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THE ASSOCIATION OF 276 G > T POLYMORPHISM IN ADIPONECTIN GENES WITH TYPE 2 DIABETES MELLITUS INCIDENCE IN IRAQI PATIENT

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INTRODUCTION

Diabetes mellitus type 2 (type 2 diabetes) is a long term metabolic disorder that is characterized by insulin resistance and elevated blood sugar, the symptoms include thirst, increased thirst (polydipsia), weight loss, urination and Polyuria, Symptoms may also include sudden vision changes, Very dry skin, more infections than usual, increased hunger, feeling tired and sores that do not heal. Often symptoms come on slowly.

Diagnosis of DM is by blood tests such as fasting plasma glucose, random blood sugar, oral glucose tolerance test and A1C. (Pasqual and mpierrez, 2014). Type 2 diabetes primarily occurs as a result of high calories intake, obesity, not enough exercise, some people are more genetically like to predispose diabetes. Many studies show the genetic predisposing factors of type2 DM in Iraq (Ahmed *et al*, 2014) and (Miriam, 2015).

Adiponectine Is an protein Compose of 244-amino-acid-long poly peptide collagen-like protein enhance insulin sensitivity (decreasing tumor necrosis factor (TNF) -alpha and resistin expression), decreased gluconeogenesis and energy metabolism control, increased glucose uptake, increase weight loss, Increase triglyceride clearance. Protection from atherosclerotic formation and Anti-inflammatory effect. (Lim *et al.*, 2014).

The adiponectin gene contains 3 exons spans 16 kb on chromosome 3q27 (Maeda *et al, 1996).* many single nucleotide polymorphisms (SNPs) have been identified in the adiponectin gene, The most studied SNPs of the *ADIPOQ* gene are 276 G > T (rs266729) substitution in

intron 2 together with SNP 45 T/G in exon 2 were significantly associated with type 2 diabetes and hypoadiponectinemia (Okada-Iwabu.2013), (Ali, 2013).

MATERIALS AND METHODS

This study was conducted during the period from November 2015 to November 2016 at University of Baghdad / Institute of Genetic Engineering and Biotechnology for post Graduate Studies, for detecting of a proposed association of polymorphisms with the incidence of type 2 diabetes mellitus (T2DM) in Iraq.

This study was consisted of two groups: (50) patients with type 2diabetes (group1) and (50) apparently healthy as a control group (group2). Recruited from the AL-Furat General hospital after medical investigation. Subject information was collected using specific questionnaire form.

DNA was extracted from the blood samples of T2DM patients and apparently healthy subjects by using ReliaPrep™ Blood g DNA Miniprep System Purification Kit. The DNA extracted from frozen blood samples yielded enough DNA concentration for PCR amplification.

By using Nano drop devise, The quantity measurement of DNA show that the DNA concentration ranged between 40-120 *ng* / µl and purity range was between (1.8 – 1.9). The procedure of DNA isolation is very efficient and showed sharp band. For genomic DNA analysis electrophoresis 7µl of DNA was mixed with 3µl of loading dye then loaded in an individual gel well carefully, after that current power supply turned at (5 volt/ cm for 30 minutes).

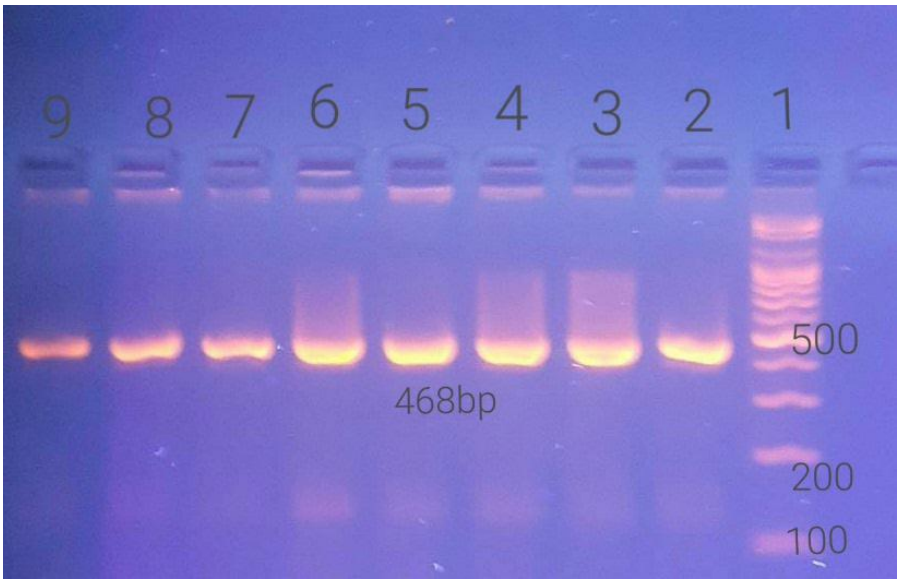
Molecular Identification of 276 G/T Polymorphism (rs1501299)

Polymerase Chain Reaction (PCR) amplified regions, which have a molecular weight of (468 bp), represent the region of the adiponectin gene that include (rs1501299) SNP. Targeted fragments of adiponectine gene (flanking G>T276 SNP) amplified by polymerase chain reaction, using the forward primer, f 5'-TCT CTC CAT GGC TGA CAG TG-3' and the reverse primer r5'-AGA TGC AGC AAA GCC AAA GT-3', 5µl DNA with The 12.5 µl master mix of PCR was mixed and 1 µl from each primer forward and reverse then the volume was completed up to 25 µl with free nucleases water, Optimal annealing temperature

of PCR reaction was find out after several trying to is 57C with a total volume of 25 µl. (Chu *et al*., 2013).

A total of 35 PCR cycles with denaturation at 94oc for 30 sec., annealing for 30 sec at 57^{o}C and extension at 72^{o}C for 30 sec. were conducted. An initial DNA denaturation at 95oc was carried out for 5 minutes and final extension at 72^{o}C were carried out for 7 minutes each. This technique was used with primers for adiponectin gene segment according to (Bieńkiewicz *et al.* 2016).

The PCR product size detected by using (100-1500) DNA ladder and the gel was photographed by a digital camera (Figures 2-2). The Same PCR product size results was obtained by Al-Daghri *et al.* (2012) Bieńkiewicz. J *et al*. (2016) and Ina Maria. k *et al* (2012).



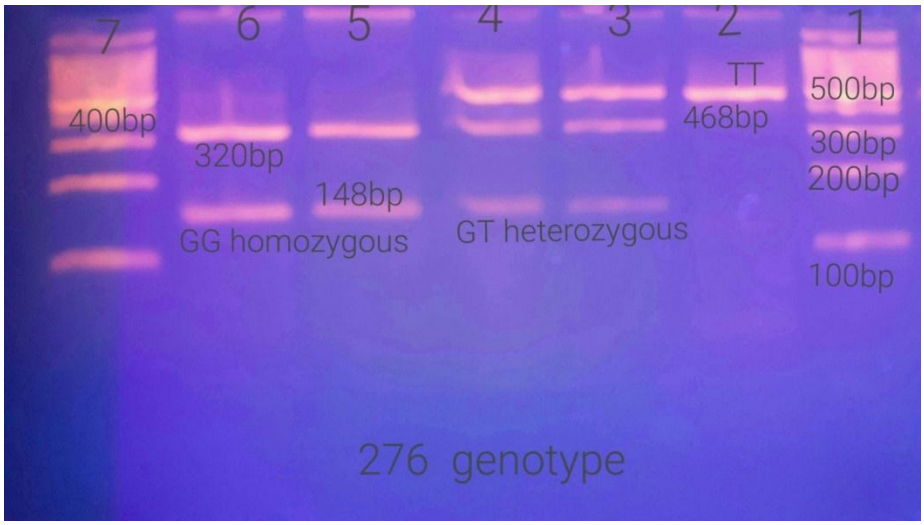
PCR products gene with size of 468 bp. The product was electrophoresis on 2% Agarose gel at 5 volt/cm^{2} for 60 minute. Visualized under U.V light after stain with Ethidium Bromide, Lane 1 DNA ladder (100-1500), Lane (2-9) PCR products of the adiponectin gene fragment.

Restriction fragment length polymorphism (RFLP) technique was used for identification SNP 276 fragment by using *BSMI* restriction enzymes. (Ina Maria *et al* 2012). Pcr product 10 µl, Enzyme 0.5 µl, Enzyme Buffer 2 µl, D.W 7.5 µl.

Bsm I restriction enzyme was used to digestion The PCR products the following fragment sizing patterns were observed by Agarose gel electrophoresis.

***Bsm I.* was cut at CTTAC↓GN**

1. homozygous 276 TT: No cleavage of the whole 468 bp segment by
2. Heterozygous for 276 TG: *Bsm I* was cut at sequence to show three fragments in Agarose gel electrophoresis (468 bp, 320 bp and148 bp).
3. Homozygous for the 276 GG show two fragments in Agarose gel electrophoresis fragments of 320 and 148-bp.



PCR product digested with *Bsm1* electrophoresis on 3%. Agarose gel at 5 volt/cm^2 for 90 minute. The (RFLP) product were Visualized under U.V light after stain with Ethidium Bromide, Lane1: DNA ladder (100-1500). Lane2: Wild type TT genotype 468 bp, Lane 3, 4: heterozygote GT 468, 320 and 148 bp., lane 5, 6 Homozygote GG 320.

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage and estimate of Odd ratio were used for determine the risk of disease in this study.

RESULTS AND DISCUSSION

As related SNP 276 The number of those with TG Polymorphism genotype in the patients group was significantly ($p<0.05$) higher than in control group (24% *versus* 19%, respectively). Individuals with the GT genotype in the patient group were significantly higher than those with the TT genotype, the number of those with TT Polymorphism genotype in the patients group show no significant difference with control group (48% *versus* 52%, respectively).

Individuals with the TT genotype in the patient group were significantly higher than those with the GT genotype.

The number of those with TT Polymorphism genotype in the control group show significant difference with patient group (40% *versus* 31%, respectively).

The polymorphism of G 276 T SNP genotype was significantly related with the risk of developing T2DM in this studied population. The reason of that 276G>T SNPs in the adiponectin gene are associated with type 2 diabetes, which might be mediated by change in the adiponectin expression level and plasma concentration of adiponectin. It can be concluded that the variation in the G276T polymorphism in adiponectin gene might play an important role in the occurrence of type 2 diabetes.

Genotype	**Patients (No.= 50)**	**Control (No.= 50)**	**O.R.**	**Chi-square (χ^2)**
TT	24 (48.00%)	26 (52.00%)	0.215	1.066 NS
TG	24 (48.00%)	19 (38.00%)	0.684	4.359 *
Chi-square (χ^2)	0.00 NS	4.106 *	---	---
O.R.	0.00	0.0498	---	---
TT	24 (48.00%)	26 (52.00%)	0.215	1.066 NS
GG	2 (4.00%)	5 (10.00%)	0.349	1.284 NS
Chi-square (χ^2)	9.263 **	9.058 **	---	---
O.R.	1.273	1.165	---	---
TG	24 (48.00%)	19 (38.00%)	0.684	4.359 *
GG	2 (4.00%)	5 (10.00%)	0.349	1.284 NS
Chi-square (χ^2)	9.263 **	8.264 **	---	---
O.R.	1.273	1.138	---	---
Allele freq.				
T	72%	71%	---	---
G	28%	29%	---	---
* (P<0.05), ** (P<0.01), NS: Non-significant.				

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