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A genetic and biochemical study of obesity in Egypt

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ABSTRACT

Background: Numerous studies have documented the association adiponectin (ADIPOQ) gene polymorphisms with obesity but the consequences are indecisive. The purpose of this work is to study the connection between adiponectin gene polymorphisms G276T and T45G with obesity and biochemical variants in Egyptian population. Material and methods: Genotyping of G276T single nucleotide polymorphisms of adiponectin gene was performed by Alleles Refractory Mutation Systems PCR(ARMS PCR) method, while Genotyping of T45G single nucleotide polymorphism of adiponectin gene was performed by RFLP PCR method. The analysis was done in 172 matched person; 89 obese patients, 43 overweight patients, and 40 normal healthy controls. Biochemical parameters were calculated through standard procedures. Results: For adiponectin polymorphism G276T, it was related to overweight under Co dominant and recessive models (P= 0.010, P= 0.036) respectively, also it was associated with obesity under recessive model (P= 0.048). Higher BMI was associated with heterozygous GT and homozygous GG carriers of G276T (P= 0.032) compared to TT carriers, For adiponectin gene polymorphism T45 G, it was associated with Obesity under Co dominant and dominance models (P= 0.046, P= 0.045) respectively. Heterozygous GT and homozygous GG carriers of T45G were related higher BMI (P= 0.029), triglyceride (P= 0.029), and VLDL (P= 0.026) compared to TT carriers at adiponectin polymorphism T45G. Conclusion: The study suggests that the ADIPOQ 276G>T and ADIPOQ 45T>G polymorphisms may be vital indicators of obesity and associated complications in several population.

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INTRODUCTION

Obesity is a great problem all over the world. it is considered a health problem. Obesity increases the risk of many common diseases, including coronary artery disease (CAD), type 2 diabetes mellitus (DM), high blood pressure, gallbladder disease, and osteoporosis[1]. As a result, obesity increases mortality [2,3]. Obesity has become a widspread epidemic [4,5]. Participants rated they were obese if their BMI was greater than or equal to 30 kg/m^2 [6]. Body mass index (BMI) was calculated by dividing the weight in kilograms per square meter in meters by approximating the results to the nearest decimal number, overweight if BMI was 25 to 29.9 and obesity if BMI was 30.0 and above, and obesity was splitted into grade 1 (BMI 30-35), grade 2 (BMI 35-40), and grade 3 (BMI ≥40)[7]. The causes of obesity are multiple genetic, environmental, socioeconomic or psychological effects with increasing rates of illness and death [8]. Obesity is the end result of a chronic energy imbalance and is regulated by a multifarious network between the endocrine and central nervous system [9, 10]. Fatty tissue was considered as an energetic endocrine system with high metabolic activity. Adipocytes produce and secrete several proteins that act as veritable hormones and are responsible for regulating energy consumption and expenditure [11]. Many of these hormones are called adipokines, which play important roles in inflammatory and arteriosclerosis processes. These comprise of tumor necrosis factor (TNF) α, leptin , interleukin (IL) 6, angiotensinogen , and plasminogen activator inhibitor-1. Increasing sign shows that adiposity participates in a proinflammatory milieu [12]. Reduction in fat bulk associated with decrease in the serum levels of many of these proinflammatory adipokines which mean that approaches designed to promote fat loss should be useful in attenuating the proinflammatory environment associated with obesity [12]. Adiponectin has recently been described as secretory product of adipose tissue [13]. Adiponectin is a relatively plentiful serum protein secreted by adipocytes [14]. Some studies have showen that level of adiponectin is related with insulin resistance [15], and with glucose and lipid metabolism[16]. According to reports, adiponectin wasn't association with metabolic syndrome (MS)[17,18] .The ADIPOQ gene consists of three exons and two introns spanning a 17-kb region and located on chromosome 3q27 [19]. It has been reported that genetic variants in the gene encoding adiponectin (*ADIPOQ*) are associated with the level of adiponectin in several genome–wide linkage and association studies [20,21]. Several polymorphisms within adiponectin gene have been identified[23].

SUBJECTS AND METHODS

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Study Design and Participants

An observational case-control study was conducted between March, 2013 to October,2013. The study was approved by the Faculty of science, Zagazig University, and analyze labs. This case_control study included 172 age and sex matched person , The age of the patients ranged from 15-55 years. They were 89 obese patients, 43 overweight patients, and 40 normal health controls. For all subjects, body weight and height were measured ; Body mass index (BMI) was computed as weight (kg) divided by height squared (m^2). Blood pressure was measured three times and the mean was taken for analysis.

Blood Sampling and biochemical Measurements

Taking 5 ml of blood from each person, 2 ml blood in EDTA tube for DNA

extraction , and 3 ml for serum collection after centrifugation. Blood glucose was measured by using (Bionime blood glucose test strip that is an electrochemical method), lipid profile was done for each sample by enzymatic colorimetric methods using a Spectrophotometer ; total cholesterol (CHOLESTEROL-LQ REF41020), triglycerides (TRIGLYCEIDES-LQ REF 41030) , HDL-C (HDL Cholesterol P REF1001095) , LDL-C is calculated using the Friedewald's equation, LDL-C (mg/dL) = total cholesterol – HDL-C -triglycerides/5 ,and VLDL was calculated by using the Friedewald's equation; VLDL = Triglycerides/5) .

Adiponectin level determination

The human total adiponectin (ADPN) ELISA kit (96 Tests ;Catalog Number: MBS720699**)** was used to determined serum adiponectin concentration**.**

DNA Extraction

DNA was extracted from blood samples and collected in tubes containing EDTA with (TIANamp Genomic DNA Kit Wizard® Genomic DNA Purification Kit). The extracted DNA was conserved at -20 °C till genotyping steps.

Genotyping of Adiponectin gene polymorphism (276 G/T) using ARMS PCR method

For each sample, 2 PCR reactions were done with two pairs of primers. The first PCR reaction is for wild allele ,while the second PCR reaction for mutant allele. Primers for 1st PCR reaction (G allele), 5`- CCT CCT ACA CTG ATA TAA ACT ATA TGA GGG-3`;and 5`- GAA TAT GAA TGT ACT GGG AAT AGG GAT G-3`. primers for 2nd PCR Reaction (T allele), 5`- GAG CTG TTC TAC TGC TAT TAG CTC TGC-3`; and 5`- TGT GTC TAG GCC TTA GTT AAT AAT GAA CGA-3`. Each PCR reaction was done in a final volume of 20 µL (5 µL DNA, 5 µL of working primer mixture, and 10 µL 2x master mix solution). Each PCR reaction was done at a final volume of 20 µL containing 5 µL DNA, 5 µL of dilute primer mixture, and 10 µL master mix solution. PCR cycling conditions were 5 min at 95º c followed by 30 cycles of 30 s at 95 º c , 27 s at 61 ºc ,and 25 s at 72, with a final step at 72°C for 5 min to allow for complete extension of all PCR fragments**.** PCR products were amplified in a 2% agarose; (**figure 1).**

Genotyping of Adiponectin gene polymorphism (+45T/G) using RFLP PCR method

For each sample, one PCR reaction was done followed by digestion with a suitable restriction enzyme. The PCR primer sequences were 5`- GAA GTA GAC TCT GCT GAG ATG-3` (forward) and 5`-TAT CAG TGT AGG TCT GTG ATG-3` (reserve).A three-step PCR was standardized and carried out with an initial denaturation at 95°C for 5 minutes followed by cycling at 95°C for 45 seconds; 60.2°C annealing for 45 seconds, 72°C for 45 seconds extension and a final extension at 72°C for 7 minutes was carried out for about 35 cycles.PCR products are amplified to RFLP using the SmaI (#R0141s NEW ENGLAND Biolabs) restriction enzyme for 37°C overnight digestion enzyme. PCR products were amplified in a 2% agarose **(figure 2).**

Statistical analysis

One-way ANOVA ,followed by post hoc test was used for numerical parameters , through the general linear models (GLM) procedure of the statistical package for social sciences version 22.0 (IBM Corp., Armonk, NY, USA). For categorical data, Chi-square test was used to compare between patients and control subjects. Allele

and genotype frequencies were calculated for each locus and for each population using the Pop gene on the web software, version 4.0.10. Genotype frequencies were tested in patients and controls for Hardy-Weinberg equilibrium (HWE) using Chi-square test (Exact Fisher's method when excepted cell frequency is less than 5). Armitage's trend test and Allele freq. difference test were used to test the significance in difference in genotype and allele frequencies distribution respectively between populations given each position. Logistic regression was performed under three genotype models (co-dominant , dominant and recessive) in each position . The strength of association was estimated by the crude odds ratios (OR),with 95% confidence interval (95% CI). The statistical significance was accepted for $p<0.05$.Also adjusted odds ratio which were adjusted for age (AOR) were also estimated and tested.
Pearson correlation coefficient was estimated to test strength and direction of association between lipid profile parameters in control, overweight and obese subjects enrolled to the study. Data were expressed as mean± standard error of mean (SE) for continuous variables, also F-statistics, p-values were reported. While results for categorical variables were reported as number and percent of patients.

RESULTS:

There were no statistically significant differences between obese people, overweight ,and control subjects in gender (X^2 = 2.243 , P=0.297) , Cholesterol (F=1.58 , P=0.21), HDL (F=1.58 , P=0.21) , LDL (F=0.50 , P=0.610), and adiponectin concentration (F=0.59 , P=0.558) , but there were highly significant differences in age (F=15.99 , P< 0.0001), BMI (F=226.94 , P< 0.0001), and glucose level (F=5.23 , P=0.006) , also significant difference in triglyceride (TG) (F=3.39, P=0.036) ,and VLDL (F=3.72 , P=0.026) **,(Table1) and (Table2).**

***For G276T polymorphism*;** The results show control subjects and obese patients are not in Hardy–*Weinberg* equilibrium (P< 0.0001, P=0.027) respectively, while overweight patients are in Hardy–*Weinberg* equilibrium (P = 0.658).
For T45G polymorphism, The results show that both genotype and allele proportions were in Hardy–*Weinberg* equilibrium in overweight, control and obese subjects (P =0.300 , P = 0.300 , P = 0.300) respectively. Armitage's trend test for G276T and T45G adiponectin polymorphism showed that there was no significant difference in genetic distribution among overweight patients and control subjects (P = 0.191 , P = 0.513) respectively, Also there was no significant difference in genetic distribution between obese patient and control subjects (P=0.128 , P= 0.118) respectively.
Allele frequencies difference test for G276T and T45G adiponectin polymorphism showed that there was no significant difference in allele frequency between overweight patients and control subjects (P = 0.147 , P = 0.465) respectively, also there was no significant difference in allele frequency between obese patients and control subjects (P=0.075,P=0.089) respectively; **(Table 3)** and **(Table 4).**

Association of adiponectin polymorphism G276T and T45G with biochemical parameters

For G276T, Results show that heterozygous GT and homozygous GG carriers were associated with higher BMI (F=3.51, P=0.032) compared to TT carriers, but there weren't significant genotyping effect on cholesterol, triglyceride, HDL, LDL, VLDL, Glucose level and adiponectin concentration. While for T45G polymorphism, heterozygous GT and

homozygous GG carriers were associated with higher BMI (F=3.60, P=0.029), triglyceride (F=3.62, P=0.029),and VLDL (F=3.73 , P=0.026) compared to TT carriers, but there was no significant genotyping effect on cholesterol, HDL, LDL, Glucose level and adiponectin concentration. (**Table 5), (Table 6)**.

Adiponectin gene polymorphism and risk of obesity

For G276T, it was associated with overweight under Co dominant and recessive models (OR=35.30, 95%CI=2.33-533.99, P =0.010), and (OR= 3.21, 95%CI=1.08-9.51, P =0.036) respectively (table4.1); while it was associated with obesity under a recessive model only (OR=2.91, 95%CI=1.01-8.41, P=0.048).

For T45G, it was not associated with overweight under Co dominant, dominant and recessive models, While it was associated with Obesity under Co dominant and dominance models (OR=4.11, 95%CI=1.03-16.50,P=0.046) and (OR=3.60, 95%CI=1.03-12.61, P=0.045) and (OR=3.60 ,95%CI=1.03-12.61, P=0.045) respectively **(Table 7), (Table 8), (Table 9), (Table 10).**

Relation between all biomedical parameters in all studied groups

In control group, there was a significant positive correlation between cholesterol and HDL (r=1.000, p=0), Cholesterol with LDL (r=0.971,p=0) ,TG with VLDL (r=0.997, p=0) , and HDL with LDL (r=0.971,p=0);as shown in **(Table 11).**

In overweight group, there was a significant positive correlation between cholesterol with HDL (r=1.000,p=0),Cholesterol with LDL (r=0.971,p=0),Cholesterol with TG (r=0.340 , p=0.026),Cholesterol with VLDL (r=0.340, p=0.026), TG with HDL (r=0.340, p=0.026),TG with VLDL (r=1,p=0), HDL with LDL (r= 0.976,p=0), and HDL with VLDL (r=0.340,p=0.026); as shown in **(Table 12).**

In obese group, there was a significant positive correlation between cholesterol with HDL (r=1.000, p=0), Cholesterol with LDL (r=0.957, p=0), cholesterol with glucose (r=0.469, p=0), TG with VLDL (r=1.000, p=0), HDL with LDL(r= 0.957,p=0), HDL with glucose (r=0.469,p=0) ,and LDL with glucose (r=0.460,p=0),and there was a significant negative correlation between BMI with adiponectin concentration (r=-0.238,p=0.025), TG with LDL (r=-0.306,p=0.004),and LDL with VLDL (r=-0.306,p=0.004); as shown in **(Table 13).**

DISCUSSION:

According to World Health Organization (WHO), obesity is one of the biggest challenges to public health in the 21st century by 2015; more than 700 million adults are obese. Adiponectin is excreted by adipose tissues and its levels are inversely associated with body mass index. In this study, it was found that the cases of weight gain and obesity is strongly associated with +45T/G and +276G/T genotype. It is suggested that ADIPOQ gene polymorphism can contribute significantly to weight gain. Furthermore, several studies showed that adiponectin is able to counter insulin resistance, atherosclerosis and inflammatory processes [23, 24, 25-27]. In 2002 Menzaghi *et al*. noted that the +45 T/G and +276 G/T polymorphisms of the adiponectin gene contributed to weight gain and the development of insulin resistance[28].

Many European, Asian and American societies have studied SNPs 45T>G and 276G>T [29]. Even though there was a lack of consistency between the studies, the results indicated that genetic variation in the ADIPOQ gene was linked to insulin resistance and diabetes mellitus type 2[30, 31]. Italy's Mediterranean population was associated with a 276T allele with higher levels of insulin [32], however other studies

have shown adverse results [33, 34]. Boumaiza *et al* [35] noted the protective role of G276T SNP among Tunisians and also the same results appeared with Menzaghi *et al.*[36] and Stumvoll *et al.* [37], but Fillipi *et al.*[38] pointed out that the T allele is linked to obesity risk and IR . It can be contrasted this discrepancy in part to ethnic Privacy. The present study showed that adiponectin polymorphism G276T was associated with overweight under Co dominant and recessive models (OR=35.30,95%CI=2.33-533.99, P=0.010), and (OR=3.21, 95%CI= 1.08-9.51,P=0.036)respectively, adiponectin polymorphism G276T was associated with Obesity under recessive model (OR=2.91, 95%CI=1.01-8.41,P=0.048) ;while The adiponectin polymorphism T45G was associated with Obesity under Co dominant and dominance models(OR=4.11, 95%CI= 1.03-16.50,P=0.046) and(OR=3.60, 95%CI= 1.03-12.61,P=0.045) respectively. SNP 276G>T can influence the sensivity of insulin as it suggested that intronic SNPs may modify the levels of gene expression [39]. Current results are consistent with previous data published by Jang and his team [40] ,where only among subjects who have a high BMI observed the phenotypic expression of the genotype for SNP G276T . In the non-diabetic Italian population, the highest IR observed among subjects with a genotype TT was more pronounced in lean compared to obese individuals [41]; The present study showed association between G276T and 45T/G genotypes with obesity parameter (BMI) and clinical biochemical parameters; there were highly significant differences in age (F=15.99,P< 0.0001), BMI (F=226.94, P< 0.0001), and glucose level (F=5.23,P=0.006) ,also significant difference in triglyceride (TG) (F=3.39,P=0.036) ,and VLDL (F=3.72,P=0.026). There was a significant negative correlation between BMI and adiponectin concentration (r=-0.238, p=0.02) ,TG with LDL (r=-0.306,p=0.004),and LDL with VLDL (r=-0.306,p=0.004); in Obese group; as shown in (table 6.3), Compared to correlation between BMI with adiponectin concentration in overweight group and control group.

CONCLUSION

The presence of alleles TT and GT at the 276 locus of the ADIPOQ gene are related to an increase in BMI, while alleles TG and GG at the 45 locus of the ADIPOQ gene are related to an increase in BMI, triglyceride, and VLDL in Egyptian people. The study proposed that the ADIPOQ G276T and ADIPOQ T45G polymorphisms may be useful indicators related to obesity and its associated complications. Early diagnosis may help to allocate of intensive interventions that may benefit children most vulnerable to obesity**.**

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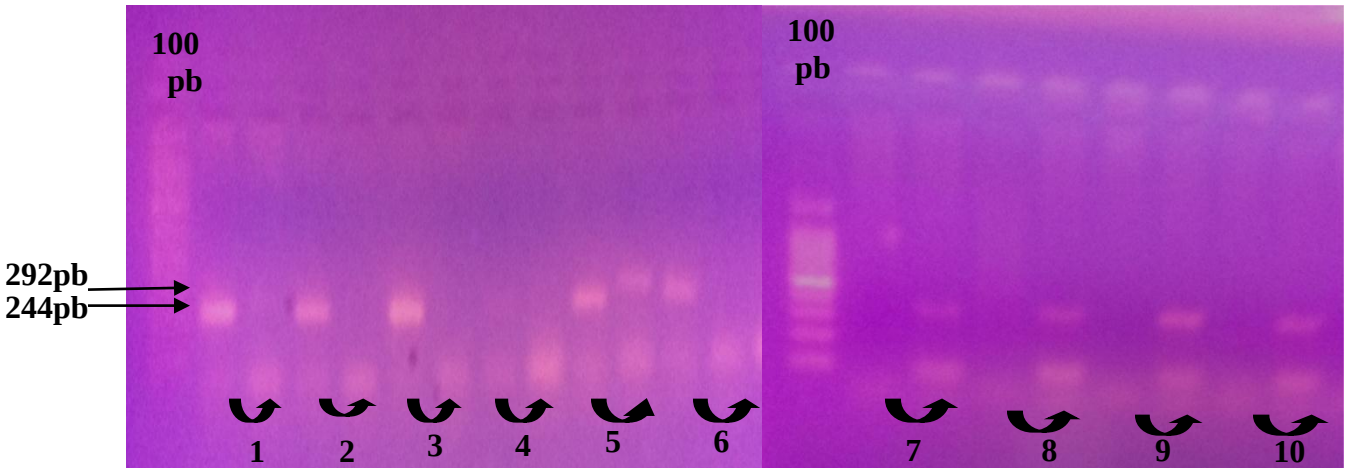

Figure 1. Genetics patterns of adiponectin polymorphism on the electrode gel with staining of ethidium bromide and UV light. Molecular weight DNA standard marker (100pb), 11:-ve ; 5and12: Heterozygous mutant (GT); 1,2,3,and6: Homozygous mutant (GG) 244pb; 7,8,9,10and13 : Homozygous wild type (TT) 292pb.

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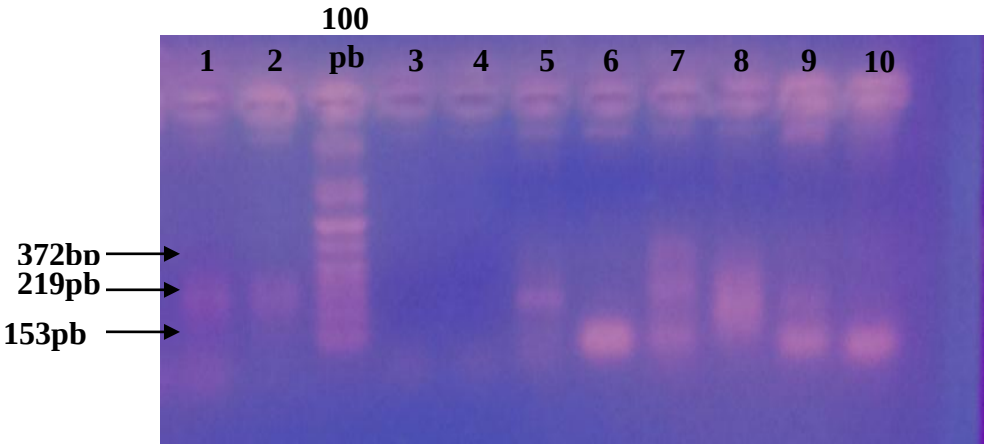

Figure 2. Restriction enzyme digestion (SmaI) of PCR products. Genetics patterns of adiponectin polymorphism on the electrode gel with staining of ethidium bromide and UV light. Molecular weight DNA marker . Lanes 7,and 14: heterozygote TG 372, 219 and 153bp. Lane1,2,5,8 and 15: Homozygous mutant (GG), Lanes 11,12,13,16,17,18,19, and20: Wild type 372 bp TT genotype Lane.

Table 1: Demographic characteristics of patients and control enrolled in the study (n=172)

		Control (n=40)	Over weight (n=43)	Obese (n=89)	F-value	P-value
Age		27.83 ± 1.62^{c} (16-59)	32.93 ± 1.76^{b} (15-56)	39.39 ± 1.2^{a} (15-57)	15.99^{**}	< 0.0001
BMI		22.63 ± 0.29^{c} (18-25)	27.04 ± 0.19^{b} (24.96-29.66)	35.39 ± 0.47^{a} (30-50)	226.94^{**}	< 0.0001
Gender	F	18 (45.0%)	18 (41.9%)	49 (55.1%)	$\chi^2=2.43^{NS}$	0.297
	M	22 (55.0%)	25 (58.1%)	40 (44.9%)		

Means within the same row carrying different superscripts are sig. different at P < 0.05 based on Bonferroni's multiple comparison test.;*: indicate significant difference (P<0.05) ;**: indicate highly significant difference (P<0.01);NS: indicate non-significant difference.

Table 2: Comparison of clinical parameters among overweight , obese patients and control

	Control (n=40)	Over weight (n=43)	Obese (n=89)	F-value	P-value
Cholesterol	164.76 ± 7.28^{a} (105.54-310.82)	180.35 ± 8.24^{a} (102.37-280.2)	181.41 ± 5.47^{a} (101.32-313.46)	1.58^{NS}	0.210
TG	90.16 ± 7.54^{b} (51.38-231.19)	116.13 ± 7.18^{ab} (59.17-238.53)	116.62 ± 6.66^{a} (63.3-521.1)	3.39^{*}	0.036
HDL	32.95 ± 1.46^{a} (21.11-62.16)	36.07 ± 1.65^{a} (20.47-56.04)	36.28 ± 1.09^{a} (20.26-62.69)	1.58^{NS}	0.210
LDL	113.97 ± 5.93^{a} (65.57-233.15)	121.08 ± 6.25^{a} (66.32-206)	121.59 ± 4.62^{a} (40.14-233.6)	0.50^{NS}	0.610
VLDL	17.83 ± 1.41^{b} (10.28-42.24)	23.2 ± 1.44^{ab} (11.83-47.7)	23.32 ± 1.33^{a} (12.66-104.22)	3.72^{*}	0.026
Glucose	101.63 ± 2.38^{b} (70-153)	106.05 ± 3.45^{ab} (78-212)	134.93 ± 9.02^{a} (74-515)	5.23^{**}	0.006
Adiponectin	19.99 ± 1.95^{a} (10.58-52.11)	17.41 ± 1.43^{a} (10.58-51.26)	18.84 ± 1.16^{a} (8.85-54.29)	0.59^{NS}	0.558

Means within the same row carrying different superscripts are sig. different at P < 0.05 based on Bonferroni's multiple comparison test.*: indicate significant difference (P<0.05); **: indicate highly significant difference (P<0.01);NS: indicate non-significant difference

Table 3: Distribution of adiponectin polymorphism G276T and T45G (Genotype and Allele) frequencies among Over weight patients and controls.

G276T	Genotypes			Allele		χ^2 (HWE)	P value*
	GG	GT	TT	G	T		
Control (n=40)	4(10.0%)	2(5.0%)	34(85.0%)	10/80	70/80	23.804	< 0.0001
Over weight (n=43)	1(2.3%)	16(37.2%)	26(60.5%)	18/86	68/86	0.663	0.658
P-value	Armitage's trend test 0.191			Allele freq. difference 0.147			
T45G	Genotypes			Allele		χ^2 (HWE)	P value
	TT	GT	GG	T	G		
Control (n=40)	36(90.0%)	3 (7.5%)	1 (2.5%)	75/ 80	5/ 80	5.184	0.124
Over weight (n=43)	36(83.7%)	6 (14.0%)	1 (2.3%)	78/ 86	8/86	1.288	0.300
P-value	Armitage's trend test 0.513			Allele freq. difference 0.465			

*: Probability value of Fisher's exact test

Table 4: Distribution of adiponectin polymorphism G276T and T45G (Genotype and Allele) frequencies among obese patients and controls.

G276T	Genotypes			Allele		χ^2 (HWE)	P value*
	GG	GT	TT	G	T		
Control (n=40)	4(10.0%)	2(5.0%)	34(85.0%)	10/80	70/80	23.804	< 0.0001
Obese (n=89)	8(9.0%)	23(25.8%)	58(65.2%)	39/178	139/178	5.33	0.027
P-value	Armitage's trend test 0.128			Allele freq. difference 0.075			
T45G	Genotypes			Allele		χ^2 (HWE)	P value
	TT	TG	GG	T	G		
Control (n=40)	36(90.0%)	75/ 80	5.184	0.124	5/80	5.184	0.124
Obese (n=89)	68(76.4%)	154/ 178	1.58	0.186	24/ 178	1.58	0.186
P-value	Armitage's trend test 0.118			Allele freq. difference 0.089			

*: Probability value of Fisher's exact test

Table 5: Association of genotypes at adiponectin polymorphism G276Twith clinical and biochemical parameters.

	GG (n=13)	GT (n=41)	TT (n=118)	F-value	P-value
BMI	29.99±1.87^{ab}	32.61±1.02^{a}	29.58±0.57^{b}	3.51*	0.032
Cholesterol	168.78±11.66	167.22±7.74	181.7±4.82	1.42^{NS}	0.245
TG	142.2±33.4	108.4±5.84	107.51±4.71	2.26^{NS}	0.108
HDL	33.75±2.33	33.44±1.55	36.34±0.96	1.42^{NS}	0.245
LDL	106.58±9.24	112.1±6.04	123.77±3.94	1.93^{NS}	0.148
VLDL	28.44±6.68	21.65±1.17	21.43±0.93	2.33^{NS}	0.100
Glucose	135.77±22.31	123.83±9.87	116.97±5.86	0.59^{NS}	0.558
Adiponectin	24.5±3.94	17.06±1.29	18.71±1.03	2.34^{NS}	0.100

Means within the same row carrying different superscripts are sig. different at P < 0.05 based on Bonferroni's multiple comparison test.;*: indicate significant difference (P<0.05) ;**: indicate highly significant difference (P<0.01);NS: indicate non-significant difference.

Table 6: Association of genotypes at adiponectin polymorphism T45Gwith clinical and biochemical parameters.

	TT (n=140)	TG (n=27)	GG (n=5)	F-value	P-value
BMI	29.74±0.51^{b}	33.29±1.44^{a}	30.89±3.76^{ab}	3.60*	0.029
Cholesterol	176.92±4.24	183.5±11.17	153.53±17.81	0.74^{NS}	0.479
TG	107.47±3.6^{b}	113.2±12.98^{ab}	175.5±87.41^{a}	3.62*	0.029
HDL	35.38±0.85	36.7±2.23	30.71±3.56	0.74^{NS}	0.479
LDL	119.97±3.39	124.16±9.3	87.72±14.22	1.66^{NS}	0.193
VLDL	21.43±0.71^{b}	22.64±2.6^{ab}	35.1±17.48^{a}	3.73*	0.026
Glucose	115.89±4.85	144.85±18.28	101.6±8.24	2.53^{NS}	0.083
Adiponectin	18.77±0.94	18.02±1.71	22.11±7.6	0.30^{NS}	0.744

Means within the same row carrying different superscripts are sig. different at P < 0.05 based on Bonferroni's multiple comparison test.;*: indicate significant difference (P<0.05) ;**: indicate highly significant difference (P<0.01);NS: indicate non-significant difference.

Table 7: Association of genotypes at adiponectin polymorphism G276Twith overweight under co dominant, dominance ,and recessive models

Co dominant model	Control (n=40)	Overweight (n=43)	[c]OR (95%CI)	P-value	[a]OR (95%CI)	P-value
GG[R]	4 (80.0%)	1 (20.0%)	1	0.006	1	0.009
GT	2 (11.1%)	16 (88.9%)	32.00 (2.29-447.83)	0.010	35.30 2.33-533.99	0.010
TT	34 (56.7%)	26 (43.3%)	3.06 (0.32-29.02)	0.330	3.86 0.37-39.99	0.258
Dominance model	Control (n=40)	Overweight (n=43)	[c]OR (95%CI)	P-value	[a]OR (95%CI)	P-value
GG[R]	4 (80.0%)	1 (20.0%)	1	0.177	1	0.134
GT+TT	36 (46.2%)	42 (53.8%)	4.67 0.50-43.66		6.02 (0.58-62.97)	
recessive model	Control (n=40)	Overweight (n=43)	[c]OR (95%CI)	P-value	[a]OR (95%CI)	P-value
TT[R]	34 (56.7%)	26 (43.3%)	1	0.016	3.21 (1.08-9.51)	0.036
GT+GG	6 (26.1%)	17 (73.9%)	3.71 (1.28-10.71)			

Reference, [c]OR; Crude odds ratio, [a]OR; adjusted odds ratio for age CI: confidence interval

Table 8: Association of genotypes at adiponectin polymorphism T45G with overweight under co dominant , dominant, recessive model.

co dominant model	Control (n=40)	Overweight (n=43)	[c]OR (95%CI)	P-value	[a]OR (95%CI)	P-value
TT[R]	36 (50.0%)	36 (50.0%)	1	0.648	1	0.635
GT	3 (33.3%)	6 (66.7%)	2.00 (0.46-8.62)	0.352	1.97 0.43-8.98	0.379
GG	1 (50.0%)	1 (50.0%)	1 (0.06-16.61)	1	1.84 0.10-32.45	0.677
dominant model	Control (n=40)	Overweight (n=43)	[c]OR (95%CI)	P-value	[a]OR (95%CI)	P-value
TT[R]	36 (50.0%)	36 (50.0%)	1	0.403	1.95 0.50-7.65	0.340
GT+GG	4 (36.4%)	7 (63.6%)	1.75 0.47-6.50			
recessive model	Control (n=40)	Overweight (n=43)	[c]OR (95%CI)	P-value	[a]OR (95%CI)	P-value
GG[R]	1 (50.0%)	1 (50.0%)	1	0.959	0.58 0.03-10.23	0.712
GT+TT	39 (48.1%)	42 (51.9%)	1.08 0.07-17.81			

Reference, [c]OR;Crude odds ratio, [a]OR ; adjusted odds ratio for age CI: confidence interval

Table 9: Association of genotypes at adiponectin polymorphism G276T with obese under co-dominant model, dominance, recessive model.

co dominant model	Control (n=40)	Obese (n=89)	[c]OR (95%CI)	P-value	[a]OR (95%CI)	P-value
GG^R	4 (33.3%)	8 (66.7%)	1	0.046	1	0.072
GT	2 (8.0%)	23 (92.0%)	5.75 (0.88-37.62)	0.068	5.42 (0.72-40.67)	0.100
TT	34 (37.0%)	58 (63.0%)	0.85 (0.24-3.05)	0.806	0.84 (0.21-3.44)	0.810
dominance model	Control (n=40)	Obese (n=89)	[c]OR (95%CI)	P-value	[a]OR (95%CI)	P-value
GG^R	4 (33.3%)	8 (66.7%)	1.13 (0.32-3.98)	0.855	-	-
GT+TT	36 (30.8%)	81 (69.2%)				
recessive model	Control (n=40)	Obese (n=89)	[c]OR (95%CI)	P-value	[a]OR (95%CI)	P-value
TT^R	34 (37.0%)	58 (63.0%)	3.03 (1.15-8.00)	0.025	2.91 (1.01-8.41)	0.048
GT+GG	6 (16.2%)	31 (83.8%)				

Reference, COR: Crude odds ratio, AOR;adjusted odds ratio for age CI: confidence interval

Table 10: Association of genotypes at adiponectin polymorphism T45G with obese under co-dominant model.

co dominant model	Control (n=40)	Obese (n=89)	[c]OR (95%CI)	P-value	[a]OR (95%CI)	P-value
TT^R	36 (34.6%)	68 (65.4%)	1	0.203	1	0.128
GT	3 (14.3%)	18 (85.7%)	3.18 (0.88-11.51)	0.078	4.11 (1.03-16.50)	0.046
GG	1 (25.0%)	3 (75.0%)	1.59 (0.16-15.83)	0.693	1.94 (0.13-28.06)	0.626
dominance model	Control (n=40)	Obese (n=89)	[c]OR (95%CI)	P-value	[a]OR (95%CI)	P-value
TT^R	36 (34.6%)	68 (65.4%)	2.78 (0.89-8.72)	0.080	3.60 (1.03-12.61)	0.045
GT+GG	4 (16.0%)	21 (84.0%)				
recessive model	Control (n=40)	Obese (n=89)	[c]OR (95%CI)	P-value	[a]OR (95%CI)	P-value
GG^R	1 (25.0%)	3 (75.0%)	0.74 (0.07-7.29)	0.793	-	-
TT+GG	39 (31.2%)	86 (68.8%)				

Reference, [c]OR; Crude odds ratio, [a]OR; adjusted odds ratio for age CI: confidence interval

Table 11: The correlation between clinical parameters in the control group.

Normal group		BMI	Cholesterol	TG	HDL	LDL	VLDL	glucose	adiponectin
BMI	r	1	-0.007	-0.083	-0.007	0.014	-0.09	-0.164	-0.256
	P		0.965	0.611	0.965	0.93	0.581	0.311	0.111
Cholesterol	r	-0.007	1	0.033	1.000**	.971**	0.05	-0.179	-0.042
	P	0.965		0.838	0	0	0.758	0.268	0.795
TG	r	-0.083	0.033	1	0.033	-0.205	.997**	-0.098	-0.07
	P	0.611	0.838		0.838	0.204	0	0.546	0.67
HDL	r	-0.007	1.000**	0.033	1	.971**	0.05	-0.179	-0.042
	P	0.965	0	0.838		0	0.758	0.268	0.795
LDL	r	0.014	.971**	-0.205	.971**	1	-0.189	-0.155	-0.026
	P	0.93	0	0.204	0		0.242	0.339	0.872
VLDL	r	-0.09	0.05	.997**	0.05	-0.189	1	-0.088	-0.065
	P	0.581	0.758	0	0.758	0.242		0.587	0.692
glucose	r	-0.164	-0.179	-0.098	-0.179	-0.155	-0.088	1	-0.023
	P	0.311	0.268	0.546	0.268	0.339	0.587		0.889
adiponectin	r	-0.256	-0.042	-0.07	-0.042	-0.026	-0.065	-0.023	1
	P	0.111	0.795	0.67	0.795	0.872	0.692	0.889	

* Significant correlation at level 0.05 (2-tailed).
** Significant correlation at level 0.01 (2-tailed).

Table 12: The correlation between clinical parameters in the overweight group.

Overweight group		BMI	Cholesterol	TG	HDL	LDL	VLDL	glucose	adiponectin
BMI	r	1	0.082	-0.259	0.082	0.147	-0.261	0.233	0.022
	P		0.599	0.093	0.599	0.346	0.09	0.137	0.89
Cholesterol	r	0.082	1	.340*	1.000**	.976**	.340*	0.147	0.248
	P	0.599		0.026	0	0	0.026	0.352	0.109
TG	r	-0.259	.340*	1	.340*	0.128	1.000**	-0.259	-0.031
	P	0.093	0.026		0.026	0.413	0	0.097	0.845
HDL	r	0.082	1.000**	.340*	1	.976**	.340*	0.147	0.248
	P	0.599	0	0.026		0	0.026	0.352	0.109
LDL	r	0.147	.976**	0.128	.976**	1	0.128	0.209	0.268
	P	0.346	0	0.413	0		0.413	0.185	0.082
VLDL	r	-0.261	.340*	1.000**	.340*	0.128	1	-0.261	-0.03
	P	0.09	0.026	0	0.026	0.413		0.095	0.848
glucose	r	0.233	0.147	-0.259	0.147	0.209	-0.261	1	-0.111
	P	0.137	0.352	0.097	0.352	0.185	0.095		0.483
adiponectin	r	0.022	0.248	-0.031	0.248	0.268	-0.03	-0.111	1
	P	0.89	0.109	0.845	0.109	0.082	0.848	0.483	

* Significant correlation at level 0.05 (2-tailed).
** Significant correlation at level 0.01 (2-tailed).

Table 13: The Correlation between clinical parameters in the obese group.

Obese group		BMI	Cholesterol	TG	HDL	LDL	VLDL	glucose	adiponectin
BMI	r	1	0.021	0.022	0.021	0.007	0.022	0.147	-.238*
	P		0.844	0.836	0.844	0.95	0.836	0.17	0.025
Cholesterol	r	0.021	1	-0.02	1.000**	.957**	-0.02	.469**	-0.086
	P	0.844		0.85	0	0	0.85	0	0.422
TG	r	0.022	-0.02	1	-0.02	-.306**	1.000**	-0.052	-0.046
	P	0.836	0.85		0.85	0.004	0	0.628	0.668
HDL	r	0.021	1.000**	-0.02	1	.957**	-0.02	.469**	-0.086
	P	0.844	0	0.85		0	0.85	0	0.422
LDL	r	0.007	.957**	-.306**	.957**	1	-.306**	.460**	-0.067
	P	0.95	0	0.004	0		0.004	0	0.534
VLDL	r	0.022	-0.02	1.000**	-0.02	-.306**	1	-0.052	-0.046
	P	0.836	0.85	0	0.85	0.004		0.628	0.667
glucose	r	0.147	.469**	-0.052	.469**	.460**	-0.052	1	-0.031
	P	0.17	0	0.628	0	0	0.628		0.773
adiponectin	r	-.238*	-0.086	-0.046	-0.086	-0.067	-0.046	-0.031	1
	P	0.025	0.422	0.668	0.422	0.534	0.667	0.773	

* Significant correlation at level 0.05 (2-tailed).
** Significant correlation at level 0.01 (2-tailed).