

Adiponectin Gene Polymorphism and the Incidence of Type 2 Diabetes Mellitus in Obese Patients in Qassim Region, Saudi Arabia

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Abstract : Obesity poses a global health care problem and is considered a major risk factor in the development of type 2 diabetes (type2DM). In the Gulf States, diabetes is reported to be an epidemic with a high prevalence in the Kingdom of Saudi Arabia (KSA) . The roles of adipose tissue and obesity are of paramount significance via secretion of adiponectin hormone , the protein product of the *APM1* gene. Adiponectin has been claimed to be associated with obesity and insulin resistance. However, contrasting results have been emerged on the genetic variability in *APM1* and characteristics of the metabolic syndrome and adiponectin plasma levels. **Objectives:** Our aim is to determine the frequency of 276G→T of the adiponectin gene and plasma adiponectin levels in obese patients with and without type 2 DM to identify the effects of this polymorphism on insulin sensitivity , type 2 DM and obesity in Saudi Society mainly in Qassim region . **Subjects:** 120 volunteers were included and divided into: **Group I** :40 healthy volunteers . **Group II** : 40 obese patients not suffered from type 2 DM . **Group III** : 40 obese patients suffering from type 2 DM . **Methods:** Fasting blood samples were collected for routine and research investigations. ELISA technique was used to estimate plasma adiponectin levels, Polymerase chain reaction (PCR) assay with restriction fragment length polymorphism (RFLP) were used to examine the adiponectin gene SNP276 G>T polymorphism . **Results:** There was a significant association of the T allele frequency in group II and group III patients when compared to controls ($X^2=12.86$, $P=0.000$), ($X^2=36.95$, $P=0.000$) respectively with more significant increase in group III when compared to group II ($X^2=8.052$, $P=0.005$) .The Fasting blood glucose (FBG) , plasma insulin levels and HOMA index were significantly higher in patients carrying the TT than in GG carriers of each group II and III ($P<0.05$). In group III patients, carriers of TT genotypes having a significant decrease in plasma adiponectin levels than GG carriers ($P<0.05$). **Conclusion :** Obese and diabetic patients had lower plasma adiponectin levels than healthy controls. It was the T allele and TT genotypes of 276G>T SNP that was associated with lower plasma adiponectin , higher risk of obesity , Insulin resistance and higher parameters of metabolic syndrome and type 2 DM.

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Introduction

According to the World Health Organization's Regional Office for the Eastern Mediterranean (WHO EMRO), obesity poses an emerging global health care problem and is considered a major risk factor in the development of diabetes and cardiovascular disease.⁽¹⁾ Presently, more than 16 million people are living with diabetes in the Eastern Mediterranean Region, which is expected to rise to almost 43 million by the year 2025.⁽²⁾ Today, more than 1.1 billion adults are overweight worldwide, and among them 312 million are considered to be obese.⁽²⁾ Diabetes has reached near-epidemic proportions in the Saudi Arabia ,the

prevalence is expected to rise to between 40-50% by 2020⁽³⁾. Insulin resistance, adipose tissue and obesity are important factors in the development of type 2 DM.⁽⁴⁾

Adipose tissue has emerged as an important endocrine organ regulating whole-body metabolism and other vital functions related to inflammation and immune responses⁽⁵⁾. These actions are mediated by a number of molecules that are secreted by adipocytes such as leptin, adiponectin, tumor necrosis factor (TNF)- α , resistin, and adiponectin⁽⁶⁾. In concert, these cytokines, or adipokines, are believed to adapt metabolic fluxes to the amount of stored energy⁽⁷⁾. Dysregulation of this

network has been implicated in the etiology of insulin resistance and other components of the insulin resistance syndrome (syndrome X), such as glucose intolerance, obesity, dyslipidemia, and high blood pressure.⁽⁸⁾

Adiponectin is a novel adipocyte-derived hormone (adipoQ) secreted by adipose tissue but present at low levels in obesity, is now widely recognized as a key determinant of insulin sensitivity and of protection against obesity-associated metabolic syndrome⁽⁹⁾.

The adiponectin gene is very polymorphic and several of its variants contribute to adiponectin level, function and are associated with metabolic syndrome phenotypes.⁽¹⁰⁾

Genome-wide scan studies have mapped diabetes susceptibility locus on chromosome 3q27, where the adiponectin gene is located.⁽¹¹⁾ It is interesting that in obesity, plasma adiponectin levels are significantly decreased⁽¹²⁾ and adiponectin seems to protect from insulin resistance and type 2 diabetes⁽¹³⁾.

Several researches explored potential associations of two single nucleotide polymorphisms (SNPs) in the adiponectin gene (*ADIPOQ*; +45T>G, and +276G>T) with circulating total and high-molecular weight adiponectin, insulin resistance (IR), and markers of obesity.^(14, 15, 16, 17, 18)

Even though several reports have shown an interaction between *ADIPOQ* SNPs and obesity⁽¹⁹⁻²⁰⁾, the mechanism is not yet known. However, possibly due to differences in metabolic or ethnic backgrounds of the participants in different studies, the results of previous genetic association studies are conflicting. In addition, most of the studies performed so far have been cross-sectional and have only included few SNPs in the region of *ADIPOQ*.⁽²¹⁾

One possible explanation might be that obesity with increased adipose tissue predispose individuals to altered adipocytokine levels (e.g., decreased adiponectin and increased tumor necrosis factor- α) and, thus, the effect of the adiponectin gene is more exaggerated in obesity. Another possibility is that lower baseline adiponectin levels in obesity might induce additional changes in adiponectin levels and amplify the diet-intervention effect⁽²¹⁾.

Subjects and Methods

This study was carried out in the Medical Laboratory department Qassim University, King Fahd Specialist Hospital (KFS), Qassim, Saudi Arabia and Medical Biochemistry department, Faculty of Medicine, Zagazig University Egypt during the period from July 2010 to Sep 2011. We studied the effect and the relationship of allelic variants of the adiponectin gene in Saudi obese patients with and without type 2 DM. A polymerase chain reaction (PCR) with restriction fragment length polymorphism (RFLP) was

used to detect gene polymorphism. All diabetic subjects were diagnosed according to World Health Organization (WHO) criteria on regular follow in diabetic Outpatients Clinics, King Fahd Specialist (KFS) Hospital. The duration of DM was determined by reviewing the clinical history and the medical records, they were suffered from Type 2 DM more than 10 years.

Subjects: 120 unrelated Saudi subjects of Qassim region have participated in the study. The subjects were recruited among the patients and the employees of the KFS Hospital Qassim region. All subjects provided a written informed consent to participate in this study. They were further divided into ;

Group I : 40 control subjects (16 females and 24 males). Their mean ages were ranged from 41.0 -67.0 years with a mean value \pm S.D of 54.52 ± 7.53 years. Who had been matched for BMI, sex, age and socioeconomic background.

They had no family history of diabetes and/or obesity. Exclusion criteria for control subjects were (1) the presence of a BMI >26 kg/m², (2) the presence of type 2 diabetes or of a first-degree relative with type 2 diabetes, and (3) the presence of Coronary Artery Disease (CAD). There was no statistical difference ($P>0.05$) in control group regarding age and sex.

Group II : included 40 patients (17 females and 23 males). Their mean ages were ranged from 43.0 – 65.0 years with a mean value \pm S.D of 51.62 ± 6.21 years. They were suffering from obesity without Type 2DM. All obese patients were selected on the basis of a body mass index (BMI) >26 kg/m². BMI was computed as weight (kg) divided by square height (m²).⁽²²⁾ Exclusion criteria were the presence of type 2 diabetes or of a first-degree relative with type 2 diabetes.

Group III: comprised 40 patients (19 females and 21 males). Their mean ages were ranged from 43.0 - 66.0 years with a mean value \pm S.D of 53.80 ± 6.52 years. They were suffering from Type 2 DM. Diagnosis of type 2 diabetes based on history of hypoglycemic treatment and/or confirmed FBG =or >126 mg/dl (7 mmol/l)⁽²²⁾. Type 2 diabetic subjects were selected on the basis of at least 5 years from diagnosis without insulin treatment, a BMI >26 kg/m², an age of diagnosis >50 years, and absence of concomitant autoimmune disease.

For all subjects, a complete medical history was obtained by questionnaire. History taking included questions about smoking habits, history of hypertension and type 2 diabetes, and current medication used.

There was no statistical difference ($P > 0.05$) between all groups regarding age and sex. Informed consent was obtained from all the participants in this study. The characteristics of all groups are tabled in table 1.

Methods :

All patients and control group were subjected to the following:

- I- Full history and clinical examination .
- II- Research investigations :
 - 1-Estimation of fasting blood glucose level by enzymatic method .⁽²³⁾
 - 2-Determination of glycated hemoglobin (HbA1c) in blood.⁽²⁴⁾
 - 3-Measurement of fasting insulin by ELISA. ⁽²⁵⁾
 - 4-Determination of insulin resistance by homeostasis model assessment (HOMA) index calculated by the following formula:⁽²⁶⁾

$$\text{HOMA index} = (\text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting plasma glucose (mg/dl)}) / 405.$$
 - 5-Total lipid profile levels :
 - Triglyceride will be determined enzymatically.⁽²⁷⁾
 - Total cholesterol will be estimated by enzymatic method.⁽²⁸⁾
 - Estimation of High Density Lipoprotein cholesterol (HDL-C) .⁽²⁹⁾
 - LDL-C concentration will be Calculated by the Friedewald formula .⁽³⁰⁾
 - 6-Determination of adiponectin gene **276 G>T** SNP by PCR-based restriction fragment length polymorphism (RFLP) .⁽³¹⁾
 - 7-Determination of plasma adiponectin levels by ELISA. ⁽³²⁾

Collection of samples:

Overnight Fasting venous blood samples were taken from every participant under complete aseptic condition in sterile EDTA containing tubes using standardized protocol and equipment., then the sample was divided into 2 portions :

- Plasma specimen was obtained by centrifugation at low speed centrifugation at $2500 \times g$; 15 min for estimating the adiponectin levels and other research investigations , were measured by standard chemical and enzymatic commercial methods in the Medical Laboratory department and KFS hospital laboratories.
- Whole blood was collected for DNA extraction and adiponectin gene detection , were done in Medical Biochemistry department , Faculty of Medicine , Zagazig University .
- Anthropometric examination (height and weight) for calculation the BMI was done for all the subjects .

DNA extraction and genotyping ⁽³¹⁾

DNA will be isolated using the DNA Isolation Kit ⁽³³⁾ in which standard DNA isolation from 500 μ l whole blood in standard 45 minutes protocol, including RNase treatment.

The average DNA concentration (127.49 ± 5.05 μ g/ml) will be determined from absorbance at 260 nm . All samples DNA will be examined at a 260/280 nm absorbance ratio .

The integrity of the DNA will be checked by electrophoresis on 1.5 % agarose gel with an ethidium bromide. Isolated DNA was used for determination of single nucleotide polymorphism (SNP) in the adiponectin gene (276 G>T).The SNP was detected by polymerase chain (Techne T-412, modelFTC 41F2D , UK Bibby Scientific LTD).

Detection details of SNP adiponectin 276 G>T.

forward primer* 5' – GGC CTC TTT CAT CAC AGA CC - 3' ⁽³⁴⁾

reverse primer* 5' – AGA TGC AGC AAA GCC AAA GT -3'

PCR conditions :95 °C/5min 1x /95 °C/60s 35x /58 °C/45s 35x/ 72 °C/45s 35x /72 °C/5min 1x

PCR product length 196 bp .Restriction enzyme Mva 1269I °RFLP conditions 37 °C/24h

Gel electrophoresis for PCR-digested products using 1.5 % agarose gel and 50x Tris-Acetate-EDTA (TAE) buffer ,ethidium bromide 5mg/ml was added to the agarose before pouring into the tray.

Sample preparation and loading:

Each digested product sample prepared and electrophoresed on 1.5% agarose gel as follows: To every 5 μ l of digested DNA product, 5 μ l of 6X gel loading dye (prepared by bromophenol blue 0.25% and sucrose 40% in 50 mM EDTA)was added .The electric current will be set at 100 mA & 70 volts for about 1-1.5 hours, and then the gel was visualized under UV transilluminator with 100 base pair ladder and photographed .

100 Base-Pair Ladder (Bioron) was 0.2 mg/ml in 10 Mm Tris (pH 8.0), 1mM EDTA.

Statistical analysis:

Results were statistically analyzed by SPSS 11.5 for Windows. The statistical data were calculated for mean and standard deviation (SD). Analysis of variance F test (ANOVA) was used to compare the results of all examined cases in all studied groups within each other. The differences between mean value for each element were tested by student's "t" test. Chi-square (X^2) test was used to compare qualitative variables. The odds ratio was used to assess the relative risk with a confidence interval (CI) at 95%. Results were

considered significant or non-significant when $P >$ or $<$ 0.05, respectively. ⁽³⁵⁾

Results:

In order to examine whether the adiponectin 276G>T SNP contribute to insulin resistance in obese subjects in qassim region of KSA, we genotyped 120 Saudi volunteers, 40 healthy controls and 80 patients suffered from obesity with and without type 2DM. After restriction digestion with restriction enzyme Mva 1269I^o, RFLP products length in presence G allele 148 a 48 bp in presence T allele 196 bp the G/T genotype resulted in 3 fragments of 196,148 and 48 bp.

Basic characteristics with ANOVA (F) test results of all different groups participated in this study are listed in table (1) :

Mean \pm SD of all biochemical parameters : age, sex, BMI, plasma insulin, FBG, HOMA index, HbAc1, plasma adiponectin and lipid profile data (TC, LDL-C, TG and HDL-C). ANOVA test revealed a significant difference of the mean values of all biochemical parameters level among different studied groups ($P=0.000$). Plasma adiponectin levels were significantly lower in group II and group III than control group ($t = -34.63$, $t = -22.168$, $P=0.000$) respectively with a significant decrease in group III than group II ($t = -61.53$, $P=0.000$). BMI was significantly increased in group II and group III more than control group ($t = -34.63$, $t = 17.56$, $P = 0.00$), respectively. There was a significant increase in BMI in group III than group II ($t = 2.58$, $P=0.014$).

Genotype and alleles frequencies for adiponectin 276 G>T are presented in table (2) and FIG(1):

-In controls (Group I): the G allele frequency was 81.25%, the T allele frequency was 18.75%. The observed level of heterozygosity GT was in 22.5%, the TT genotype was 7.5% and the GG genotype was the most common in controls (70%).

-In obese non diabetic patients (Group II): the homozygous GG was found in 22 patients (55%), the heterozygous GT in 12 patients (30%) and the homozygous TT in 6 patients (15%). Chi square (X^2) for group II = 2.149 with non-significant association when compared to the controls ($P = 0.342$). G allele frequency was 70% and T allele frequency was 30%, when compared to controls there a significant association ($X^2 = 12.86$, $P = 0.000$) (OR = 0.294 with 95% CI of 0.148- 0.583) table(3).

-In obese diabetic patients (Group III): the GG pattern was found in 9 patients (22.5%), the GT in 18

patients (45%) and the TT pattern in 13 patients (32.5%), there was a significant association when compared to the controls ($X^2 = 19.00$, $P=0.000$). G allele frequency was 45% and the T allele frequency is 55%, with significant association when compared to controls ($X^2 = 36.95$, $P = 0.000$) (OR = 0.130 with 95% CI of 0.065-0.260).

-When comparing between two obese patients with and without diabetes, there was a significant association of genotype frequency in obese diabetic patients when compared to the obese non-diabetic patients ($X^2 = 6.353$, $P = 0.042$). Frequency of T allele was more frequent in obese diabetic patients with significant association when compared to obese non-diabetics ($X^2 = 8.052$, $P = 0.005$ with OR = 0.442 with 95% CI of 0.250- 0.780).

-The relation between different biochemical parameters with genotype variants of adiponectin 276 G>T SNP in diseased groups I and II (table 3,4):

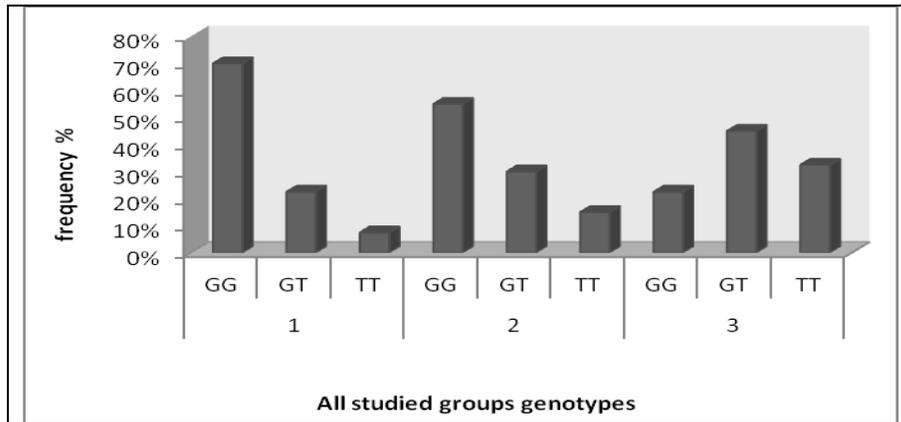
-There were no significant differences in terms of sex, age, BMI and lipid profile data (TC, LDL-C, TG and HDL-C) according to 276 G>T SNP different genotypes in group II and III patients ($P > 0.05$). However, FBG, plasma insulin levels and HOMA index were significantly higher in patients carrying the GT and TT than in GG carriers of each group II and III ($P < 0.05$). In group II patients we noticed a significant decrease in the plasma adiponectin levels in TT genotype carriers when compared with GG carriers ($t = 3.450$, $p = 0.018$). In group III patients, carriers of GT and TT genotypes having a significant decrease in plasma adiponectin levels than GG carriers ($P < 0.05$).

-The relationship between changes in plasma adiponectin level and metabolic parameters in all studied groups :

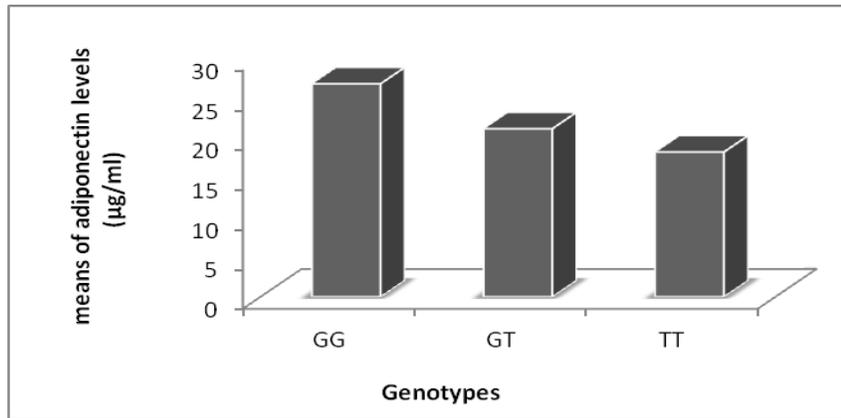
-Overall, changes in plasma adiponectin levels were negatively and significantly correlated with FBG levels ($r = -0.67$), insulin levels ($r = -0.73$), HOMA index ($r = -0.72$) (FIG .3), Hb Ac1 ($r = -0.66$), BMI ($r = -0.81$), TC ($r = -0.68$), LDL-C ($r = -0.64$) and TG ($r = -0.90$) with positive significant correlation with HDL-C ($r = 0.809$) with (p value = 0.000) respectively.

-The relationship between changes in BMI and metabolic parameters in all studied groups :

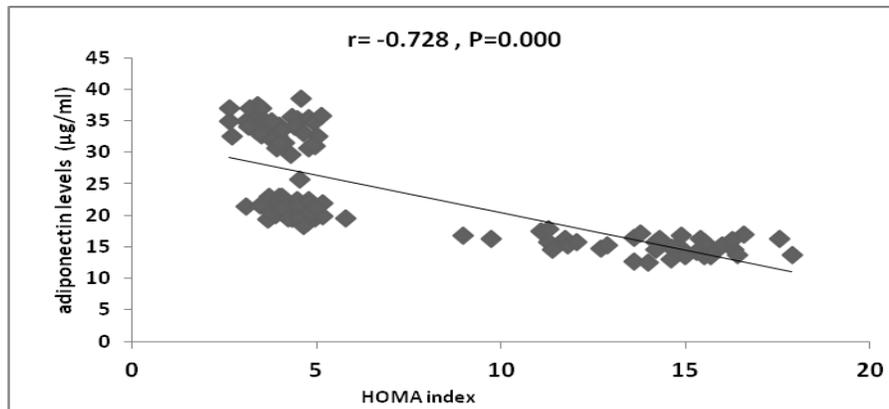
-There was a significant positive correlation between BMI changes and each of FBG ($r = 0.462$), Insulin levels ($r = 0.533$), HOMA-IR ($r = 0.532$), HbAc1 ($r = 0.482$), TC ($r = 0.572$), TG ($r = 0.776$) with significant negative correlation with each of plasma adiponectin ($r = -0.81$) and HDL-C ($r = -0.676$) $P = 0.000$ respectively.



FIG(1) :Frequency of adiponectin 276G>T genotypes in all studied groups .



FIG(2). Mean of plasma adiponectin levels in 276G>T genotypes in all studied groups .



FIG(3):The correlation between plasma adiponectin levels and HOMA index in all studied groups .

Table (1): Comparison of clinical and laboratory data of the all studied groups (mean \pm SD and range , with anova (F) test) .

	Group I	Group II	Group III	F value P value
Sex	-16 females -24 males	-17 females -23 males	-19 females -21 males	F=0.233 P=0.793
Age (years) Range	54.52 \pm 7.53 41.0 -67.0	51.62 \pm 6.21 43.0 - 65.0	53.80 \pm 6.52 43.0- 66.0	F=1.944 P=0.148
BMI (kg/m ²)	22.85 \pm 1.50 19.7 -25.3	31.95 \pm 3.52 25.7 -39.5	33.83 \pm 3.97 26.7 -40.5	F=135.94 P=0.000
Plasma adiponectin (μ g/ml)	33.92 \pm 2.053 29.60-38.60	20.98 \pm 1.41 18.40-25.60	15.23 \pm 1.30 12.50-17.80	F=1388.494 P=0.000
FBG (mg /dl)	93.85 \pm 9.6 68.0-107.0	95.23 \pm 9.82 68.0-115.0	140.15 \pm 12.99 123.0-192.00	F= 236.027 P=0.000
Insulin (μ U/L)	17.08 \pm 1.76 13.50-19.60	18.39 \pm 1.02 16.60-20.40	40.96 \pm 5.14 29.60-56.20	F=705.147 P=0.000
HOMA index	3.77 \pm 0.66 2.63-4.98	4.31 \pm 0.54 3.10-5.79	14.11 \pm 2.10 8.98-17.90	F=768.419 P=0.000
HbA _{1c}	6.62 \pm 1.00 4.2 -8.2	6.79 \pm 0.98 5.10 -8.2	10.9 \pm 1.09 8.9 - 13.4	F=223.768 P=0.000
Total chlolesterol (TC) (mg/dl)	188.50 \pm 36.48 110-226.00	229.5 \pm 7.60 213.0-240.0	242.01 \pm 14.633 214.0-301.0	F=58.711 P=0.000
Triglycerides TG (mg/dl)	108.18 \pm 7.55 99.5-132.0	213.9 \pm 28.82 123.0-245.0	224.78 \pm 6.11 213.0-234.0	F=537.909 P=0.000
HDL-C (mg/dl)	57.02 \pm 6.77 43-70.0	40.75 \pm 3.60 34.0-48.0	38.20 \pm 2.95 32.0-45.0	F=139.988 P=0.000
LDL-C(mg/dl)	114.52 \pm 40.21 24.32 -160.40	154.79 \pm 54.52 120.96-183.56	169.83 \pm 18.38 142.12-240.68	F= 46.914 P=0.000

Table (2) : Allele and genotype frequencies for adiponectin 276 G>T polymorphism in all studied groups.

Groups	Genotype frequency n (%)			Allele frequency n (%)	
	GG	GT	TT	G	T
Group I	28 (70%)	9 (22.5%)	3 (7.5%)	65 (81.25%)	15 (18.75%)
GroupII	22 (55%)	12 (30%)	6 (15%)	56(70%)	24 (30%)
GroupIII	9 (22.5%)	18 (45%)	13 (32.5%)	36(45%)	44(55%)

Table (3) : Chi square and OR (95% CI) of adiponectin 276 G>T genotypes and allele frequency in all studied groups .

Groups	Genotype	G and T Allele frequency	OR of G and T allele frequency 95% CI
Control group Vs Group II	$X^2 = 2.149$ $P=0.342$	$X^2 = 12.86$ $P=0.000$	0.294 0.148-0.588
Control group Vs Group III	$X^2 = 19.00$ $P=0.000$	$X^2 = 36.95$ $P= 0.000$	0.130 0.065-0.260
GroupII Vs Group III	$X^2 = 6.353$ $P = 0.042$	$X^2 = 8.052$ $P=0.005$	0.442 0.250-0.780

Table (4): Mean \pm SD of laboratory data and different genotypes in controls (groupI).

GroupI	GG n=28	GT n=9	TT n=3	t- value	P
FBG (mg /dl)	91.91 \pm 9.15	96.38 \pm 10.05	104.0 \pm 3.78	*-0.521 **-2.87	0.521 0.102
Insulin (μ U/L)	17.096 \pm 1.79	17.01 \pm 1.85	17.16 \pm 1.81	*-0.155 **-1.473	0.881 0.279
HOMA index	3.89 \pm 0.68	3.94 \pm 0.67	4.34 \pm 0.59	*-0.255 **-1.23	0.805 0.343
Adiponectin (μ g/ml)	34.39 \pm 2.0	33.01 \pm 1.98	32.23 \pm 0.92	*1.614 **0.776	0.145 0.519
BMI (kg/m2)	22.78 \pm 1.6	23.13 \pm 1.29	22.60 \pm 1.01	*1.44 **0.396	0.188 0.731
HbA1c	6.62 \pm 1.03	6.75 \pm 1.06	6.16 \pm 0.50	*-0.699 **-0.577	0.504 0.622
Total cholesterol (mg/dl)	190.5 \pm 38.5	181.33 \pm 34.06	191.33 \pm 31.53	*1.651 **-0.373	0.137 0.745
TG (mg/dl)	108.91 \pm 7.88	106.88 \pm 7.45	105.33 \pm 5.03	*0.016 **-0.225	0.987 0.843
HDL-C (mg/dl)	57.41 \pm 8.58	57.38 \pm 7.36	52.33 \pm 8.43	*0.910 **0.372	0.389 0.746
LDL-C(mg/dl)	114.46 \pm 42.0	106.84 \pm 36.69	138.14 \pm 15.32	*-1.914 **-0.988	0.092 0.427

*referred to t -value of comparing GG with GT,**referred to t -value of comparing GT with TT

Table (5): Mean \pm SD of laboratory data and different genotypes in obese non-diabetics (groupII).

GroupII	GG n=22	GT n=12	TT n=6	t- value	P value
FBG (mg /dl)	91.31 \pm 9.69	96.45 \pm 6.23	107.16 \pm 5.46	*-0.933 **-3.412 ***-3.077	0.371 0.019 0.028
Insulin (μ U/L)	18.20 \pm 0.929	18.49 \pm 1.02	18.86 \pm 1.338	*-0.799 **-1.498 ***-2.973	0.441 0.194 0.031
HOMA -IR index	4.09 \pm 0.49	4.44 \pm 0.39	4.98 \pm 0.461	*-1.216 **-2.903 ***-3.56	0.249 0.034 0.016
Adiponectin (μ g/ml)	21.49 \pm 1.48	20.64 \pm 1.03	19.83 \pm 0.95	*1.766 **0.951 ***3.450	0.112 0.385 0.018
BMI (kg/m2)	31.51 \pm 3.136	32.13 \pm 3.50	33.16 \pm 5.07	*-0.350 **-0.457 ***-1.88	0.265 0.362 0.118
HbA1c	6.75 \pm 0.956	6.83 \pm 1.13	6.85 \pm 0.92	*-0.430 **-0.124 ***-0.132	0.675 0.906 0.900
Total cholesterol (TC) (mg/dl)	231.13 \pm 7.53	227.5 \pm 7.47	228.0 \pm 8.09	*0.788 **-0.568 ***-0.047	0.447 0.595 0.964
Triglyceride (TG) (mg/dl)	213.45 \pm 30.20	212.83 \pm 33.63	217.66 \pm 11.80	*0.188 **-1.442 ***-1.487	0.854 0.209 0.197
HDL-C (mg/dl)	40.33 \pm 3.76	41.00 \pm 3.83	41.83 \pm 2.62	*-0.105 **-0.293 ***-0.544	0.918 0.782 0.610
LDL-C(mg/dl)	154.81 \pm 10.7	156.5 \pm 12.05	151.33 \pm 15.56	*-1.573 **0.508 ***0.408	0.144 0.633 0.700

*referred to t -value of comparing GG with GT,** t -value of comparing GT with TT,***t value of GG compared to TT genotype.

Table (6): Mean \pm SD of laboratory data and different genotypes in obese diabetic patients (Group III) .

Group III	GG n=9	GT n=18	TT n=13	t- value	P value
FBG (mg /dl)	138.83 \pm 21.46	140.27 \pm 10.59	141.83 \pm 8.87	*-0.419 **2.468 ***-0.078	0.686 0.030 0.940
Insulin (μ U/L)	33.76 \pm 2.35	43.3 \pm 4.15	42.71 \pm 2.76	*-8.80 **0.641 ***-11.11	0.000 0.533 0.000
HOMA –IR index	11.50 \pm 1.68	14.84 \pm 1.73	14.91 \pm 1.28	*-0.635 **0.199 ***-3.95	0.013 0.131 0.004
Adiponectin (μ g/ml)	16.14 \pm 1.05	15.51 \pm 0.96	14.22 \pm 1.27	*2.688 **3.490 ***2.667	0.028 0.004 0.028
BMI (kg/m ²)	34.35 \pm 4.56	34.85 \pm 3.71	32.06 \pm 3.56	*-0.044 **5.366 ***-1.190	0.966 0.057 0.268
Hbc1	10.71 \pm 1.22	10.77 \pm 1.17	11.23 \pm 0.85	*-0.334 **0.546 ***-0.978	0.747 0.595 0.357
Total cholesterol (TC) (mg/dl)	253.94 \pm 26.56	238.39 \pm 7.36	238.73 \pm 3.26	*1.40 **0.131 ***1.64	0.199 0.898 0.139
Triglyceride (TG) (mg/dl)	226.11 \pm 7.02	223.36 \pm 5.81	225.84 \pm 5.93	*1.337 **1.76 ***-0.242	0.218 0.104 0.815
HDL-C (mg/dl)	37.33 \pm 3.11	38.86 \pm 2.91	37.88 \pm 2.90	*-0.152 **1.405 ***0.603	0.883 0.183 0.563
LDL-C (mg/dl)	166.98 \pm 4.53	174.96 \pm 26.37	164.69 \pm 4.99	*-0.757 **1.799 ***0.916	0.471 0.097 0.387

* referred to t -value of comparing GG with GT, ** t -value of comparing GT with TT, ***t value of GG compared to TT genotype.

Discussion :

Statistics and early epidemiological studies in the Gulf States have shown that diabetes is taking an epidemic form and is a public health risk at national level ⁽²⁾. Adipose tissue is now considered an important endocrine organ that produces numerous factors affecting food intake, lipids and carbohydrates metabolism and other processes in human body. ⁽³⁶⁾ Some of adipose tissue-derived hormones such as adiponectin and resistin can represent a possible link between obesity and insulin resistance ⁽³⁷⁾. Adiponectin (ADP) is considered one of the few insulin-sensitizing factors produced by adipose tissue. ⁽⁹⁾ The genetic background of insulin resistance is likely to be polygenic, but the responsible genes are mostly unknown ⁽³⁸⁾.

Our data suggests that adiponectin may be one of the genes having a vital role in insulin resistance metabolic syndrome. We studied the effect of adiponectin 276G>T SNP in Saudi subjects who were divided into three main groups, control volunteers, obese patients not suffered from type 2 DM and obese patients suffered from type 2 DM.

We noticed a significant association of T allele frequency in obese diabetic patients when compared to controls and obese non-diabetic patients. More precisely a significant association between 276G>T SNP and IR was observed, Carriers of 276 T allele with genotypes GT and TT exhibiting higher FBG levels, Insulin levels, HOMA-IR index and in contrast having lower plasma adiponectin levels than those carrying 276 G allele in obese patient groups.

Interestingly, these results were significant in obese diabetic patients but in obese non diabetic patients the plasma adiponectin level was significantly decreased in TT carriers comparing to GG carriers but non significantly changed between GG and GT carriers, moreover, in healthy volunteers plasma adiponectin levels and other biochemical parameters were not significantly changed according to different 276 G>T genotypes except for insulin and HOMA-IR that were significantly increased in TT carriers.

We could speculate that it was the T allele and TT genotypes having the higher risk of obesity, higher FBG levels, increase the HOMA –IR, lower plasma adiponectin levels and type 2 DM.

Our results also were in harmony with those of Melistas et al⁽¹⁸⁾, Yang et al⁽³⁹⁾ Yang et al,⁽⁴⁰⁾ Filippi et al⁽⁴¹⁾ and Salmenniemi et al⁽⁴²⁾ Who revealed that subjects carrying the *SNP276* T-containing genotypes (GT or TT) and who were also centrally obese had the greatest risk of being hyperglycemic, suggesting that obese carriers of T containing variants were at an additive risk of being hyperglycemic and the presence of the G allele plays a protective role against the development of overweight and obesity .They confirmed the correlation between the T allele and lower serum adiponectin levels , higher insulin levels , HOMA-IR and greater IR. Our findings confirm this suggestion.

Other studies have also found an association between the mutant T allele and adverse glucose outcomes, as well as lipid profiles⁽⁴³⁻⁴⁴⁻⁴⁵⁾. The T allele has been associated with elevated LDL-C and reduced HDL-C in Canadians⁽⁴⁶⁾ and higher risk for cardiovascular diseases in Americans⁽⁴⁷⁾. But our results did not find an association between *SNP276* G>T and any of lipid profile parameters (P>0.05).

-It has been reported that intronic SNPs may modulate adiponectin gene expression levels , that may explain the exact molecular mechanisms of *SNP 276* G>T which could influence insulin sensitivity and aggravating the IR and type 2 DM in obese subjects.⁽⁴⁸⁾

In contrast to our results Mohammad zadeh et al⁽⁴⁹⁾ could not find any significant difference in allele and genotype frequencies of *SNP 276* comparing control group with T2DM group .

Opposing also to our findings , other studies have provided that the 276T allele was found to be protective for IR and the G/G genotype of *SNP276* is a putative risk genotype which associated with lower plasma adiponectin levels and higher IR .⁽⁴⁻¹⁶⁻⁵⁰⁻⁵¹⁻⁵²⁻⁵³⁾ These conflicting association results in various populations suggesting a complex relationship between *ADIPOQ* gene variation and IR . Possible explanation for this discrepancy also can be referred to differences in family history , environmental factors, anthropometric and ethnic factors that may interfere with the results and causing different findings .⁽⁵⁴⁾

Another possible explanation for these conflicting results is the presence of two types of circulating adiponectin , total and HMW adiponectin . Assays should differentiate between types of circulating adiponectin, as not all the circulating forms may be functional. However, in our study we did not differentiate between these two types we have just estimated the total adiponectin plasma levels .

In study done on northern European⁽⁵⁵⁾ and Pima Indian⁽⁵⁶⁾ populations , both total and HMW adiponectin, as well as the ratio of HMW to total adiponectin, were not different across the two *SNP* genotype groups. In addition, two genomic scans have

shown that the *ADIPOQ* gene has only a small effect on plasma adiponectin levels.

Different metabolic environments, including obesity and diabetic or prediabetic state, could affect the regulation of the *ADIPOQ* gene and the influence of SNPs within it. With regard to this notion, we found that the impact of *ADIPOQ 276* genotypes variations on HOMA-IR index , plasma Insulin and adiponectin levels are associated in obese patients but these correlations are not found in control normal weight subjects .

Our findings are in accordance with previously published data by Jang et al.⁽⁵³⁾ and Jang et al.⁽⁵⁷⁾ where the phenotypic expression of the T allele was observed only among subjects only with elevated BMI. They stated that the contribution of these *ADIPOQ 45 - 276*SNPs on IR is too small to be detected in lean subjects, but significant in more obese states where the risk of diabetes is greater.⁽⁴¹⁾

We observed a significant decrease of plasma adiponectin levels in both patient groups comparing to control group, with more significant decrease in obese diabetic patients more than obese non –diabetic patients . A significant negative correlation was observed between plasma adiponectin levels and each of FBG , plasma insulin , HOMA-IR , HbA1c , BMI , TC , LDL-C and TG with positive significant correlation with HDL-C levels in all studied subjects . These results were in harmony with⁽¹⁰⁻⁵⁸⁻⁵⁹⁻⁶⁰⁾ who stated that adiponectin presents at low levels in obesity ,is recognized as a key determinant of insulin sensitivity and of protection against obesity-associated metabolic syndrome.

In the current study ,the observed T allele frequency (GT and TT carriers) of adiponectin 276 G>T in type 2 obese diabetic and obese non-diabetic patients which were significantly higher than in control subjects , suggests the effect of adiponectin 276 -T allele on increased the risk of type 2 DM through obesity and insulin resistance . We have also suggested possible roles of adiponectin in the development of insulin resistance. The underlying mechanism is unclear, although direct effects of adiponectin on the transversely striated muscles and the liver are considered^{(61) (62) (63)} . Adiponectin increases lipid oxidation and directly affects the insulin signaling pathway both at and beyond the receptor level⁽⁶³⁾. In addition, adiponectin inhibits gluconeogenesis and the release of Tumor necrotic factor- α (TNF- α) by adipose tissue^{(21) (64)(65)} .

Conclusions:

The results of the current study represent the association of *SNP 276* G>T genotypes of the adiponectin gene with plasma adiponectin levels , Insulin levels , FBG and HOMA-IR levels in obese

Saudi patients in Qassim region . Additionally, the T allele at SNP 276 was associated with lower plasma adiponectin and higher HOMA-IR in obese patients than in normal control volunteers . Moreover, we concluded that plasma adiponectin is an important molecular link between obesity , insulin resistance and type 2DM . It is possible that plasma adiponectin levels may be a convenient marker for identifying subjects with the metabolic syndrome who may progress to impaired glucose tolerance and type2 DM. Multiple further studies are required to clarify this clinical application of adiponectin . Such knowledge of the association of identified variants with obesity and its consequences, insulin resistance and type 2 diabetes helping patients at higher risk of developing obesity complications to be identified and getting the benefit from more targeted therapy and prevention.

Limitations and recommendations :

The main limitations in the current study that is this study had a relatively small sample size, therefore, the results of this study may not be representative of all volunteers in Qassim region of KSA . Thus a larger sample size with examining the frequency of different polymorphisms and linkage disequilibrium among these SNPs are needed to confirm our present results. Furthermore, extension from these studies provide the opportunity to discover new molecular targets for future therapeutic interventions.

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