR: 0.56; CI: 0.25-1.26; p=0.23).

### Impact of Genotypes Distribution on Clinical Characteristics

Clinical characteristics of the T2DM cases according to adiponectin rs266729 genotypes are shown in Table 3. There was significant impact of rs266729 polymorphism on circulatory adiponectin level (p<0.001). Serum adiponectin level was significantly lower in GG genotype as compared to CC & CG genotypes. Serum creatinine levels were also gradually raised from 14% to 16% for rs266729 genotypes CG and GG, respectively as compared to CC, but it was not significantly raised.

Study of SNP rs266729 of ADIPOQ gene with gender distribution showed that GG genotype was almost two folds higher in females (12.9%) than males (6.5%), but it was not significantly associated (Table 4).

A multiple linear regression was performed so as to find the predictor of SNP rs266729. Model showed adiponectin as the only strong predictor ( $\beta = -0.233$ , p=0.000, CI: -0.15 to -0.05), (Table 5).

Receiver operating characteristics (ROC) curve of adiponectin illustrated that area under curve was 0.446. When we take the cut-off value of adiponectin as 1.7 $\mu$ g/ml (sensitivity 48% and specificity 50%), then 52% T2DM patients have adiponectin levels below 1.7 $\mu$ g/ml.

### DISCUSSION

In present study, we investigated the possible association of ADIPOQ gene (rs266729 SNP) with levels of adiponectin and other clinical and anthropometric parameters in patients with T2DM in North India population. The baseline characteristics of clinical parameters i.e. blood sugar, HbA1c, SCr, triglyceride and VLDL were significantly raised while serum adiponectin level was significantly lower in T2DM cases as compared to healthy controls. Similarly, anthropometric parameters i.e. WC, WHR, SBP and DBP were significantly higher in T2DM cases than healthy controls which are consistent with other reports from various ethnic groups<sup>20, 21</sup>. Studies have also reported that serum adiponectin level was lower in T2DM patients than healthy controls and hypoadiponectinemia was strongly associated with

**Table 4: Genotype and Allele frequency distribution of SNP rs266729 of ADIPOQ gene in Genders (M/F) of T2DM and controls**

rs266729 Polymorphism	Male (M)N (%)	Female (F)N (%)	OR (95% CI)	P-value
Co dominant				
CC	101 (54.9%)	62 (53.4%)	1.00	-
CG	71 (38.6%)	39 (33.6%)	0.89 (0.54-1.48)	0.76
GG	12 (6.5%)	15 (12.9%)	2.04 (0.89-4.64)	0.13
Dominant				
CC	101 (54.9%)	62 (53.4%)	1.00	-
CG+GG	83 (45.1%)	54 (46.6%)	1.06 (0.66-1.69)	0.90
Recessive				
CG+CC	172 (93.5%)	101 (87.1%)	1.00	-
GG	12 (6.5%)	15 (12.9%)	2.13 (0.96-4.73)	0.09
Alleles				
C	273 (74.2%)	163 (70.3%)	1.00	-
G	95 (25.8%)	69 (29.7%)	1.22 (0.84-1.75)	0.34

Values are expressed as Number (N) and Percentage (%)

OR: Odd Ratio, CI: Confidence Interval

Significant considered as P<0.05



T2DM, insulin resistance, obesity and other metabolic diseases<sup>22, 23</sup>. wild type<sup>26</sup>.

The frequencies of the CC, CG and GG genotypes of SNP rs266729 of ADIPOQ gene, had no significant differences in the homozygous CC and heterozygous CG genotypes in T2DM patients and healthy controls. We did not find any association between SNP rs266729 with C and G allele. Other research groups also found similar genotypic and allelic frequencies<sup>15, 24, 25</sup>. Whereas, a study on Iraqi population reported that the frequency of the G allele of rs266729 (C/G) polymorphism was significantly higher in diabetic subjects (28%) compared to that in normal subjects (14%). The homozygous genotype (GG) significantly increased the risk of T2DM by three folds and the heterozygous CG genotype significantly raised the risk of T2DM by two folds with respect to those of

SNP rs266729 of ADIPOQ gene demonstrated that it has a significant association with lower circulatory adiponectin levels in T2DM and we also found that GG genotype of SNP rs266729 was significantly associated with lower adiponectin levels in T2DM than healthy control. About half of the T2DM patients had adiponectin level below 1.7µg/ml in our diabetic group. Contrary results were reported by other previously published reports in different population<sup>14, 27</sup> as well as Indian population<sup>25</sup>. A study from US on multi-ethnic population reported that G allele was significantly associated with hypoadiponectinemia but there was no significant association of this SNP with adiponectin<sup>27</sup>. A very recent study on Chinese population also observed no significant association of this polymorphism with circulatory adiponectin level<sup>15</sup> but various other Asian studies support our

**Table 5: Multiple linear regression analysis to show the dependence of SNP rs266729 of ADIPOQ gene on study parameters in T2DM cases (N=150)**

Model	Unstand- ardized B	Coefficients Std. Error	Standardized Coefficients Beta	t	p-value	95% Confidence Interval for B Range (CI)	
(Constant)	-1.674	0.604		2.770	0.006	0.484	2.863
HbA1c	-0.010	0.028	-0.031	-0.351	0.726	-0.064	0.045
FBS	0.002	0.001	0.164	1.227	0.221	-0.01	0.004
PPBS	0.000	0.001	-0.147	-1.083	0.280	-0.003	0.001
TC	0.000	0.002	-0.033	-0.212	0.832	-0.004	0.004
TG	-0.001	0.004	-0.161	-0.267	0.790	-0.009	0.007
HDL	0.000	0.004	-0.010	-0.153	0.879	-0.008	0.007
LDL	0.000	0.002	-0.031	-0.228	0.820	-0.004	0.003
VLDL	0.010	0.022	0.276	0.442	0.659	-0.033	0.052
BMI	-0.005	0.011	-0.039	-0.449	0.618	-0.027	0.016
WHR	0.354	0.727	0.038	0.486	0.627	-1.078	1.786
SCr	0.038	0.025	0.099	1.505	0.133	-0.012	0.088
ADP	-0.106	0.027	-0.233	-4.014	0.000*	-0.159	-0.054
SBP	7.007E-5	0.003	0.003	0.028	0.978	-0.005	0.005
DBP	-0.002	0.004	-0.050	-0.565	0.573	-0.010	0.006

Dependent variable: SNP rs266729 of ADIPOQ gene

\* p<0.05 is considered significant at 95% confidence interval

SBP – systolic blood pressure, DBP – diastolic blood pressure, FBS - Fasting Blood Sugar; Post - Prandial Blood Sugar; HbA1c - glycated haemoglobin; TC – total cholesterol, HDL - High-Density Lipoprotein; LDL - Low-Density Lipoprotein; VLDL - Very Low-Density Lipoprotein, SCr – serum creatinine, WHR – waist hip ratio, ADP- Adiponectin

results<sup>25, 26, 28, 29</sup>. Serum creatinine levels were also gradually raised for rs266729 genotypes CG and GG, respectively as compared to CC. The higher serum creatinine levels were reported in diabetic and diabetic nephropathy patients who carry GG genotype and have higher frequency of G allele in various ethnic populations<sup>30</sup>. HbA1c was significantly elevated in T2DM as compared to healthy controls, but HbA1c was not associated by SNP rs266729 of ADIPOQ gene. A recent study observed that serum adiponectin was decreased in T2DM and negatively correlated with HbA1c<sup>22</sup>. Adiponectin was significantly associated with altered glucose metabolism and independently contributed to the variance of HbA1c in a population<sup>31</sup>.

In our study, T2DM patients have higher WC, WHR, SBP, DBP, TG, FBS and PPBS than healthy controls. A study from UK reported that the C11377G SNP in the adiponectin gene promoter is associated with blood pressure and waist-hip ratio. Replacing C by G caused an increase in DBP by 1.63%, SBP by 1.83% and WHR by 1.61%, per gene copy of which DBP and WHR had significant association<sup>32</sup>. However, a meta-analysis did not find any association of the ADIPOQ polymorphism (rs266729) with blood lipids and blood pressure in East Asian population<sup>33</sup>. Adiponectin has shown a negative correlation to SBP and DBP levels in healthy control<sup>34</sup>. Adiponectin level was positively associated with HDL cholesterol and negatively associated with BMI, WHR, TG, FBS, fasting insulin, SBP and DBP. The rs266729 minor G allele was associated with lower adiponectin<sup>27</sup>. Contrary, Adiponectin levels were inversely correlated with WC, WHR, weight, BMI and physical activity, but were less influenced by the polymorphisms of ADIPOQ studied<sup>35</sup>. Lipid profile was correlated with circulatory adiponectin level<sup>36</sup>. A significant association was observed for gender with diabetes. Significantly higher WC and WHR were found in diabetic males of our study group. Results showed that SNP rs266729 was significantly associated with body weight, WC, BMI and percentage of total body fat<sup>37</sup>.

Genotypic analysis showed that GG genotype was more common in females (12.9%) as compare to male (6.5%). Though it was not

significant yet it may double the risk for diabetes in females as compare to males. Zhong et al. studied rs266729 SNP and found no significant difference in allelic frequency between the two genders but they observed that females with G allele at rs266729 had higher risk for coronary artery disease compared to females with C allele<sup>38</sup>.

## CONCLUSION

There was no significant association of CC, CG genotype and C, G allele frequencies of the rs266729 with T2DM cases as compared to healthy controls. However, it was observed that GG genotype of rs266729 has significant impact on circulatory adiponectin levels in T2DM cases. GG genotype was more prevalent in females than males in T2DM cases in our study group. Therefore we can say that GG carrier females have two fold increased risk for diabetes than men and subjects with adiponectin below 1.7 µg/ml doubles the risk for developing the T2DM.

## Recommendations

We have studied one SNP of promoter region of ADIPO gene. Haplotypes and block study with another SNP of promoter region of ADIPOQ gene are warranted in this population. Linkage disequilibrium of this SNP with various variants of ADIPOQ gene is also needed. Our sample size might be smaller to validate this SNP. Study should be replicated in larger sample size of this population.

## Key messages

Lower circulatory adiponectin and higher serum creatinine were observed. GG genotype and G allele frequency are strongly associated with hypoadiponectinemia in T2DM patients. Most of the T2DM patients have hypoglycaemia, hypertension and hypoadiponectinemia which are leading to cause CKD, CVD and other comorbidities of metabolic diseases.

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