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# Investigation of the PON1(C>T rs705379) SNP and its Correlation with Physiological Adiponectin Levels in Insulin Resistance T2D Patients Induced by Obesity

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**Abstract**

Body mass index (BMI) more than 30 kg/m$^2$ is considered obese. Obesity is a pathophysiologic component of type 2 diabetes, and there is a common relationship between insulin resistance (IR) and obesity (T2D). The aim of this study was to investigation of the PON1 (C>T rs705379) SNP and its correlation with adiponectin (ADNT) levels in insulin resistance T2DM patients induced by obesity. This study involved on 60 patients with insulin resistance IRT2D (34-59 Y) and whom obese status group (IRT2D)**.** Also, 40 subjects (32-60 Y) as healthy control group (CNT) were assessed of all parameters in this work. The ELISA method was used for assessment of ADNT (ng/ml) in both IRT2D and CNT groups**.** PCR-RFLP were used to investigation of PON1 (C>T rs705379) SNP genotyping by using the *MbiI* as RE with specific primers. The results of this study showing statistically significant differences (p-value< 0.05) in levels of ADNT (ng/ml) between IRT2D and CNT groups. The PCR-RFLP amplification of PON1 gene showing one band for specific genotypes CC, CT, and TT were 240 bp, 212 bp, and 28 bp respectively in both groups IRT2D and CNT. The results showed that frequency of C allele of PON1 gene was (0.65) (0.52) in IRT2D and CNT group respectively, and found significant difference between C allele in IRT2D and CNT (OR=5.7 ), CI $_{95\%}$ (1.5-6.9). Measurement of ADNT levels and assessment of C>T rs705379 SNP of PON1 gene can be consider as indicator of IRT2D patients whom obese status.

**Keywords:** Obesity, T2D, PON1 gene, Adiponectin, Insulin resistance

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## 1. Introduction

An initiating factor for diabetes linked to insulin resistance is obesity. Adipose tissue in obese people releases increased levels of non-esterified fatty acids, glycerol, hormones, and pro-inflammatory cytokines that may contribute to the emergence of insulin resistance. In addition, genetic predisposition, adipose tissue hypoxia, oxidative stress, lipodystrophy, and endoplasmic reticulum stress all contribute to insulin resistance. (1-3). Although the process is unclear, obesity may result in long-lasting, low-grade local and systemic inflammation that promotes the development of insulin resistance and diabetes mellitus. (4Diabetes mellitus (DM), usually referred to as diabetes, is the most common illness in Westernized, affluent nations, and its incidence rises with age. It is responsible for 8.4% of all fatalities globally. (5). Type 2 diabetes mellitus (T2DM), which belongs to the second group and is the more common type of diabetes, is brought on by a confluence of insulin resistance and an insufficient compensatory insulin secretory response. (6). In addition, the development of obesity may be influenced by insulin resistance and hyperinsulinemia (7) .

The etiology of T2DM is accelerated by obesity by increasing insulin resistance. Because of our limited understanding of insulin resistance, T2DM therapeutic options are limited. However, several investigations have noted the connection between insulin resistance and mitochondrial dysfunction, inflammation, hyperinsulinemia, and lipotoxicity. (8). Through the establishment of insulin resistance, endoplasmic reticulum stress, oxidative stress, genetic background, aging, hypoxia, and lipodystrophy are all mentioned in the pathogenesis of T2DM. (9). Through the establishment of insulin resistance, endoplasmic reticulum stress, oxidative stress, genetic background, aging, hypoxia, and lipodystrophy are all mentioned in the pathogenesis of T2DM. (10-12). This illness state involves several adipokines, including adiponectin (ADNT), TNF-, resistin, and IL (13). As a result, rising levels of adiponectin improve insulin sensitivity whereas rising levels of resistin have insulin-opposing effects. (14). Human PON1 is a 354 amino acid glycoprotein with a molecular weight of 43000 Daltons that associates HDL in the bloodstream. (15). A tissue-specific protein of 247 amino acids, adiponectin (ADNT) is very similar to complement protein C1q, collagen VIII, and collagen X. Reduced levels of this protein are a major factor in disorders linked to obesity, such as diabetes and cardiovascular conditions. (16). Nearly 200 single nucleotide polymorphisms (SNPs) exist in the PON1 gene, including the 108 C/T (rs705379) polymorphisms found in the promoter region on chromosome 7. The purpose of this study was to investigate the relationship between adiponectin (ADNT) levels and the PON1 (C>T rs705379) SNP in individuals with insulin resistance type 2 diabetes brought on by obesity.

## 2. Materials and methods

### *2.1. Study design:*

Group 1: 60 patients with insulin resistance type 2-DM (IRT2D)
Group 2: 40 subjects as control healthy group (CNT)

### *2.2. Determination of ADNT levels:*

ELISA method was used to assessment of ADNT (ng/ml) in both IRT2D and CNT groups depending on protocol and standard curve provided by manufacture as shown in figure 1. PON1 (C>T rs705379) SNP analysis of genotypes
All research participants (IRT2D and CNT) had peripheral whole blood drawn, and genomic DNA was extracted using the AccuPrep® genomic DNA micro kit (Bioneer, Korea), which offers an effective approach for obtaining total DNA from whole and frozen blood. For the examination of PON1 genotyping, allele specific PCR was carried out using certain primers, as given in table 1. Taqman polymerase was used in a total volume of 50 l of the reaction mixture, and the thermocycler (Exycycler 96, Bioneer, Korea) carried out the PCR. The reaction mixture was subjected to denaturation at 95 Co for 4 min, 40 cycles of 95 Co for 30 sec, 62.7 Co for 45 sec, and the final extension phase at 72 Co for 7 min. The final PCR product was electrophoresed on a 2 percent agarose gel and photographed.

## 3. Results and Discussions

Clinical characteristics of IRT2D group is shown in table 2. Statistically significant differences in ADNT levels (ng/ml) between the IRT2D and CNT groups were found in this investigation, as shown in table 3. The PCR-RFLP amplification of PON1 gene showing one band for specific genotypes CC, CT, and TT were 240 bp, 212 bp, and 28 bp respectively in both groups IRT2D and CNT. As shown in table 4, the findings revealed that the frequency of the C allele of the PON1 gene was (0.65) (0.52) in the IRT2D group and found to be significantly different between IRT2D and CNT (OR=5.7), CI 95% (1.5-6.9).

In the current investigation, we compared the levels of circulating ADNT and CC, CT, and TT C>T rs705379 SNP of PON1 gene in patients with IRT2D brought on by obesity as well as in controls who were of normal weight. One of the most prevalent proteins found in adipose tissue is ADNT. On chromosome 3q27, the ADNT gene, which encodes it, may be found. It has crucial functions in maintaining energy balance, cholesterol and glucose metabolism, and vascular system anti-inflammatory responses. It is probably going to affect how sensitive the body is to insulin and affect how both human and animal models of insulin resistance are modeled. Type 2 diabetes mellitus has a key component of insulin resistance, which is frequently linked to obesity. (17). Even if the exact process is unclear, obesity may result in long-lasting, low-grade local and systemic inflammation that eventually gives rise to insulin resistance and diabetes mellitus. Additionally, insulin resistance and hyperinsulinemia can help people become obese. (19). The findings of this investigation revealed statistically significant variations in ADNT levels (ng/ml) between the IRT2D and CNT groups (p-value 0.05). This indicates that the levels of ADNT (ng/ml) in the IRT2D group are statistically greater than those in the CNT group. Additionally, the current conclusion is consistent with several studies that showed decreased LEP/ADNT ratios in metabolically unsound obese people. According to reports, the ADNT/LEP ratio was related to insulin sensitivity, and this study, in keeping with other research, showed that this ratio was a factor in the development of the metabolic syndrome. (20). For the particular genotypes CC, CT, and TT, the allele-specific PCR amplification of the PON1 gene showed one band at 240 bp, 212 bp, and 28 bp, respectively, in both groups IRT2D and CNT. The findings revealed a significant difference between the C allele in the IRT2D and CNT groups (OR=5.7), CI $_{95\%}$, and the frequency of the C allele in the PON1 gene was (0.65)(0.52) in each group, respectively (1.5-6.9). revealed decreased PON1 activity toward paraoxon (PONase) substrate in CAD patients compared to controls, with findings comparable to the prospective epidemiological Caperhilly (21) research. Independent of all other known risk factors for CAD, including age, sex, smoking, alcohol use, and HDL-C levels, low PONase activity is a risk factor for the disease. These results suggest that PONase activity is crucial to the development of CAD. However, the Q192R polymorphism had a considerable impact on PONase activity. In both groups, it was considerably greater in RR homozygotes and lower in QQ homozygotes. This finding is consistent with other investigations. (22-27).

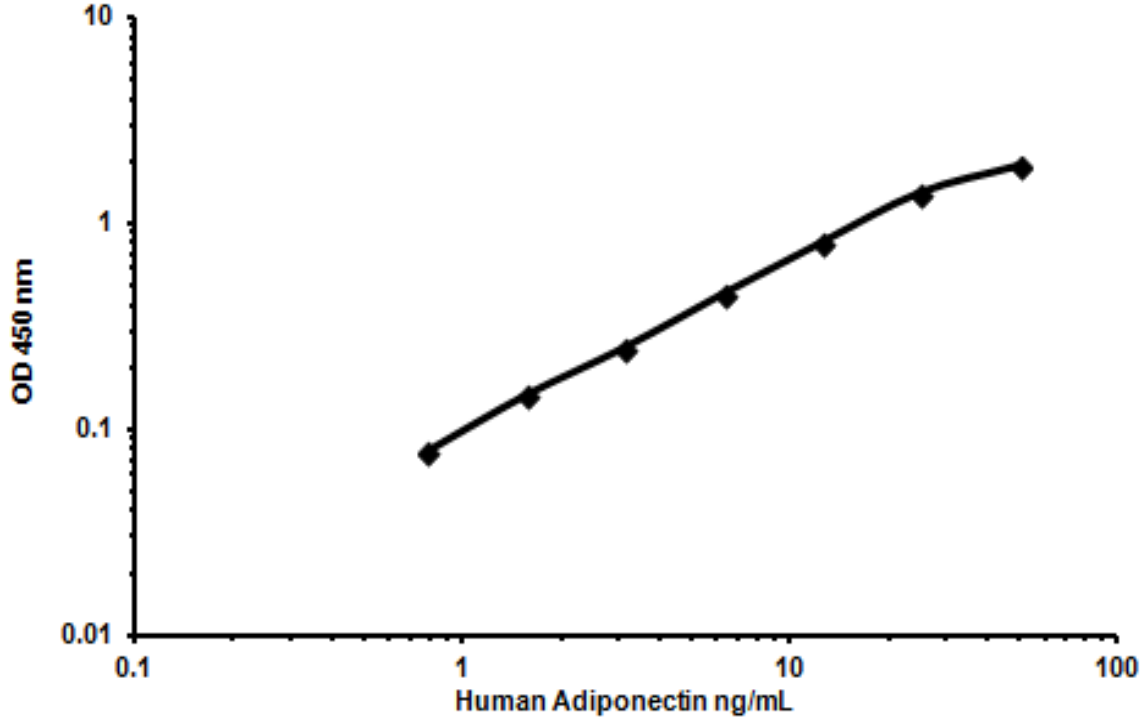


**Fig. 1.** ADNT (ng/ml) standard curve

**Table 1.** Primers used in genotyping analysis.

| GENOTYPE | F:GGCTGCAGCCCTCACCACAACCC<br>R:AGCTAGCTGCCGACCCGGCGGGG | RE | Amplicon (bp) |
|---|---|---|---|
| C>T rs705379<br>PON1 gene | | *MbiI* | 240 bp for T allele (TT)<br>28 (CC)+ 212 (TC) bp for C allele |

**Table 2.** Clinical characteristics of Obese Subject group

| ARAMETERS | IRT2D<br>*N=60* | (%) | P-value<br>$X^2$ |
|---|---|---|---|
| Age | | | |
| - <40 | 40 | 67 | 0.001** |
| - ≥40 | 20 | 33 | |
| Gender | | | |
| - Male | 36 | 60 | 0.01* |
| - Female | 24 | 40 | |
| BMI | | | 0.01* |
| - 30-35 | 42 | 70 | |
| - 25-30 | 18 | 30 | |
| Education status | | | 0.087 |
| - Yes | 32 | 53 | |
| - No | 28 | 57 | |
| Dwelling | | | 0.056 |
| - Rural | 27 | 45 | |
| - Urban | 33 | 55 | |

**Table 3.** LEP and ADNT (ng/ml) levels in study groups

| Groups | ADNT (ng/ml) mean± SD | P-value |
|---|---|---|
| IRT2D n=30 | 34.8±2.8 | 0.0001 |
| CNT n=30 | 19.6±3.1 | |

**Table 4.** A comparison of three genotypes incidence in IRT2D and CNT groups

| Genotypes | IRT2DM N=60 | CNT N=40 | OR(CI 95%) | P-Value |
|---|---|---|---|---|
| CC | 15 (50%) | 11 (27%) | REF. | - |
| CT | 10 (33%) | 19 (48%) | 3.8(1.5-5.6) | 0.008* |
| TT | 5 (17%) | 10 (25%) | 3.9 (1.6-6.3) | 0.003* |
| Alleles C | 39 (65%) | 21 (52%) | 5.7 (1.5-6.9) | 0.0001** |
| T | 21 (35%) | 19 (48%) | 1.2 (0.7-1.9) | |

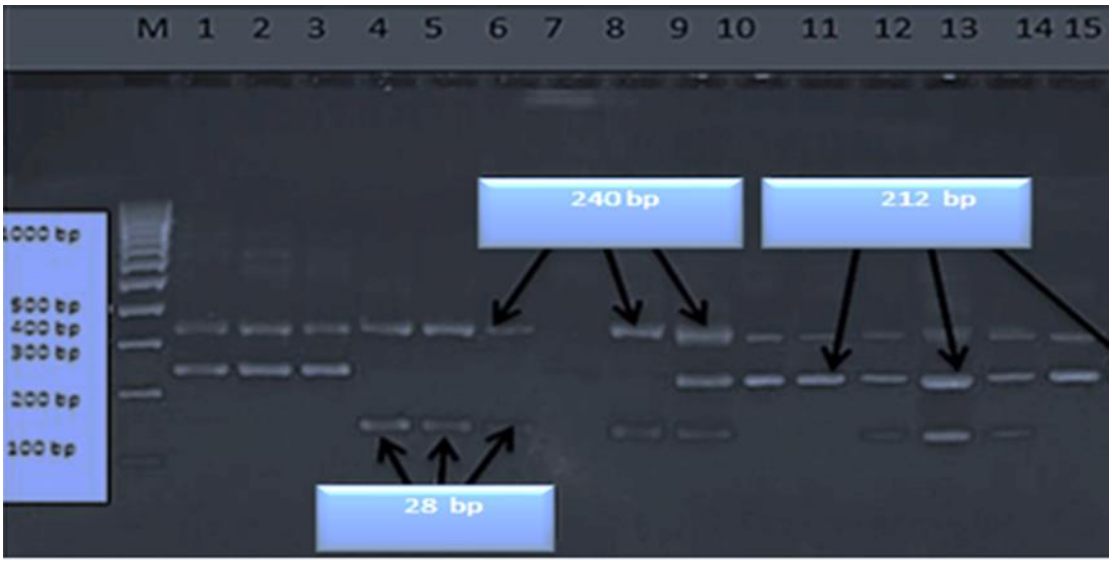


**Fig. 1.** Electrophoretic pictures represents the genotypes, CC, CT, and TT C>T rs705379 SNP of PON1 gene

In conclusion, measuring serum ADNT might become a noninvasive and practical way to determine the risk of developing diabetes mellitus in healthy obese people due to the increasing serum ADNT levels' correlation with clinical symptoms and biochemical aspects of IRT2D.

## 4. Conclusions

In the present investigation, we compared the levels of circulating ADNT and CC, CT, and TT C>T rs705379 SNP of PON1 gene in patients with IRT2D brought on by obesity as well as in controls who were of normal weight. One of the most prevalent proteins found in adipose tissue is ADNT. Indicators of IRT2D patients were established in this study as being ADNT levels and the C>T rs705379 SNP of the PON1 gene.

## Conflict of interest

There were no declared potential conflicts of interest pertaining to this paper.

## Ethical approved

The World Medical Association Declaration of Helsinki was followed in conducting this research in an ethical manner. The Institutional Review Board of Al-Qasim Green University in Iraq accepted Research Project No. QGU:9876, and all participants completed an informed consent form.

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