

Adiponectin (+276 G/T), Tumor Necrosis Factor-alpha (-308 G/A) and Interleukin-6 (-174 C/G) Genes Polymorphisms in Egyptian Type 2 Diabetic Patients

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Abstract

Type 2 diabetes mellitus (T2DM) is a metabolic pro-inflammatory disorder characterized by chronic hyperglycemia and increased levels of circulating cytokines. Adiponectin, tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), are important cytokines mediators in the pathogenesis of T2DM. The single nucleotide polymorphisms (SNPs) present in the regulatory regions of cytokine genes often have an impact on their expression levels.

Aim: Explore potential associations between SNP+276 G/T of adiponectin, SNP -308 G/A of TNF- α and SNP -174 C/G of IL-6 genes with T2DM and to assess its influence on their serum levels.

Subjects and Methods: From the Egyptian population, we enrolled 95 T2DM patients and 85 non-diabetic controls. Serum adiponectin, TNF- α and IL-6 were measured. Genotyping for three SNPs of the adiponectin, TNF- α and IL-6 genes was performed by polymerase chain reaction-restriction fragment length polymorphism.

Results: Subjects with the GT/TT genotype of SNP 276 were at increased risk for T2DM (OR=15.88, CI=7.56-33.31, $P \leq 0.01$) and associated with hypoadiponectinemia compared with the GG genotype. Furthermore, the allelic frequency of the A allele of SNP 308 was significantly different between T2DM patients compared to controls ($\chi^2=30.54$, $P \leq 0.0001$). Moreover, Individuals with T2DM carrying the GA/AA genotypes had significantly higher serum TNF- α levels than those carrying GG genotype. In addition, Carriers of G allele of IL-6 were significantly more likely associated with T2DM.

Conclusion: Genetics variations in Adiponectin +276 G/T, TNF- α 308 G/A and IL-6 -174 C/G may contribute to the disposition for T2DM in Egyptian patients.

Keywords: Single nucleotide polymorphism; Polymerase chain reaction-restriction fragment length polymorphism; Adiponectin +276 G/T; Tumor necrosis factor- α 308 G/A; Interleukin-6-174 C/G

Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder that causes significant morbidity and mortality. It affects about 258 million adults worldwide and is expected to increase to 439 million adults by 2030 indicating a growing burden of diabetes, particularly in developing countries [1]. In 2010, it was estimated that 4.787 million Egyptians (10.4% of the Egyptian population) had diabetes and that diabetes will increase to 8.615 million Egyptians by the year 2030 [2]. Insulin resistance (IR) is a major contributor to the pathogenesis of T2DM and plays a crucial role in the development of its associated complications [3]. Adipose tissue represents an active endocrine organ by releasing the large number of bioactive mediators (adipokines) that plays an important role in modulating glucose metabolism and inflammation [4]. Certain cytokines, like interleukin-6 (IL-6), tumor

necrosis factor (TNF- α), and some members of the adipocytokine family, namely adiponectin may represent the link between insulin resistance, T2DM and cardiovascular disease [5]. The single nucleotide polymorphisms (SNPs) present in the regulatory regions of cytokine genes often have an impact on their expression levels and can be disease modifiers [6]. The degree of inflammation is controlled, thereby leading to the progression of various immunological diseases, including T2DM [7].

Adiponectin is the most abundant gene product in adipose tissue also known as adipocytes complement-related protein of 30 KDa. It is involved in the homeostatic control of circulating glucose and lipid levels [8]. Adiponectin is encoded by adipocyte, C1q, and collagen domain containing (ACDC), which is located on chromosome 3 at q27, where genome-wide scans have revealed a susceptibility locus for T2DM, obesity, and metabolic syndrome [9]. One of the most commonly reported variants is the +276 G/T polymorphism in intron 2 [10]. Several researches explored potential associations of SNPs in

the adiponectin gene +276G/T with circulating plasma adiponectin levels, IR, and metabolic syndrome or diabetes [11].

The role of TNF- α in the pathogenesis of T2DM has been investigated in several studies. The TNF- α gene is located within the HLA III region in chromosome 6p21, and involved in inflammatory responses. This gene encodes a potent cytokine that has been implicated as an important factor in obesity-associated insulin resistance and pathogenesis of T2DM [12]. TNF- α -308 is a multifunctional cytokine primarily produced by macrophages and fat cells. It can directly inhibit phosphorylation of insulin receptors substrate and reduce glucose uptake by peripheral tissues [13]. Many studies have shown that SNP at position -308 G/A was associated with various inflammatory conditions including T2DM [14]. It is reported that -308 G/A polymorphism associates with either increased or decreased insulin resistance in patients with T2DM and patients without diabetes [15]. Because of controversial reports from several populations, there is still no definitive evidence of the association between this variation and risk for T2DM [16]. Hence, further studies in different population are needed to clarify the precise role of TNF- α -308 G/A polymorphism in susceptibility to T2DM.

Interleukin-6 is a proinflammatory cytokine secreted by immune cells, adipose tissue and muscles. The direct effect of IL-6 may be on glucose homeostasis and metabolism or it might act indirectly by action on adipocytes and pancreatic β -cells [17]. Augmented levels of IL-6 are associated with T2DM indicating a potential role of this cytokine in its etiology. There is a significant correlation between adipose IL6 mRNA expression and insulin resistance, These data suggest that IL6 is an appealing candidate gene for T2DM [18]. In humans, the gene for IL-6 maps to chromosome 7p15-p21. Studies on SNPs in the promoter region of IL-6 gene in different populations worldwide suggested its possible role in T2DM susceptibility [19]. Most studies focused on a single 5' promoter polymorphism -174C/G, which has been associated with an increased expression of IL-6 and also with inflammatory response and insulin sensitivity [20]. However, the reported associations between polymorphism -174C/G and diabetes risk are conflicting. Therefore, we attempted to analyze the association of IL-6 promoter polymorphisms -174 C/G with T2DM patients.

Considering the distinct ethnic feature of the Egyptian population, the high prevalence of T2DM in the population, and the absence of any report on adiponectin, TNF- α , and IL-6 gene polymorphisms in Egyptian T2DM patients, the present work was undertaken to explore whether adiponectin +276G/T, TNF- α -308G/A, and IL-6-174 C/G polymorphisms are associated with T2DM and their serum levels in Egyptian diabetic patients.

Subjects and Methods

Subjects

A total of 180 participants (96 male and 84 female) were enrolled in the study: 95 patients with T2DM and 85 age and sex matched non-diabetic healthy control subjects. 95 T2DM obese patients without complications were recruited from admitted patients of the internal medicine department of Suez Canal University Hospitals. 85 non diabetic subjects were randomly recruited from the local population Cairo city to act as control. They had normal fasting blood glucose levels, were not suffering any health problems, and had a negative history for T2DM and CVD. All participants gave their informed

consent prior to participation, and the study was conducted in accordance with the approval of the Ethics Committee of the Faculty of Pharmacy, Suez Canal University, Egypt. A detailed medical history and drug treatment(s) were collected for all subjects. The following exclusion criteria were used for all study participants: Patients suffering from T1DM, insulin treatment, thyroid, hepatic, acute infectious diseases, acute or chronic inflammatory disease, autoimmune disease, any hematologic disorder (assessed by complete blood count for every participant), and cancer were excluded from this study. Both the non-diabetic control group and the diabetic group were selected to have matching BMI and had a similar distribution of sex and age. The characteristics and biochemical data of patients and healthy controls are summarized in Table 1.

Variables	Control	T2DM
Sex	85 (45 M / 40 F)	95 (51 M / 44 F)
Age (years)	50.61 \pm 0.66	51.28 \pm 0.55
BMI (Kg/m ²)	32.52 \pm 0.22	33.16 \pm 0.30
D.M Duration (years)		3.7 \pm 0.16
FBG (mg/dL)	87.02 \pm 1.13	200.7 \pm 5.82 ^a
Serum Insulin (μ IU/mL)	7.62 \pm 0.28	16.55 \pm 0.48 ^a
HOMA-I.R index	1.62 \pm 0.06	8.38 \pm 0.38 ^a
Serum TAG (mg/dL)	113.6 \pm 2.20	163.9 \pm 3.05 ^a
Serum TC (mg/dL)	164.3 \pm 1.80	227.7 \pm 3.19 ^a
Serum HDL-C (mg/dL)	50.53 \pm 0.57	35.71 \pm 0.92 ^a
Serum LDL-C (mg/dL)	89.37 \pm 1.81	157.6 \pm 3.4 ^a
Serum Adiponectin (μ g/mL)	24.78 \pm 0.61	12.7 \pm 0.40 ^a
Serum IL-6 (pg/mL)	3.21 \pm 0.10	6.56 \pm 0.11 ^a
TNF- α (pg/mL)	12.77 \pm 0.31	25.83 \pm 0.48 ^a
T2DM: Type 2 Diabetes Mellitus; BMI: Body Mass Index; FBG: Fasting Blood Glucose; TAG: Tri Acyl Glycerol; TC: Total Cholesterol; HDL-C: High-Density Lipoprotein; LDL-C: Low-Density Lipoprotein Cholesterol; IL-6: Interleukine-6; TNF- α : Tumor Necrosis Factor-Alpha.		
Data are given as mean + SEM		
^a Significant difference from control group at P<0.05		

Table 1: Clinical and hemodynamic characteristics of participants and comparing Adiponectin, TNF- α and IL-6 in the studied groups.

Blood sampling and laboratory assays

About 10 mL of fasting venous blood was obtained from all participants. Aliquots of blood were collected on EDTA for estimation of plasma glucose and extraction of DNA. The remaining portion of the blood samples were collected in serum separation tubes for determination of insulin, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and triacylglycerol (TAG) using standard laboratory methods. Serum adiponectin, IL-6, and TNF- α levels were estimated using enzyme-linked immunoassay kits. (Adipo Gen Inc., Incheon, Korea; RayBio, Parkway, USA; and ALPCO Diagnostics, Keewaydin,

USA, respectively). The homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated from fasting insulin and glucose levels as described by Matthews [21].

Genotyping of polymorphisms

Genomic DNA was extracted from whole blood using DNA extraction kit and stored at -80°C in aliquots until required. This was done using TIANamp Genomic DNA kit (Beijing, China) according to the manufacturer's instructions. The concentration of the extracted DNA was determined by using Qubit 2.0 Fluorometer.

Genotyping of Adiponectin SNP 276 G/T Polymorphism

The genotype for adiponectin polymorphism 276 G/T was determined by PCR-RFLP. PCR products and the digest product were resolved by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. PCR primer sequences were used as following: forward primer, 5'- GGC CTC TTT CAT CACAGA CC -3' and the reverse primer, 5'- AGA TGC AGC AAA GCC AAAGT -3'. Each reaction contained 25 µL final volumes consisting of, 250 ng genomic DNA, 200 µM dNTPS, 0.5 unit of DNA polymerase (DyNAZyme II, FINZYMES) and 20 pmol of each primer. The thermocycling conditions consisted of initial denaturation at 94°C for 10 minutes, followed by 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and the final extension at 72°C for 10 minutes. The PCR products were digested with the restriction enzyme, Mva 1269I at 37°C for 15 minutes (Fermentas, Germany) [Fragment size: 196 bp for wild type allele (GG); 196, 148 and 48 bp for heterozygous allele (GT) and 148 and 48 bp for homozygous mutant type allele (TT)] (Figure 1).

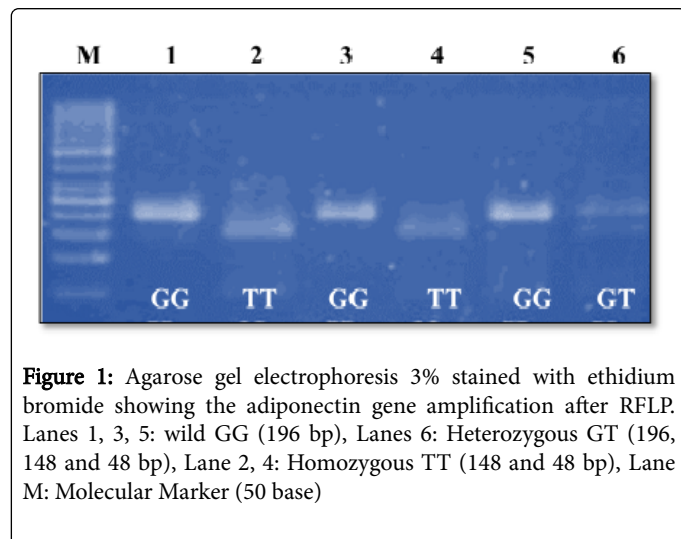


Figure 1: Agarose gel electrophoresis 3% stained with ethidium bromide showing the adiponectin gene amplification after RFLP. Lanes 1, 3, 5: wild GG (196 bp), Lanes 6: Heterozygous GT (196, 148 and 48 bp), Lane 2, 4: Homozygous TT (148 and 48 bp), Lane M: Molecular Marker (50 base)

Genotyping of TNF-α-308 (G/A) Polymorphism

Genotyping of TNF-α -308 G/ A polymorphisms in genomic DNA was performed using PCR and RFLP method using the primer pair 5'- AGGCAATAGGTTTTGAGGGCCAT-3' (forward) and 5'- TCCTCCCTGCTCCGATTC CG-3' (reverse). PCR assay was performed in a 25 µL volume with 100 ng DNA, 100 µM dNTPs, 20 pmol of each primer, 1.5 mM MgCl₂, 1X PCR buffer with (NH₄)₂SO₄ (Fermentas, Vilnius, Lithuania,), 10% DMSO and 2U Taq DNA polymerase (Fermentas, Vilnius, Lithuania). Amplification was performed on an automated Thermal Cycler (Techne Flexigene,

Cambridge, UK). PCR conditions were: 2 min for initial denaturation at 95°C; 35 cycles at 95°C for 45 sec for denaturation, 1 min at 60°C for annealing and 90 sec at 72°C for extension, followed by 7 min at 72°C for final extension. After the amplification, PCR products were digested by 10U NcoI restriction endonuclease (Fermentas, Vilnius, Lithuania) for 14 h at 37°C, and genotyping of the TNF-α gene was performed by fragment separation at 120 V for 40-50 min on a 3.5% agarose gel containing 0.5 µg/ml ethidium bromide. PCR for the TNF-α gene gave a 107-bp fragment for the 308 G/A polymorphism in the TNF gene, generating a restriction pattern of 87 bp and 20 bp when the cleavage site is present (Figure 2).

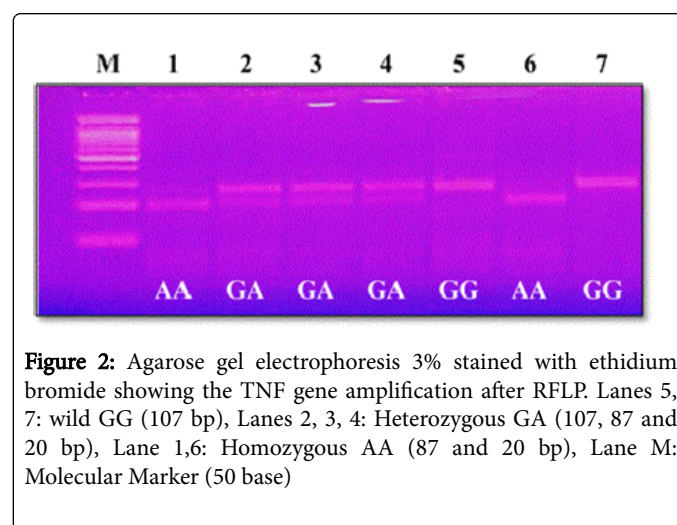


Figure 2: Agarose gel electrophoresis 3% stained with ethidium bromide showing the TNF gene amplification after RFLP. Lanes 5, 7: wild GG (107 bp), Lanes 2, 3, 4: Heterozygous GA (107, 87 and 20 bp), Lane 1,6: Homozygous AA (87 and 20 bp), Lane M: Molecular Marker (50 base)

Genotyping of IL-6 174 C/G polymorphism

Genotyping of IL-6 174 C/G polymorphisms in genomic DNA was performed using PCR and RFLP. To identify the -174G/C polymorphism, a 198 bp fragment was amplified using the forward primer 5'TGACTTCAGCTTTACTCTTTGT 3' and the reverse primer 5' CTGATTGGAAACCTTATTAAG 3'; the conditions of the reaction were as follows: denaturalization at 94°C for 60 seconds, followed by 35 cycles of annealing at 53°C for 1 minute and 20 seconds, extension at 72°C for 1 minute and 20 seconds, and a final elongation step extension at 72°C for 5 minutes. The PCR fragments (198 bp) were digested using the restriction enzyme Nla III. Each PCR product was electrophoresed on a 3% polyacrylamide gel stained with silver nitrate. After enzymatic digestion of the amplified fragment for the -174 C/G polymorphism, we were able to identify the different GG (148 pb and 50 bp), CG (198 bp, 148 bp, and 50 bp), and CC (198 bp) genotypes (Figure 3).

Statistical Analysis

The distribution of the alleles of SNP Adiponectin +276 G/T, TNF-α 308 G/A and IL-6-174 C/G was tested for Hardy-Weinberg equilibrium (P>0.05). Proportions of genotypes of alleles were compared by X² analysis, Odd ratios (ORs) and 95% confidence intervals (CI). Descriptive statistics were computed for all variables. The results were expressed as means ± SEM. To determine the statistical significance of laboratory findings, multiple comparisons were achieved using analysis of variance (ANOVA) followed by Bonferroni post hoc analysis. Differences in serum adiponectin, TNF-α and IL-6 concentrations between individuals with different genotypes

were tested by Student's t-test. The significance level was set at 0.05 or less. All statistical analyses were performed with SPSS, version 17.0 (SPSS Inc.).

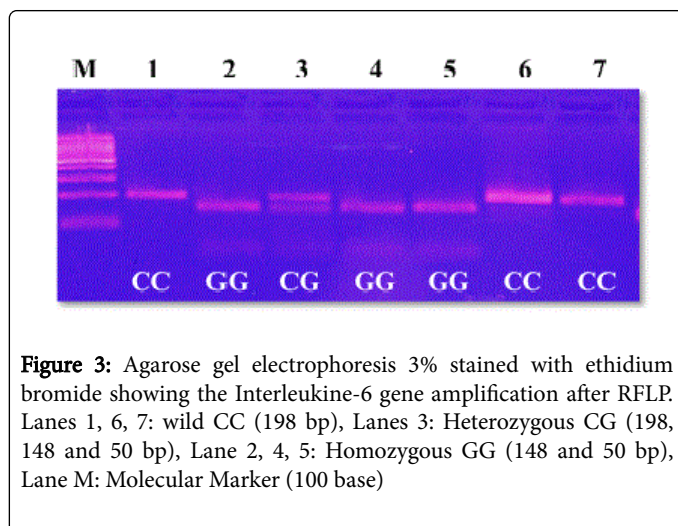


Figure 3: Agarose gel electrophoresis 3% stained with ethidium bromide showing the Interleukine-6 gene amplification after RFLP. Lanes 1, 6, 7: wild CC (198 bp), Lanes 3: Heterozygous CG (198, 148 and 50 bp), Lane 2, 4, 5: Homozygous GG (148 and 50 bp), Lane M: Molecular Marker (100 base)

Results

Clinical and biochemical characteristics of the study subjects

As shown in Table 1, there was no significant difference in the age, BMI, or sex distribution between the different study groups. T2DM showed significantly higher levels of FBG, insulin, and HOMA-IR compared with healthy individuals. Among the lipid profiles, the levels of TAG, TC, and LDL-C were significantly higher in T2DM patients than controls. Whereas levels of HDL-C were lower in T2DM patients than controls.

In the present study the serum adiponectin level in T2DM patients showed significant decrease to $12.7 \pm 0.40 \mu\text{g/mL}$ than the healthy control group $24.78 \pm 0.61 \mu\text{g/mL}$ ($P \leq 0.05$). Regarding interleukin levels, T2DM group had significantly elevated levels of interleukin compared to non-diabetic control group ($7.42 \pm 0.14 \text{ pg/mL}$ and $3.21 \pm 0.10 \text{ pg/mL}$, respectively) at $P < 0.05$ (Table 1). Furthermore, our study showed that the mean serum levels of TNF- α were significantly increased in the T2DM patients group ($25.83 \pm 0.48 \text{ pg/mL}$) than the controls ($12.77 \pm 0.31 \text{ pg/mL}$) at $P \leq 0.05$.

In Pearson's correlation analyses, adiponectin levels were negatively correlated with interleukine-6 ($r = -0.672$, $P = 0.0001$) and TNF- α ($r = -0.773$, $P = 0.0001$). However, interleukine-6 levels were positively correlated with TNF- α ($r = 0.588$, $P = 0.0001$) as shown in Figure 4.

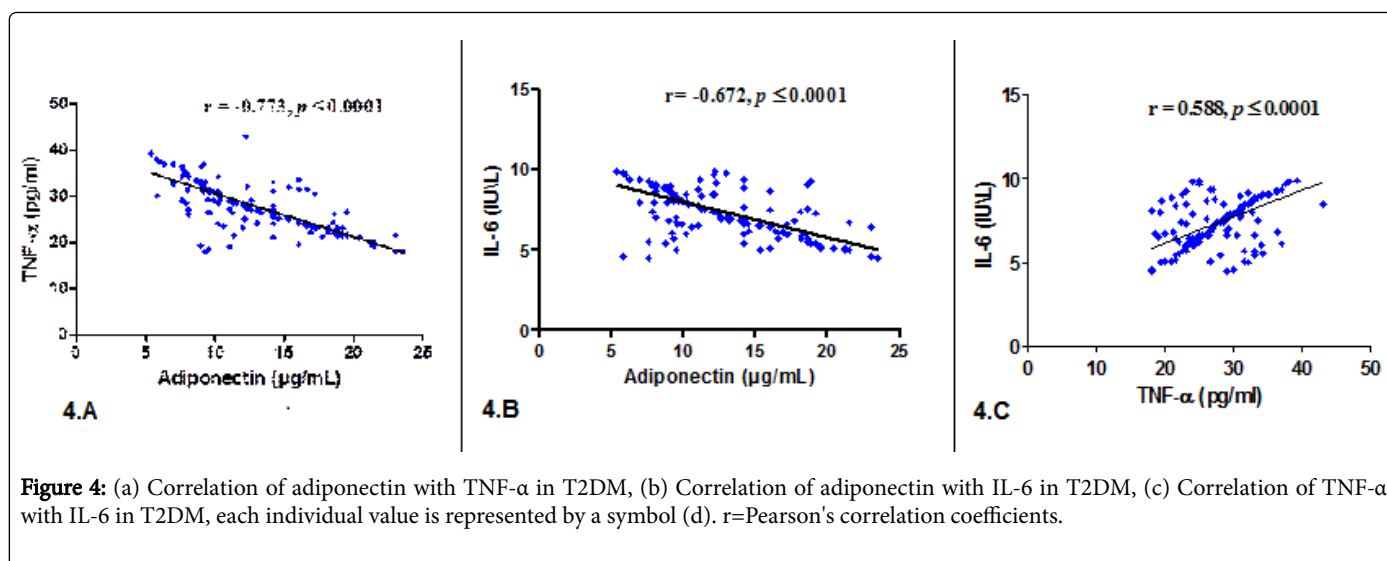


Figure 4: (a) Correlation of adiponectin with TNF- α in T2DM, (b) Correlation of adiponectin with IL-6 in T2DM, (c) Correlation of TNF- α with IL-6 in T2DM, each individual value is represented by a symbol (d). r = Pearson's correlation coefficients.

Genotypes and allele distribution of Adiponectin 276 G/T

The observed genotype distributions of Adiponectin 276 G/T SNP agreed with those expected from Hardy-Weinberg equilibrium in all study groups. Results presented in Table 2 indicated that the frequencies of adiponectin 276 GT/TT genotype and T allele were significantly higher in T2DM patients than controls (75.8% versus 16.5%) $\chi^2 = 94.87$, $P \leq 0.0001$ and (41.6% versus 10%) $\chi^2 = 59.87$, $P \leq 0.0001$ respectively.

Association between Adiponectin polymorphisms with Type 2 diabetes mellitus

As indicated in Table 2 Subjects with the GT/TT genotype and T allele were at increased risk for T2DM ($OR = 15.88$, $CI = 7.56-33.31$, $P \leq 0.01$) and ($OR = 6.41$, $CI = 3.59-11.42$, $P \leq 0.01$) respectively compared with those having the GG genotype and G allele.

Groups	n	Genotype frequency		OR	95% CI	Allele frequency		OR	95% CI
		GG	GT + TT			G	T		
Control	85	71 (83.50%)	14 (16.50%)			153 (90%)	17 (10%)		
T2DM	95	23 (24.20%)	72 (75.80%)	15.9	7.56-33.31	111 (58.40%)	79 (41.60%)	6.41	3.59-11.42
						$\chi^2= 94.87, P=0.0001$			
Abbreviations: OR: Odds Ratio; 95% CI: 95% Confidence Interval									
Results are expressed as number and in parentheses percent; P < 0.05 is considered statistically significant after adjusting for age, sex, and BMI.									
χ^2 -test was performed to compare categorical data.									

Table 2: Differences in allele distribution and genotype frequency of adiponectin gene single nucleotide polymorphism (SNP) Adiponectin 276 G/T between control and TDM groups.

The relation between Adiponectin 276 G/T polymorphism and serum adiponectin levels

As illustrated in Figure 5A Serum adiponectin levels were significantly lower, according to the presence of the T allele in different study groups. The present study revealed that serum level of adiponectin in patients with GT/TT genotype carriers (n=72) in the T2DM without complications was significantly lower than those with the GG genotype (n=23) (11.44 ± 0.35 µg/mL and 16.53 ± 0.82 µg/mL, respectively) (P ≤ 0.05). Likewise the serum adiponectin level of GT/TT genotype carriers (n=14) was significantly lower from that of the GG genotype (n=71) in the healthy control (21.54 ± 2.27 µg/mL and 25.43 ± 0.56 µg/mL, respectively) (P ≤ 0.05).

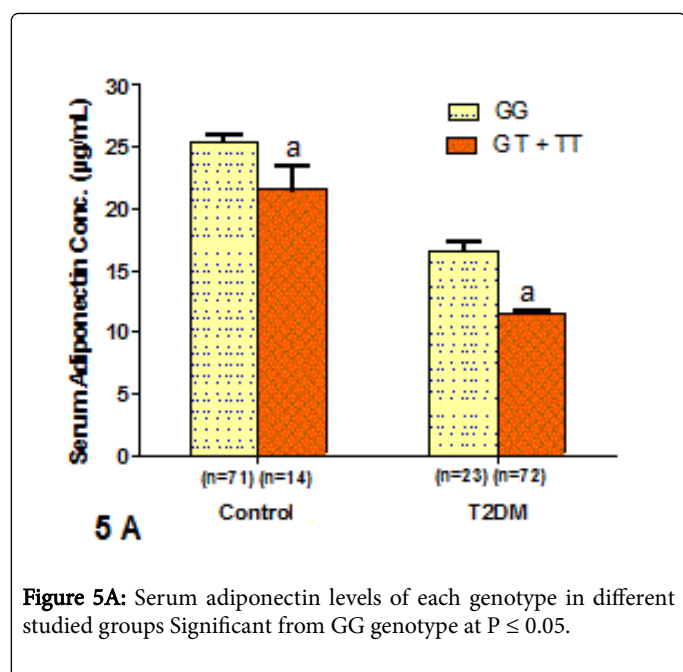


Figure 5A: Serum adiponectin levels of each genotype in different studied groups Significant from GG genotype at P ≤ 0.05.

Genotypes and allele distribution of TNF-α 308 G/A

Results presented in Table 3 revealed significantly higher frequencies of TNF-α 308 GA/AA genotype and A allele in T2DM patients without complications and with CVD compared to controls (58.9% versus 18.8%) $\chi^2=38.66, P \leq 0.0001$ and (34.2% versus 11.8%)

$\chi^2=30.54, P \leq 0.0001$ respectively. The observed genotype distributions of TNF-α 308 G/A SNP agreed with those expected from Hardy-Weinberg equilibrium in all study groups.

Association between TNF-α 308 G/A polymorphisms with Type 2 diabetes mellitus

Carriers of GA/AA genotype and A allele of TNF-α were significantly more likely associated with T2DM (OR=6.19, CI=3.13-12.23, P ≤ 0.001) and (OR= 3.9, CI= 2.24- 6.79, P ≤ 0.001) respectively as shown in Table 3.

Group s	n	Genotype frequency		OR	95% CI	Allele frequency		OR	95% CI
		GG	GA+AA			G	A		
Control	85	69(81.20%)	16 (18.80%)			150(88.20%)	20(11.80%)		
T2DM	95	39(41.10%)	56(58.90%)	6.19	3.13-12.23	125 (65.80%)	65 (34.20%)	3.9	2.24-6.79
						$\chi^2= 38.66, P= 0.0001$			
						$\chi^2= 30.54 P= 0.0001$			
Abbreviations: OR: Odds Ratio; 95% CI: 95% Confidence Interval									
Results are expressed as number and in parentheses percent. P<0.05 is considered statistically significant after adjusting for age, sex, and BMI. Chi squared (χ^2) test was performed to compare categorical data.									

Table 3: Differences in allele distribution and genotype frequency of adiponectin gene single nucleotide polymorphism (SNP) TNF-α 308 G/A between control and T2DM groups.

The relation between TNF-α 308 G/A polymorphism and serum TNF-α levels

GA/AA genotype carriers (n=56) of T2DM patients without complications exhibited significantly higher serum TNF-α values (27.75 ± 0.56 pg/mL) than GG (n=36) (23.06 ± 0.63 pg/mL). Similarly, controls with GA/AA genotype (n=16) showed increased levels of serum TNF-α (15.51 ± 0.66a pg/mL) compared with GG (n=69) (12.14 ± 0.31 pg/mL) as shown in Figure 5B.

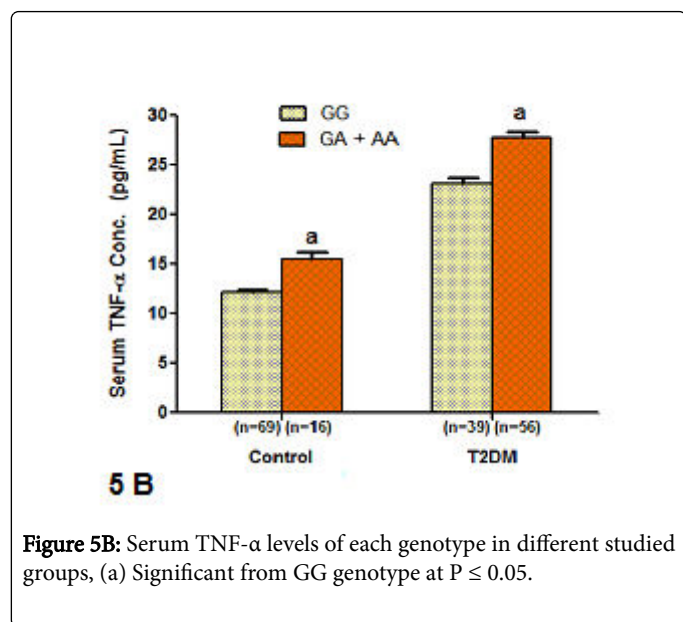


Figure 5B: Serum TNF- α levels of each genotype in different studied groups, (a) Significant from GG genotype at $P \leq 0.05$.

Genotypes and allele distribution for SNPs Interleukin-6 174 C/G

Table (4) revealed significantly higher frequencies of interleukin-6 174 CG/GG genotype and G allele in T2DM patients without complications compared to controls (63.2% versus 30.6%) $X^2=35.27$, $P \leq 0.0001$ and (37.9% versus 18.3%) $X^2=23.58$, $P \leq 0.0001$ respectively. The observed genotype distributions of 174 C/G Interleukin-6 SNP agreed with those expected from Hardy-Weinberg equilibrium in all study groups.

Association between IL-6 polymorphisms with Type 2 diabetes mellitus

Carriers of CG/GG genotype and G allele of IL-6 were significantly more likely associated with T2DM (OR= 3.89, CI=2.08-7.24, $P \leq 0.001$) and (OR=2.73, CI=1.68- 4.45, $P \leq 0.001$) respectively as shown in Table 4.

Groups	n	Genotype frequency		OR	95% CI	Allele frequency		OR	95% CI
		CC	CG + GG			C	G		
Control	85	59 (69.4%)	26 (30.6%)			139 (81.7%)	31 (18.3%)		
T2DM	95	35 (36.8%)	60 (63.2%)	3.89	2.08-7.24	118 (62.1%)	72 (37.9%)	2.7	1.68 -4.45
						$X^2= 35.27$, $P = 0.0001$		$X^2= 23.58$ $P = 0.0001$	

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval

Results are expressed as number and in parentheses percent. $P < 0.05$ is considered statistically significant after adjusting for age, sex, and BMI. Chi squared (X^2) test was performed to compare categorical data.

Table 4: Differences in allele distribution and genotype frequency of interleukine gene single nucleotide polymorphism (SNP) IL-6 174 C/G between control and T2DM groups.

The relation between IL-6 174 C/G polymorphism and serum IL-6 levels

As demonstrated in Figure 5C, serum IL-6 levels were higher but not significantly, according to the presence of the G allele in different study groups. The highest serum IL-6 values (6.71 ± 0.13 pg/mL) were observed in CG/CC genotype carriers (n=60) among T2DM patients group. While the CC (n=35) genotypes showed lower value (6.30 ± 0.18 ng/mL). Meanwhile, controls with CG/CC genotype (n=26) showed increased levels of serum IL-6 (3.27 ± 0.19 pg/mL) followed by CC (n=59) (3.19 ± 0.12 pg/mL).

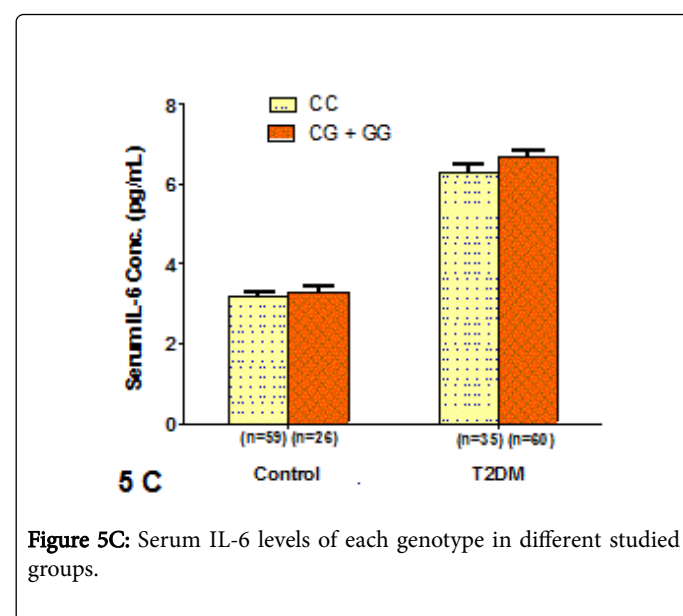


Figure 5C: Serum IL-6 levels of each genotype in different studied groups.

Discussion

In the present study, the relations between adiponectin 276 G/T, TNF- α 308 G/A and IL-6 174 C/G SNPs and T2DM were studied in Egyptian diabetic patients. Adipose tissues express a variety of secretory proteins of potential importance for metabolic and vascular diseases. Adiponectin is an adipose specific cytokine that is abundant in blood. Adiponectin plays important roles in the regulation of insulin action and metabolisms of glucose and fatty acid [22]. Increasingly evidences indicate that adiponectin may also have anti-inflammatory and antiatherogenic properties [23]. And recently, data support a strong linkage between the SNP of adiponectin gene and the risk of T2DM and the 276 G/T polymorphism is one of the most commonly concerned one [24]. In the current study, our results showed significantly higher frequencies of adiponectin 276 GT/TT genotype and T allele in T2DM patients compared to the controls. Furthermore, association of the GT/TT genotype and T allele with T2DM was observed in Egyptian diabetic patients, suggests the effect of adiponectin 276-T allele on increased the risk of T2DM 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 [25]. Adiponectin increases lipid oxidation and directly affects the insulin signaling pathway both at and beyond the receptor level [26]. In addition, adiponectin inhibits gluconeogenesis and the release of TNF- α by adipose tissue [27].

Our results were in harmony with those of Melistas et al. [11], Yang et al. [28], Salmenniemi et al. [29], and Mackawy et al. [26], 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. Opposing also to our findings, other studies have provided that the 276 T 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 [30,31]. These conflicting association results in various populations suggesting a complex relationship between adiponectin 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 [26].

In the present study, patients with T2DM are carrying the T allele of SNP 276, a putative at risk allele, exhibited much lower plasma adiponectin concentrations than T2DM patients carrying the G allele. This suggests that the SNP 276 polymorphism may affect IR, possibly through changes in mRNA stability, levels of adiponectin and eventually reduced plasma adiponectin concentrations. Our findings are similar those reported by Filippi et al. [32]. Gui et al. [10] and recent study of Ramya et al. [33]. revealed that the TT genotype of +276 G/T variant was observed to be significantly associated with hypoadiponectinemia. In contrast to these results other studies showed association of the TT allele of the SNP 276G/T with increased adiponectin levels has previously been reported in diabetic or non-diabetic individuals [34]; however this association is not universally accepted [35]. Diabetic subjects showed that plasma adiponectin levels are significantly higher in TT homozygous T2DM patients, in accord to similar findings in the literature [36]. In addition other studies dealt

with 276 G/T allele, it was found that T allele of this variant was related with higher levels of serum adiponectin and was a protective factor for diabetes, coronary heart disease, hypertension, or dyslipidemia in American [37], Finland [38], Japanese, and Korean [39] populations. However, the conclusions in different populations are somehow controversial.

Inflammation has been widely known as an important feature of T2DM, with high levels of proinflammatory cytokines, including IL-1, IL-6, and TNF- α . Because TNF- α can impair insulin signal pathways and lead to β cell destruction, increased TNF- α is considered to play a central role in the development of T2DM [40]. So far, many studies have focused on the association between -308 G/A TNF- α promoter polymorphism and T2DM, but the results were still controversial [15,16].

In our study our results revealed significantly higher frequencies of TNF- α 308 GA/AA genotype and A allele in T2DM patients compared to controls. Furthermore, association of the GA/AA genotype and A allele with T2DM was observed in Egyptian diabetic patients. The current results agreed with the findings of previous studies that revealed significantly higher GA/AA genotype and A allele frequencies among Chinese [41], French [42], Scandinavian [15], Germans [43] and Australians [44] populations.

There are some points should be concerned for the inconsistent results. Furuta et al. [45] did not find any difference in allele frequencies of the -308 G/A TNF- α polymorphism among patients with noninsulin-dependent diabetes mellitus and healthy controls in Japanese patients [45] and Greek population [7]. In another study, there was not any significant difference in the A-allele of this variant between case and control groups [13] Kubaszek et al. [46] reported, in their study among the Finnish population, that the A-allele of the -308 G/A TNF- α variation was associated with twofold higher risk for T2DM, and was also a predictor for the conversion from impaired glucose tolerance to T2DM.

In our study, patients with T2DM carrying the GA + AA genotypes of -308 G/A TNF- α variation had significantly higher serum TNF- α levels, as compared with the carriers of GG genotype. In agreement with our results, Heijmans et al. [47] reported that individuals carrying the AA homozygous genotype (TNF hyper producer) have a 4.6-times greater risk of presenting with T2DM than an individual with the GG homozygous genotype (TNF hypo producer). In addition other studied revealed that the -308A allele had been shown to increase the transcription and expression of TNF- α and was also found to inhibit insulin signaling and impair insulin secretion [48]. However, our results are in contrast with the results from the Taiwanese population [49] we also found that the dominant model of -308 G/A TNF- α polymorphism be significantly associated with T2DM. This result was in accordance with the results from Caucasian and Asian populations [16].

There is compelling evidence that augmented levels of IL-6 are associated with type 2 diabetes [18]. The main finding of this study reveals significantly higher levels of IL-6 in diabetic group than the control subjects Similar to previously reported studies. It suggests that IL-6 being an inflammatory mediator might be responsible for insulin resistance which may contribute for the development of T2DM [50].

Earlier studies on the association of -174 C/G SNP with T2DM and IR have demonstrated varied results among different populations. Analyses of small cohorts of native Americans and Spanish Caucasians [51], Brazilian [52], Polish [53] and German [54] populations, showed

the 'G' allele of -174 C/G SNP to be associated with higher risk of T2DM, but this SNP was not linked with diabetes in the Finnish Diabetes Prevention Study [46] and Australian population [55].

In the current study, higher IL-6 -174 C/G genotype frequency and G allele distribution were observed in T2DM patients than in healthy control. Besides, analysis of the association of IL-6 -174 C/G polymorphism with T2DM revealed that CG/GG genotype and G allele were significantly associated with the T2DM among Egyptian diabetic patients compared with controls. The current results agreed with the findings of Qi et al. [56] and the recent study of Saxena et al. [19] that reported that the G allele of this polymorphism was associated with an increased risk of type 2 diabetes. In contrast to these results, a previous study found that genotype GG might be protective for the disease [57].

It has been documented that polymorphism 174 C/G affects the transcriptional activity of IL6 gene. However, data on the effects of this polymorphism on IL-6 levels in vivo have led to conflicting results [58]. Our results do not support an effect of polymorphism 174 C/G on plasma IL-6 levels. Patients with T2DM carrying the CG + GG genotypes of IL-6 174 C/G variation had not significantly higher serum IL-6 levels as compared with the carriers of CC genotype which is in harmony with [56]. Nevertheless, we cannot exclude the possibility that this polymorphism may affect the adipose abundance of IL-6, as suggested by Yang et al. [59].

Our data suggest that IL-6 gene polymorphisms play a prominent role in determining susceptibility to T2DM. The present study provides a lead to the contribution of cytokine gene heterogeneity to the susceptibility and development of T2DM but it is essential to find out the degree of association in an individual population. Also, we did not find significant associations between IL6 polymorphisms and plasma IL-6 levels.

Conclusion

It was found that the incidence of T2DM is higher in T allele carriers of adiponectin SNP 276 associated with hypoadiponectinemia compared to the GG genotype. Besides, higher frequencies of T2DM cases were carriers of the TNF- α 308 A allele than controls, these cases are high producers of TNF- α as the -308 A allele had been shown to increase the transcription and expression of TNF- α and was correlated with the prevalence of T2DM. Moreover, carriers of G allele of IL-6 SNP 174 C/G is associated with the risk of development of T2DM in Egyptian population, while the serum IL-6 concentration of CG+GG genotype carriers was not significantly different from that of the CC genotype in the diabetic group suggesting that this polymorphism does not affect IL-6 transcription.

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