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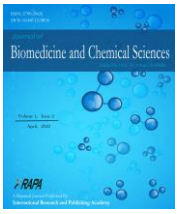

Association of ADIPOQ (rs 2241766) Gene Polymorphism with Type 2 Diabetes Mellitus Patients:

A Case-Control Study

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ABSTRACT

The adiponectin gene (ADIPOQ) is the main genetic determinant of plasma adiponectin amounts from (30 - 70%) of the inherited genetic components. Multiple nucleotide polymorphisms (SNPs) rs 2241766 for the ADIPOQ gene correlate with metabolic syndromes such as insulin resistance, abdominal obesity, impaired glucose tolerance, hyperlipidemia, hypertension, rapid glucose excess, and plasma adiponectin level. A polymorphism study was conducted based on PCR 2241766 in Al-Diwaniyah city in Iraq, and this study included (300) people aged (30-45) years who were distributed into two groups, the first group (G1) included (150) control, and the second group (G2) included (150) type 2 diabetes mellitus patients. The purpose of the current study was to reveal the ADIPOQ polymorphism and its role in the pathophysiology of type 2 diabetes mellitus. The overall genotype of the ADIPOQ rs2241766 gene was significantly different between the type 2 diabetes mellitus patients (G2) and control (G1) for the genotype GG (x2 = 13.45, p-value = 0.002), G allele (x2 = 10.324, p-value = 0.001), and TG & TT compared to the GG genotype (x2 = 13.266, p-value = 0.0001). In conclusion, the results of the genotype and allele distribution of ADIPOQ rs2241766 gene in type 2 diabetes patients group showed that there is an association between ADIPOQ rs2241766 gene and type 2 diabetes mellitus.

1. Introduction

Type 2 Diabetes Mellitus (T2DM) is a complex chronic disease associated with a state of high blood glucose level, or hyperglycemia, occurring from deficiencies in insulin secretion, action, or both (Bigagli & Lodovici, 2019). T2DM happened when β-cell secretion is unable to overcome insulin resistance (Hussain & Ali, 2016), insulin sensitivity, blood glucose imbalance, where there is an excess of the insulin secretion cycle in the β-cells found in Langerhans islets in the pancreas, which leads to poor regulation of blood sugar levels and an increased risk of complications from T2DM. Also, hyperglycemia, hypertriglyceridemia, and hyperinsulinemia lead to T2DM (Farr & Khosla, 2016). T2DM can lead to death as it greatly affects some parts of the body due to its complications (Owusu Adjah, et al., 2018).

Complications include cardiovascular disease, cerebrovascular disease, and peripheral arterial disease, and microvas cular complications such as nephropathy, retinopathy, neuropathy, myocardial infarction, coronary artery disease (Zheng, Ley & Hu, 2018), physical inactivity, weight loss, insulin resistance, infectious complications, Postoperative cardiac events peripheral artery disease (Mezza et al., 2019), peripheral neuropathy, and organ resection, morbidity, paralysis, and amputation of limbs such as leg amputation (Murray & Coleman, 2019). Adiponectin is a protein hormone that modulates several metabolic processes, including glucose regulation and fatty acid oxidation (Tura et al., 2017). High-molecular-weight adiponectin was further found to be associated with a lower risk of diabetes with a similar magnitude of association as total adiponectin (Fiaschi, 2019). Polymorphisms of adiponectin is associated with high blood pressure,

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cardiovascular disease, and has anti-inflammatory and antiviral effects (Kao and Sabin, 2016). Multiple nucleotide polymorphisms (SNPs) rs 2241766 for the ADIPOQ gene correlate with metabolic syndromes such as insulin resistance, abdominal obesity (Yaribeygi et al., 2019), impaired glucose tolerance, hyperlipidemia, hypertension, rapid glucose excess, and plasma adiponectin level (Wang et al., 2019).

The current study aimed to studying the ratios of alleles and genotypes of the ADIPOQ gene in samples of healthy subjects (Control) and patients with type 2 diabetes mellitus and identifying the alleles and genotypes that increase the risk of developing type 2 diabetes mellitus.

2. Materials and Methods

2.1. Study Population

The study population consisted of (300) people of both genders (males and females), non-smokers and non-obese, their ages ranged between (30-45) years, who attended Al-Diwaniyah Teaching Hospital in Al-Diwaniyah, Iraq. The study population consisted of 150 patients with type T2DM (G2), and 150 healthy controls (G1). The gender of the people in both groups(G1 and G2) was 75 of them males and 75 of them females.

2.2. Extraction of DNA

Genomic DNA was extracted from whole blood using a DNA Extraction Kit (Bioneer/Korea), were equal to or less than 20 µg/ml. The concentration of extracted DNA and its purity were estimated by measuring the absorbance at A 260 nm and A 280 nm by the Nanodrop device. The concentration of the DNA samples was (50 ng/µL), and the purity of the DNA samples (1.8 µg/ml).

2.3. PCR Amplification

Isolated DNA is amplified with specific primers, as shown in "Table.1".

Table 1
The name and sequence and melting point of prepared ADIPOQ (rs 2241766) gene polymorphism

Gene Name	SNP name	Sequence (5'–>3')	Peak	Tm(°C)
(ADIPOQ)	rs 2241766	F 5- GCAGCTCCTAGCCGTAGACTCTGCTG -3 R 5- GGAGGTCTGTGATGAAAGAGGCC -3	372	54.2
		FG 5- TGCTATTAGCTCTGCCCGAG -3 FT 5- TGCTATTAGCTCTGCCCGAT -3	226	

F: Forward, R: Reverse, FT: Forward of the T allele, and FG: Forward of the G allele.

The prefixes were designed by the Korean company Pioneer for all genes used in this study as shown in "Table.1". All were dried and prepared with high purity H2O (Bioneer, Korea) according to the manufacturer's instructions, and all were split and kept at - 20 °C. The PCR products were run on 2% agarose gel electrophoresis. The different fragments obtained were homozygous TT (FT) genotypes (372 & 226 bp); heterozygous TG (FT+FG) genotypes (372, 226 & 226 bp); and homozygous GG (FG) genotypes (372 & 226 bp).

2.4. Statistical Analysis

Statistical analysis between allele frequencies and genotype distributions for the four groups was confirmed by descriptive statistic at (p-value < 0.05) using SPSS 21 software by mathematical calculation of odds ratio (OR), as well as for confidence intervals (95% CI) and for x2 values. The comparison between groups for biochemical and immunohistochemical tests was performed by means, of a double sample, T-test at (p-value < 0.05) as it was a significant difference. The statistical analysis between the allele frequencies and genotype distributions of ADIPOQ (rs 2241766) gene Polymorphisms in the two groups (Control G1, and T2DM patients G2) was confirmed by the descriptive statistics at (p-value < 0.05).

3. Results & Discussion

3.1. Amplification of ADIPOQ (RS 2241766) Gene

Adiponectin plays a major role in the development of T2DM due to its special relationship to increased insulin response, enhanced β-cell dysfunction in pancreatic, Langerhans (Davis et al., 2015). The ADIPOQ gene consists of the primers (F, R, FG, and FT), and when performing a PCR assay for these primers and for a set of eight DNA samples, Gel documentation showed that the primers (FG, FT) give the same bands that appeared at (226 bp). To distinguish between primer bundle (FG) and primer bundle (FT), an assay was carried out for primer (FG) in isolation from primer (FT) by taking primers for the gene as follows: Outer + FT (F, R, and FT) and Outer + FG (F, R, and FG) when conducting an examination of all DNA samples and their purpose to prevent the interference of primer-generated (FT) bands with primer-generated (FG) bands. Amplification of ADIPOQ (rs 2241766) gene polymorphism is shown in "Fig. 1, and 2".

3.1.1. The Genotype of the ADIPOQ (rs 2241766) Gene in the Control Group (G1)

The genotype frequencies and allele distributions of rs 2241766 polymorphisms of ADIPOQ gene for the Control (G1) group that are shown in "Table. 2", are calculated from Figure 1.

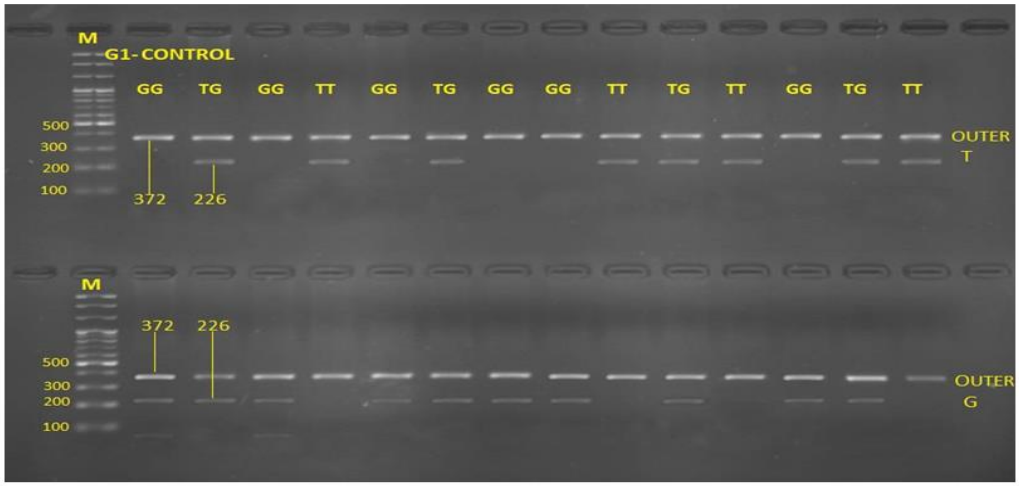

Fig. 1. Gel electrophoresis of ADIPOQ (rs 2241766) gene polymorphism amplified with a specific pair of primers using conventional PCR for control group (G1). M: DNA ladder (100-3000 bp). The PCR products were stained with safe stain dye. homozygous TT genotypes (372 and 226 bp) of FT; heterozygous TG genotypes (372, 226, and 226 bp) of FT+FG; homozygous GG genotypes (226 bp) of FG.

3.1.2. The Genotype of the ADIPOQ (rs 2241766) Gene in the Type 2 Diabetes Mellitus Group (G2)

The genotype frequencies and allele distributions of rs 2241766 polymorphisms of ADIPOQ gene for the Type 2 diabetes mellitus (G2) group that are shown in Table (3.1) are calculated from "Figure 2".

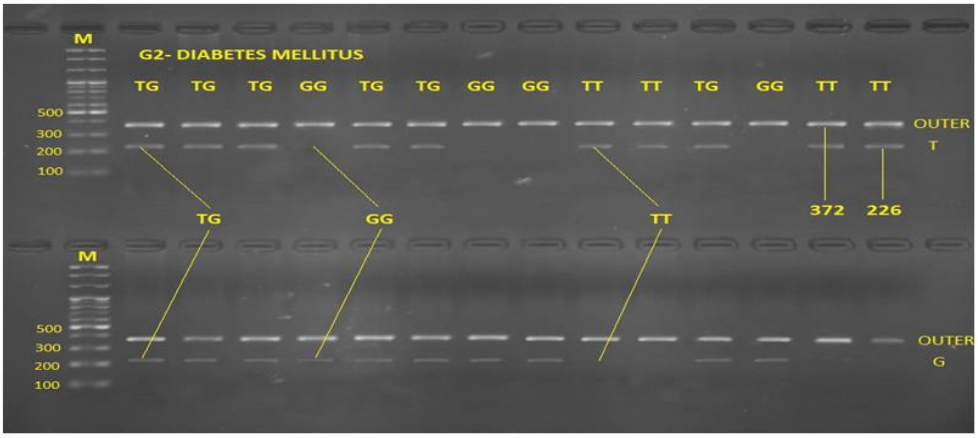

Fig. 2. Gel electrophoresis of ADIPOQ (rs 2241766) gene polymorphism amplified with a specific pair of primers using conventional PCR for T2DM group (G2). M: DNA ladder (100-3000 bp). The PCR products were stained with safe stain dye. homozygous TT genotypes (372 and 226 bp) of FT; heterozygous TG genotypes (372, 226, and 226 bp) of FT+FG; homozygous GG genotypes (226 bp) of FG.

The amplification product of the ADIPOQ gene polymorphism (rs 2241766) is three alleles, the values of whose bands were calculated from the "Figures 1, and 2", and those values are shown in "Table.2".

Table 2
Size of bands of ADIPOQ (rs 2241766) gene polymorphism

Genotype		No. of bands	Size of bands (bp)
Homozygous	TT (FT)	2	372, 226
Heterozygous	TG (FT+FG)	3	372, 226, 226
Homozygous	GG (FG)	2	372, 226

There are three alleles in ADIPOQ (rs 2241766) gene polymorphism, and they are homozygous TT, heterozygous TG, and homozygous GG. When the bands appear at (372 bp, and 226 bp), (372 bp, 226 bp, and 226 bp) and (372 bp, and 226 bp), respectively. If the bands appear in (Outer + FT) at (372 bp, and 226 bp), this means the presence of the allele TT. And if the bands appear in Inner-T and Inner-G and at the (372 bp, 226 bp, and 226 bp), this means the presence of the allele heterozygous TG. Also, if the bands appear in (Outer + FG) at (372 bp, and 226 bp), this means the presence of the allele homozygous GG. Based on the obtained results from these alleles, the percentage of all alleles were calculated for control (G1), and T2DM patients (G2), as shown in "Figures 1, and 2", respectively. When studying the Figures of comparison of genotype distribution and allele frequencies that appeared of ADIPOQ gene, it was found that this gene has two types of alleles: T and G, where the percentage of allele T was greater than the percentage of allele G in (T2DM patients) group, it was found that the percentage of allele T was 125 (62.5%) in T2DM patients,

while the percentage of allele G was 75 (37.5%) in T2DM patients. Also, the percentage of allele G was greater than the percentage of allele T in the (Control) group, it was found that the percentage of allele G was 107 (53.5%) in control, while the percentage of allele T was 93 (46.5%), in control as shown as in "Figure 3".

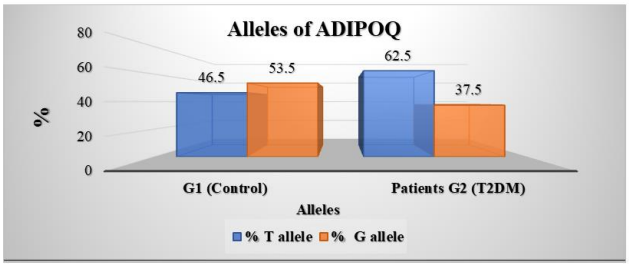

Fig. 3. The percentage of alleles T/G of ADIPOQ in blood samples of control, and T2DM patients.

The genotype distribution of ADIPOQ rs2241766 (T>G) showed three polymorphisms: TT, TG, and GG. The values of the percentages of the first polymorphism TT were 32 %, and 41% for control, and T2DM patients, respectively. The values of the percentages of the second polymorphism TG were 29%, and 43% for control, and T2DM patients, respectively. The values of the percentages of the third polymorphism GG were 39 %, and 19% for control, and T2DM patients, respectively ("Figure 4"). The GG genotype was the major genotype at control, while the TG genotype was the major genotype at T2DM patients in rs 2241766 (ADIPOQ).

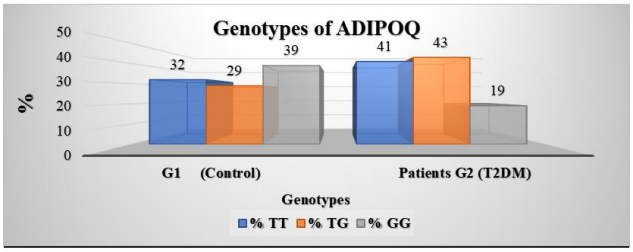

Fig. 4. The percentage of TT, TG, and GG genotypes of ADIPOQ in samples of control, and T2DM patients.

The values of percentage of T/G alleles, TT, TG, and GG genotypes of ADIPOQ were calculated from the "Figures 1 and 2".

3.2. Association Between ADIPOQ (rs 2241766) Gene Polymorphism and Risk of T2DM (G2) as Compared with Control (G1)

The genotype frequencies and allele distributions of T/G polymorphism of ADIPOQ (rs 2241766) for control (G1) and T2DM patients (G2) groups are shown in "Table. 3", The results revealed a significant association between ADIPOQ (rs 2241766) T, G-alleles, and T2DM where x2 is 13.45 and (p-value = 0.001 < 0.05). This indicate that there was a significant relationship between this SNP and the risk of T2DM. Also, the results show that the frequencies were 32% for TT, 29% for TG, and 39% for GG in G1 (Control). The frequencies were 41% for TT, 43% for TG, and 16% for GG in G2 (T2DM patients). There was significant association in rs 2241766 polymorphism of ADIPOQ between G1 healthy (Control) and G2 (T2DM patients) (P-value < 0.05) as shown in "Table. 3", therefore GG allele was a significant (p-value = 0.002 < 0.05). In G1, the T allele frequency was 93 (46.5%) and the G allele was 107 (53.5%) and in G2 the frequencies were 125 (62.5%) and 75 (37.5%) for T and G alleles, respectively. Descriptive statistics analyses revealed of the rs 2241766 polymorphism, in the dominant model, when comparing TG and GG genotypes with the TT genotype there was no significant difference (p-value = 0.240 > 0.05), while in the recessive model, when comparing the TG and TT genotypes with the GG genotype there was a significant difference (p-value = 0.0001 < 0.05). There was also a statistically significant relationship between the G allele and the T allele (p-value = 0.001 < 0.05), these results agree with the findings by (Murray, C. E. & Coleman, C. M., 2016; Hoidy et al., 2019) The genotype frequencies and allele distributions of rs 2241766 polymorphism of ADIPOQ for Control (G1) and T2DM Patients (G2) groups that showed in "Table. 3" calculated from "Figures 1 and 2".

Table 3
The genotypes and allele distribution of ADIPOQ (rs 2241766) polymorphism in G1 (Control) and G2 (T2DM)

Polymorphisms ADIPOQ (rs 2241766)	G1 Control)(N=100(%)	G2 (T2DM) N=100(%)	X2	p value	OR (95%CI)	P value
TT	32	41	13.45		1.0ref (1.0ref)	
TG	29	43		0.001*	0.864 (0.447-1.672)	0.664
GG	39	16			3.123 (1.485-6.567)	0.002*
T allele	93 (46.5%)	125(62.5%)			1.0ref (1.0ref)	
G allele	107 (53.5%)	75 (37.5%)	10.324	0.002*	1.918 (1.287-2.858)	0.001*
TT	32	41			1.0ref (1.0ref)	
TG&GG	68	59	1.747	0.240	1.477 (0.828-2.635)	0.186
GG	39	16			1.0ref (1.0ref)	
TG&TT	61	84	13.266	0.0001*	3.357 (1.720-6.552)	0.0001*

Based on the values in "Table. 3" we found that individuals carrying the TG genotype of rs 2241766 manifested no effect on the increased risk of T2DM in comparison with those carrying the TT genotype (OR = 0.864, 95%Cl = 0.447 - 1.672, p-value = 0.664 > 0.05), these results are in agreement with the findings by (Mofarrah, et al., 2016). While the GG genotype of rs 2241766 manifested an increased risk of T2DM compared with those carrying the TT genotype (OR = 3.123, 95%Cl = 1.485-6.567, p-value = 0.002 < 0.05), these results are in agreement with the findings by (Mofarrah, et al., 2016), and (Hussain *et al.*, 2018). In the dominant model, when comparing TG & GG genotype with the TT genotype there was no significant difference, therefore, no effect on increasing risk of T2DM

(OR = 1.477, 95%Cl = 0.828 - 2.635, P-value = 0.186 > 0.05), these results are in agreement with the findings by (Cui *et al.*, 2020), while in the recessive model, when comparing the TG & TT genotypes with the GG genotype there was significant difference therefore, effect on increasing risk of T2DM (OR = 3.357, 95%Cl = 1.720 - 6.552, P-value = 0.001 < 0.05), these results are in agreement with the findings by (Davis et al., 2015; Jabir & Hoidy, 2018).

4. Conclusions

In summary, the results showed there is association between ADIPOQ (rs 2241766) gene polymorphisms and T2DM, we found that individuals carrying the GG genotype of T/G had an increased risk of Infection with T2DM compared to the TT genotype (OR = 3.123, 95% Cl = 1.485-6.567, p-value = 0.002 < 0.05), also individuals carrying G allele of T/G had an increased risk of Infection with T2DM (OR = 1.918, 95%Cl = 1.287- 2.858, p-value = 0.001 < 0.05), and individuals carrying TG & TT had an increased risk of Infection with T2DM compared to the GG genotype (OR = 3.357, 95%Cl = 1.720- 6.552, p-value = 0.0001 < 0.05).

Competing Interests

The authors have declared that no competing interests exist.

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