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Association Between RETN Gene Polymorphism and Serum Resistin Levels in Type 1 and Type 2 Diabetes Patients

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ABSTRACT

Resistin is a cysteine-rich polypeptide, which is encoded by the RETN gene on chromosome 19p13. It has been established that human adipocytes, pancreatic cells, muscle, and mononuclear cells all express this 108 amino acid polypeptide. Up to 70% of the variability in serum resistin in Caucasians can be attributed to hereditary causes. Therefore, the goal of this study is to investigate the association between RETN gene polymorphism and serum resistin levels in type 1 and type 2 diabetes patients. Frequencies of the polymorphism were determined in 51 type 1 diabetes, 52 type 2 diabetes patients, and 50 control group using (Enzyme-linked immunosorbent assay), and conventional polymerase chain reaction. The AA genotype has differed significantly between patients and the control group while genotypes, AC and CC, have not differed significantly between patients and the controls in the type 1 diabetes group. The (AA) was found in 100% of the type 1 diabetes patients and 80% of the control group (Pc=2.7 x 10-4). The AA, AC, and CC genotypes have not differed significantly in type 2 diabetes. A positive correlation was detected between the AA genotype and serum resistin levels in the type 1 diabetes group compared to type 2 diabetes and the control groups, but there was a negative correlation between AC, CC genotypes and them. Also, there was a negative correlation between the AA, AC, and CC genotypes and serum resistin levels in the type 2 diabetes group compared to the control group. Conclusion: The AA genotype of RETN gene 3′UTR polymorphism rs115073262 was found to be significantly associated with type 1 diabetes incidences. The findings suggest that the AA genotype and A allele in this gene increase the risk of type 1 and type 2 diabetes. The AA genotype of polymorphism associated with higher serum resistin levels in type 1 diabetes patients samples.

Keywords: *T1DM, T2DM, RETN gene 3'UTR, Gene polymorphism, Resistin*

INTRODUCTION

Diabetes mellitus (DM) is a group of disorders of carbohydrate metabolism, whose main feature is a chronic hyperglycemia that results from defects of insulin secretion, insulin resistance, or a combination of those [1].

Both type 1 (T1DM) and type 2 diabetes mellitus (T2DM) are caused by a failure to maintain glucose homeostasis, even though these two types of diabetes are fundamentally while insulin resistance causes the inability to keep up with insulin demand in T2DM.

ß-cell apoptosis is the fundamental event causing dysglycemia in T1DM. Under several circumstances present in both T1DM and T2DM, endoplasmic reticulum (ER) stress and oxidative stress are significant factors in ß-cell malfunction and death [2]. According to the comprehensive survey's findings, 10.9% of Iraq's population overall had T2DM [3]. Diabetes mellitus affects both industrialized and developing nations. 422 million people worldwide have diabetes, which caused 1.5 million fatalities in 2012; according to the world health organization's global assessment of the disease [4]. Both T1DM and T2DM have a genetic component, with over 60 chromosomal regions related to T1DM and over 200 connected with T2DM at significant genome-wide levels. Examining areas linked to both types of the disease may reveal colocalization-related signals that simultaneously change the risk of both diseases. Finding co-localizing signals may offer biological information about similar illness mechanisms and may also point to prospective treatment targets. In five areas surrounding CENPW, CTRB1/BCAR1, GLIS3, BCL11A, and THADA, a recent investigation revealed that the same genetic mutation affects the risk of both T1DM and T2DM [5]. Since the linked loci for T1DM and T2DM were previously believed to be almost entirely distinct despite having a substantially shared phenotype, a genetic connection between them is of particular interest. However, more recent investigations discovered more common risk genes or genetic polymorphisms [5][6][7]. Resistin, a cysteine-rich polypeptide also known as FIZZ3 or adipose tissue-specific secretory factor (ADSF). The hormone is thought to be a key player in the emergence of insulin resistance; it was initially identified in a screen for targets of thiazolidinediones (TZDs) in white adipose mice. It has been established that human adipocytes, pancreatic cells, muscle, and mononuclear cells all express this 108 amino acid polypeptide. It is important to note that resistin is primarily produced by peripheral blood mononuclear cells (PBMCs) in humans [8]. Numerous studies have noted genetic variations in the resistin protein, which is encoded by the RETN gene on chromosome 19p13. Up to 70% of the variability in serum resistin in Caucasians can be attributed to hereditary causes. For instance, single nucleotide polymorphisms in the RETN gene have been linked to insulin resistance indices [9]. Insulin resistance which can be caused by the RETN gene, can negatively impact how insulin works. Numerous both in vivo and in vitro studies have demonstrated that the RETN gene can impair glucose tolerance and cause insulin resistance. Therefore, it is believed that the RETN gene represents a key link between obesity and T2DM [10]. Furthermore, we examined possible associations between RETN gene polymorphism and serum resistin levels related to T1DM and T2DM patients.

METHODS

The participants in this study were split into three groups: 51 T1DM and 52 T2DM patients recruited from the National Center for Diabetes Treatment and Research/ Al-Mustansiriya University/ Baghdad Governorate, and the consulting clinics in Al-Muqdadiya District and Baquba District/ Diyala Governorate in Iraq, and 50 age and gender-matched seemingly healthy people acting as the control group.

Sample collection

About (5) ml of venous blood was obtained from each T1DM and T2DM patient, and healthy people. (3) mL of the blood sample were placed in a gel tube and left for 10-30 minutes at room temperature and then placed in a centrifuge for 5 minutes at a speed of 3000 r.p.m to separate the serum for resistin levels estimation, and (2) mL were put into an EDTA vacutainer, inverted several times, and inspected to exclude the possibility of clots then stored at −20°C until DNA extraction and detection were done.

Measurement of serum resistin

Serum resistin levels were estimated by using ELISA (Enzyme-linked immunosorbent assay) kit from Al-shkairate Company for each T1DM and T2DM patients, and healthy people

DNA extraction and polymerase chain reaction (PCR)

Extraction of DNA from blood samples which were taken from patients with T1DM and T2DM and the control group, was done by using the favorPrep tmBlood/ cultured cells genomic DNA extraction mini system hghjd kit. The concentration the extracted DNA was calculated using the electrophoresis method and was conducted to detect DNA through an agarose gel. Primers were used to detect the RETN gene, and two special primers were used, as in the following nucleotide sequences shown:

Forward primer
5′-GGTATGTCATTCTCACCCAG-3′
Reverse primer
5′-CTCAGCTAACCAAATCCGG-3′

The stock solution for both the forward and reverse primers was prepared by adding (210) µL of distilled water to the powder particles at the bottom of the ampoule to reach a concentration of (100) pmol/µL according to the instructions of the attached leaflet for the primers, and preparation of working solutions for primers was done by transferring (10) µL of the stock solution and (90) µL of distilled water was added to it to obtain a working solution at a concentration of (10) pmol/µL. For each sample, a PCR reaction was set up. The following reaction volume was set: I-taq master mix (5) µL, forward primer (1) µL, reverse primer (1) µL, DNA template (5) µL, and distilled water (13) µL. The samples were placed in a thermal cycler for DNA amplification. For amplification, this procedure was used: Denaturation of the template at 95°C for 5 minutes, initial denaturation at 95°C for 30 seconds then annealing at 62°C for 30 seconds, and extension at 72°C for 30 seconds for 40 cycles. Final extension at 72°C for 10 minutes and The final stage of incubation at 4.0°C for 5 minutes for 1 cycle.

Statistical analysis

SPSS program statistics was used to analyze the data. The normality, homogeneity, and randomization were checked for the tested data before they were statistically analyzed. For parametric data, the mean and standard deviation were calculated, independent-T test and ANOVA table (Duncan test) were used to calculate the significance of probability; the probability was considered significant when it was less than 0.05. For Non-parametric data, fisher's exact probability and Bonferroni corrected probability were calculated for genotyping and alleles' frequencies. Also, the online Hardy-Weinberg equilibrium calculator was used to calculate the probability between the observed and expected genotypes of the studied SNPs.

RESULTS

The study included (51) T1DM and (52) T2DM patients. The gender percentages for T1DM, T2DM patients, and control group were of males 28 (54.9%), 18 (34.6%), and 24 (48.0%) respectively, and females 23 (45.1%), 34 (65.4%), and 26 (52.0%) respectively. The age mean ± SD for T1DM, T2DM patients, and control groups were not significant (27.94 ± 10.04), (60.27 ± 10.08) and (41.98 ± 16.30) respectively, as shown in table (1).

TABLE 1: Distribution of T1DM, T2DM patients, and control groups according to gender and age

Studied parameters		T1DM	T2DM	Control	Probability
Gender No. (%)	Males	28 (54.9)	18 (34.6)	24 (48.0)	0.110
	Females	23 (45.1)	34 (65.4)	26 (52.0)	
	Total	51 (100.0)	52 (100.0)	50 (100.0)	
Age mean ± SD (Years)		27.94±10.04	60.27±10.08	41.98±16.30	

No significant differences at ($P<0.05$).

The process of extracting DNA from blood samples of patients with T1DM and T2DM and a control group was carried out, and the purity and concentration of the isolated DNA required in the polymerase chain reaction were measured, as in figure (1).

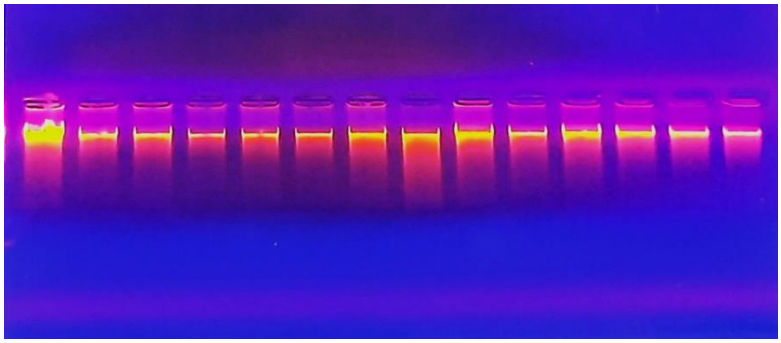

FIGURE 1: DNA bundles extracted from a sample of patients with T1DM and T2DM, and the control group, and relayed using agarose gel at a concentration of 1% and under a voltage of 100 volts for half an hour.

An experiment was conducted to find out the degree of binding of primers to DNA, and the best temperature was 62°C, which is the appropriate temperature for conducting the PCR reaction in this study. The results of the electrophoresis of the amplified RETN gene by PCR technique in a samples of patients with T1DM and T2DM and the control group showed that the molecular weight of the resulting bundles for all samples was 533 bp, after comparing it with the molecular marker ladder consisting of 100-1000 bp, as in figure (2).

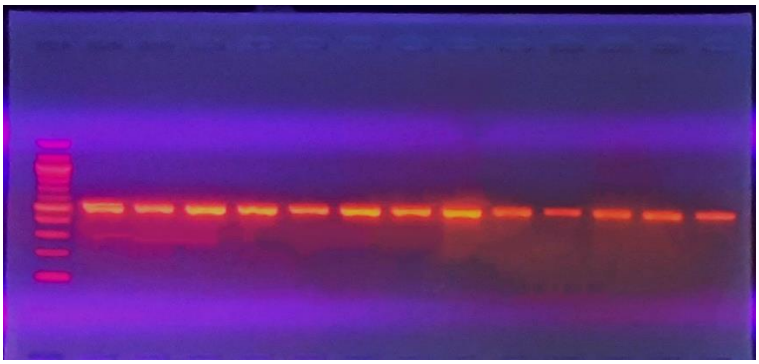

FIGURE 2: Electrophoresis to amplify the DNA RETN gene in a sample of patients with T1DM and T2DM. Electrophoresis was carried out using agarose gel at a concentration of 1.5% and under a voltage of 90 volts for an hour.

The results of the study showed that the genotypes of SNPs rs115073262 for the RETN gene in the T2DM group and the control group were compatible with Hardy-Weinberg equilibrium, as shown in table (2).

TABLE 2: The genotyping frequencies percentages of RETN gene SNP rs115073262

Genotypes of rs115073262	T1DM patients' frequency (%)		T2DM patients' frequency (%)		Control frequency (%)	
	Observed	Expected	Observed	Expected	Observed	Expected
AA	73 (100.0)	51 (100.0)	70 (88.5)	46.2 (88.8)	40 (80.0)	38.7 (77.4)
AC	2 (0.0)	0 (0.0)	5 (11.5)	5.7 (10.9)	8 (16.0)	10.6 (21.1)

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CC	0 (0.0)	0 (0.0)	0 (0.0)	0.2 (0.3)	2 (4.0)	0.7 (1.4)
Total	51 (100.0)	51 (100.0)	52 (100.0)	52 (100.0)	50 (100.0)	50 (100.0)
P-HWE	-		0.6589		0.0865	

P-HWE: The probability of Hardy-Weinberg equilibrium, %: Percentage

The results showed in table (3) that the observed number of patients with homozygous AA genotype was (51) and the A allele was (102), a significant increase was recorded in the T1DM group (100% and 100% respectively) compared to the control group, as it recorded (80.0% and 88.0% respectively) according to Pc (2.7 x 10-4 and 7.1 x 10-5 respectively) among the group T1DM compared to the control group, therefore the homozygous genotype AA and allele A are considered a causative factor, according to OR (26.70% and 28.95% respectively). The homozygous CC genotype showed no significant difference, while the C allele showed a significant difference in the T1DM group, where it recorded (0.0% and 0.0% respectively) compared to the control group which recorded (4.0% and 12.0% respectively) according to Pc (0.118 and 7.1 x 10-5 respectively), therefore, the genotype CC and allele C are a protective factor for the disease according to the OR of (0.19% and 0.03% respectively). The heterotypic AC and allele C recorded a significant decrease in the T1DM group by the value of (0.0% and 0.0% respectively) compared to the control group, where it recorded (16.0% and 12.0% respectively), and according to Pc (0.001 and 7.1 x 10-5 respectively), therefore, the AC genotype and the C allele are considered a protective factor for the disease with an OR of (0.05% and 0.03% respectively).

TABLE 3: The genotypes and alleles frequencies of RETN gene SNP rs115073262 of the T1DM and control groups

Genotypes of rs115073262	T1DM patients group frequency (%)	Control group frequency (%)	OR (95% CI)	Fisher's exact probability	Pc
AA	51 (100.0)	40 (80.0)	26.70 (1.56 – 456.60)	5.3 x 10-4	2.7 x 10-4
AC	0 (0.0)	8 (16.0)	0.05 (0.0 – 0.84)	0.003	0.001
CC	0 (0.0)	2 (4.0)	0.19 (0.01 – 3.91)	0.243	0.118
Total	51 (100.0)	50 (100.0)			
Allele's frequency					
A	102 (100.0)	88 (88.0)	28.95 (1.71-489.25)	1.5 x 10-4	7.1 x 10-5
C	0 (0.0)	12 (12.0)	0.03 (0.0 – 0.58)		

OR: Odds ratio, 95% CI: 95% confidence intervals, Pc: Bonferroni correction probability

Additionally, it was noted from table (4) that the observed number of patients with T2DM who carry the homozygous genotype AA was (46) and allele A was (98), they did not record a significant increase according to the mentioned percentages (88.5% and 94.0% respectively) compared to the control group it recorded (80.0% and 88.0% respectively) according to Pc (0.191 and 0.093

respectively) among the group of patients with T2DM compared to the control group, therefore the homozygous genotype AA and allele A are considered as causative factors, according to OR (1.92% and 2.23% respectively). The homozygous genotype CC and allele C showed no significant difference in the T2DM group, as it recorded (0.0% and 6.0% respectively) compared to the control group, which recorded (4.0% and 12.0% respectively) and according to Pc (0.114 and 0.093 respectively), therefore, the genotype CC and the C allele are protective factors for the disease according to OR of (0.18% and 0.45% respectively). The heterotypic AC and the C allele recorded a significant decrease in the T2DM group by the value of (11.5% and 6.0% respectively) compared to the control group, as it recorded (16.0% and 12.0% respectively) according to Pc (0.093 and 0.412 respectively). Therefore, the AC genotype and the C allele are considered a protective factor for the disease, with an OR of (0.68% and 0.45% respectively).

TABLE 4: The genotypes and alleles frequencies of RETN gene SNP rs115073262 of the T2DM and control groups

Genotypes of rs115073262	T2DM patients group frequency (%)	Control group frequency (%)	OR (95% CI)	Fisher's exact probability	Pc
AA	46 (88.5)	40 (80.0)	1.92 (0.65 – 5.68)	0.284	0.191
AC	6 (11.5)	8 (16.0)	0.68 (0.22 – 2.11)	0.574	0.412
CC	0 (0.0)	2 (4.0)	0.18 (0.01 – 3.83)	0.238	0.114
Total	52 (100.0)	50 (100.0)			
Allele's frequency					
A	98 (94.0)	88 (88.0)	2.23 (0.81 – 6.15)	0.142	0.093
C	6 (6.0)	12 (12.0)	0.45 (0.16 – 1.24)		

OR: Odds ratio, 95% CI: 95% confidence intervals, Pc: Bonferroni correction probability

In addition, the T1DM, T2DM, and control groups were divided into three subgroups according to the genotypes (AA, AC, and CC) (table 5). No significant differences were recorded between the genotypes among them for the group T1DM, the group T2DM and the control group separately, while a significant increase was recorded in the group T1DM carries the genotype AA, the highest mean for resistin (2.40 ± 0.34) (pg/ml) compared to the T2DM group and the control group. No significant difference was recorded in the mean resistin for any genotype in the group T2DM and the control group.

TABLE 5: Resistin levels among the compared groups according to the genotyping of RETN gene SNP rs115073262

Genotyping of rs115073262	Resistin means level ± SD (pg/ml)		
	T1DM	T2DM	Control
AA	2.40 ± 0.34 A	1.45 ± 0.09 Ba	1.30 ± 0.65 Ba
AC	-	1.55 ± 0.68 a	0.60 a
CC	-	-	-

The superscript capital different letters referred to significant differences ($P<0.05$) among the compared groups, while the subscript small different letters referred to significant differences ($P<0.05$) among the compared genotypes of the same group.

DISCUSSION

Resistin is detectable in the serum of mammals and may be regulating body fat mass by peripheral signaling and negative feedback. Plasma resistin levels have been linked to metabolic and inflammatory factors, according to a study [11]. Genetic factors such as single nucleotide polymorphisms are thought to contribute to the increasing incidence of diabetes mellitus through insulin resistance. SNPs in the RETN gene encoding the resistin protein have been reported to play a role in causing abnormalities in blood glucose and lipid metabolism. RETN gene SNPs may require interaction with other factors or genes to induce insulin resistance or act by indirect glucose–fatty acid metabolic cycle mechanisms [12]. Our findings showed that the RETN gene polymorphism at the AA genotype was significantly correlated with T1DM. In addition, the high frequency of the A allele in T1DM patients may influence susceptibility to T1DM. According to several types of research, resistin may affect glucose tolerance, insulin action, and skeletal muscle glucose intake. Moreover, in diet-induced obese mice, the neutralization of resistin with an anti-resistin antibody led to the development of insulin action. In humans, macrophages primarily generate resistin, a released cytokine that may have an impact on several tissues [13]. According to another study, people with insulin resistance tend to have an AA genotype with an A allele polymorphism, and their levels of TNF-α are much greater than those in the healthy group [14]. However, our findings showed that the RETN gene polymorphism at the AA genotype was not significantly correlated with T2DM. In addition, the high frequency of the AA genotype and A allele in T2DM patients may influence susceptibility to T2DM. The studies showed that the RETN gene variants have been linked strongly to the incidence of type 2 diabetes [15]. According to the study's findings, there are no appreciable differences in Indonesia between the genotype and allele frequency distribution of the RETN gene rs3745368 polymorphism and the levels of resistin and obesity. Resistin level and BMI are inversely correlated [16]. Based on the results of various studies, it was determined that metabolic syndrome (MetS) in the Chinese population is not caused by mutations in the RETN genes [17]. Moreover, research revealed that resistin does not appear to worsen insulin resistance in people of African descent [18].

Our findings demonstrated a correlation between resistin levels and the RETN gene's SNP rs115073262. This lends credence to the idea that resistin plays a role in the pathophysiology of T1DM. We found that the AA genotype was significantly associated with higher resistin levels in T1DM, but did not find an association between AC, CC genotypes, and resistin levels in the T1DM group compared to T2DM group and the control group. According to the studies, genetic variables such as SNPs are believed to be a factor in the rising incidence of DM. According to Dany et al. SNPs in the RETN gene, which codes for the resistin protein [12], may contribute to anomalies in lipid and blood glucose metabolism as well as inflammatory markers [11]. Resistin plays a part in the pro-inflammatory process, and IL-1, TNF-α, and IL-6 have an impact on how it expressed in peripheral blood mononuclear cells from several types of research. In patients with severe inflammatory illnesses, a correlation between the production and secretion of resistin and other inflammatory indicators such as IL-6, leptin, and CRP has also been documented. When macrophages are incubated with recombinant human resistin, the production of the pro-inflammatory cytokines TNF-α and IL-12 is increased [16]. To cause insulin resistance, RETN gene SNPs may need to interact with other genes or factors, or they may work through covert mechanisms involving the glucose-fatty acid metabolic cycle [12]. In SNP rs115073262, we did not observe any association between serum resistin levels and AA, AC, and CC genotypes in the T2DM group compared to the control group. Another study found a strong correlation between serum resistin level and insulin level in the

Indonesian population and polymorphism of +299G>A as a risk factor for insulin resistance [19]. Additionally, according to another study by Fawzy et al., the RETN+299 G>A SNP and an increase in serum resistin may have enhanced insulin resistance and made T2DM susceptibility in the children of T2DM patients in Egypt more likely [20]. Studies that quantify resistin levels and use custom genotyping in the 3′UTR of the RETN gene in T1DM and T2DM are currently rare. To the best of our knowledge, no research has been done on the relationship between T1DM and the RETN gene SNP rs115073262. More research is needed to determine how markers of inflammation in T1DM interact with the RETN gene and its polymorphism.

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