Chemerin Gene Polymorphisms and Their Association with Risk of Diabetes Mellitus Type2 in A Sample of Iraqi Patients

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Abstract
Type 2 diabetes mellitus was first identified as a component of the metabolic syndrome in 1988.Genetic, environmental, and behavioral risk factors interact to cause it. The most prevalent form of diabetes, Type 2 DM, affects between 90 and 95 percent of diabetics. A number of potential gene polymorphisms that may influence susceptibility to T2DM, obesity, and type-2 diabetes mellitus (T2DM) are associated with dysregulation of adipokines. These candidate genes include adipokines. Chemerin is a novel adipokine that regulates inflammation, adipogenesis, and glucose metabolism by acting as an endocrine signaling molecule. This research included 100 blood samples collected from type 2 diabetes patients divided into normal-weight T2DM patients (n = 50) and obese patients with T2DM (n = 50) and 50 apparently healthy subjects as a control, which was collected from Al-Jadriya International Medical Center during the month of November 2019 until October 2020.The results showed that serum fasting blood sugar were in apparently healthy subjects significantly ($p< 0.01$) lower than those of normal weight T2DM patients and obese T2DM patients. In addition, serum HbA1c levels were significantly (p 0.01) lower in apparently healthy subjects than in normal weight T2DM patients and obese T2DM patients. DNA samples for the three study groups were genotyped for chemerin gene SNP (rs17173608). Normal weight T2DM patients had a significantly higher percentage of the G allele (p 0.05) than obese T2DM patients and apparently healthy subjects, and normal weight T2DM patients had a significantly higher frequency of the GG genotype (p 0.05) than obese T2DM patients and apparently healthy subjects. In conclusion, a risk factor for type 2 diabetes mellitus in Iraqi patients of normal weight is the GG genotype and G allele of the rs17173608 SNP at the chemerin gene.
Keywords: Chemerin, type 2 diabetes mellitus, Adipokine, SNP

1. Introduction

Type 2 diabetes is a serious chronic disease that affects many people along with other risk factors like obesity and a sedentary lifestyle. Genes and the environment interact in a complex way to cause it. Type 2 diabetes and its entanglements comprise a significant overall general medical condition, influencing practically all populaces in both created and non-industrial nations with high paces of diabetes-related horribleness and mortality. (Yanling and others, 2014). The pathogenesis of type 2 diabetes involves two fundamental abnormalities: resistance to insulin's biologic functions in glucose and lipid metabolism and inadequate insulin secretion from pancreatic ß cells (Saltiel and Kahn, 2001)
Multiple lines of evidence (Tabatabaei et al.) suggest that genetic polymorphism plays a role in the pathogenesis of numerous human diseases, including diabetes, kidney disease, cancer, diabetes, cardiovascular diseases, and neurodegenerative diseases.,2017).Numerous polymorphisms in candidate genes, such as adipokines, that may influence T2DM susceptibility have been discovered in recent years (Zhang et al.,2013). The human body's largest endocrine organ is now known to be adipose tissue.Numerous adipocytokines are secreted by this tissue, all of which play multiple roles in the metabolic profile and immune system (Shin et al.,2012).Adipokine dysregulation is linked to obesity and diabetes type 2 . Roman et al. discovered that the novel adipokine chemerin, which acts as an endocrine signaling molecule, controls adipogenesis, inflammation, and glucose metabolism. Chemerin is a recently discovered adipocytokine that controls immune function as well as adipocyte development and metabolic function.2007;Goralseki and others,2007). The purpose of this study was to find out if the chemerin gene's genetic variations are linked to an increased risk of type 2 diabetes, how these genetic variations affect the levels of some relevant parameters in blood serum, and how obesity affects the relationship between these genetic variants and the incidence of type 2 diabetes.

2. Materials and Methods

Subjects

In total, 150 people participated in this study: 50 apparently healthy control subjects and 100 T2DM

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patients. Type 2 diabetes patients divided into normal-weight T2DM patients (n = 50) and obese patients with T2DM (n = 50).

According to the age results revealed that most normal weight patients with T2DM were aged between 40-50 years (40%), while, the most obese patients with T2DM were aged more than 50 years (42%). For the date of disease diagnosis, the results showed that the highest percentage of T2DM patients of normal weight and the highest percentage of T2DM patients of obesity were both diagnosed at or after one year (72 percent).Male to female ratios were 48 percent:52%), (72% :28%) and (38% :62% for T2DM patients of normal weight, T2DM patients of obesity, and apparently healthy subjects (control), respectively. The COBAS analyzer was used to measure glucose concentrations and glycated hemoglobin (HbA1c).Using a genomic DNA purification kit, total genomic DNA was extracted.

Genotyping of Polymorphisms

The chemerin SNP polymorphisms (rs17173608) was detected by TaqMan SNP genotyping using real time thermocycler and the following primers and probes: forward primer (5′-TGGCTC AGCTGTCACCTATT-3') and Reverse primer (5′-TCTAGTTCCCG CTCTGTGAG-3') and VIC–BHQ dye (5′-CCGTGAGCCGACAGAGG-3') and Fam–BHQ (5′CTTGAGCCGACAGAGGGAA -3'). Real time Taq Man PCR was performed in a 25 µl total volume, Primer forward 1 µl(10 pmol) , Primer reverse 1 µl (10 pmol) ,Template DNA 3 µl, (4µg/ml) and 12.5 µl Taq Man master mix . Fam – BHQ 1 µl (10 pmol), VIC – BHQ 1 µl (10 pmol) and 4.5 µl free nuclease water.

3. Statistical Analysis

The Statistical Analysis System (SAS) program from 2012 was used to determine how difference factors affected the study's parameters. Significant comparisons between means were made using the Least Significant Difference (LSD) test of the analysis of variance (ANOVA). The chi-square test was used to estimate the CI and odds ratio of the study to make a significant comparison between percentages (0.05 and 0.01 probability.(

4. Results and Discussion

Table 1 represent The values of serum fasting blood sugar were in apparently healthy subjects significantly ($p< 0.01$) lower than those of T2DM patients of normal weight and obese T2DM patients (128.16 ± 3.2 *versus* 237.96 ± 18.2 and 231.76 ± 10.9 mg / d L, respectively). In addition, the values of serum HbA1c were in apparently healthy subjects significantly ($p< 0.01$) lower than those with T2DM who are normal weight and those with T2DM who are obese (5.49 ± 0.1 *versus* 8.96 ± 0.4 and 8.98 ± 0.3, respectively). These results are consistent with those of Sale et al.Chemerin supplementation decreased insulin-stimulated glucose uptake in skeletal muscle cells, according to a 2009 study. Tan and others (2009) as well as Sell et al. (2009) reported on decresed insulin sensitivity and inhibition of glucose uptake in response to chemerin exposure in skeletal muscle cells. (2011) and Hu and Feng (2011) showed that levels of chemicals in the blood were significantly increased in patients with T2DM compared to apparently healthy subjects. According to Takahashi et al. (2011), According to Takahashi et al (2013), chemerin levels in T2DM patients were significantly lower than in control subjects. MafA, a transcription factor found in pancreatic beta cells that also positively regulates the expression of glucose transporter (GLUT)-2, is also positively regulated by chemerin / CMKLR1 signaling.

Table (1).Serum FBS and HbA1c in Iraqi patients with type 2 diabetes who weigh normal but are obese. (mean ± SE)

Parameters (mg / d L serum)	Study groups			LSD value
	Apparently healthy control	T2DM patients normal weight [1]	T2DM patients obese weight [2]	
FBS	128.16 ± 3.2 b	237.96 ± 18.2 a	231.76 ± 10.9 a	34.606 **
HbA1c	5.49 ± 0.1 b	8.96 ± 0.4 a	8.98 ± 0.3 a	1.976 **

1 BMI= 18-25; 2 BMI more than 30; ** means a significant difference at 0.01 level,; LSD: least square difference; FBS: fasting blood sugar; HbA1c: glycated haemoglobin .

Genotype and allele frequency of chemerin Gene (rs17173608 T/G)

Results existing in Table 2, as related with TG and TG +GG genotypes, no significant differences in frequency percentage were noted between apparently healthy subjects and normal weight T2DM patients. Whereas, the frequency of the GG genotype was significantly ($p<0.05$) higher in patients with T2DM who were of normal weight than in those who appeared to be in good health (20% *versus* 8 %, respectively, X^2= 0.0463, OR = 1.17, $p<0.05$). Also, the proportion of G allele was significantly ($p< 0.05$) higher in normal weight T2DM patients than in apparently healthy subjects (0.44 *versus* 0.36, respectively, X^2 = 0.0497, OR = 1.039, $p<0.05$).

Table (2). Genotypes and alleles frequency of rs17173608 SNP at chemerin gene in apparently healthy subjects versus patients of normal weight who have type 2 diabetes.				
Rs17173608 [1]	Frequency, n (%)		X^2	OR (CI)
	Control[2]	T2DMN[3]		
Co-dominant				
TT	18 (36%)	16 (32%)	--	1.00 (reference)
TG	28 (56%)	24 (48%)	0.258 NS	0.347 (0.11-0.64)
GG	4 (8%)	10 (20%)	0.0463 *	1.17 (0.86-1.76)
Dominant				
TT	18 (36%)	16 (32%)	--	1.00 (reference)
TG + GG	32 (64%)	34 (68%)	0.611 NS	0.835 (0.31-1.82)
Recessive				
TT + TG	46 (92%)	40 (80%)	--	1.00 (reference)
GG	4 (8%)	10 (20%)	0.0463 *	1.17 (0.86-1.76)
Allele				
T	0.64	0.56	--	1.00 (reference)
G	0.36	0.44	0.0497 *	1.039 (0.68-2.41)
[1] SNP in chemerin gene; [2] apparently healthy subjects; [3] type 2 diabetes mellitus (normal weight) ; X^2 :chi square; OR: odd ratio; CI: confidence interval. N=50 for each group.				

There were no significant differences in frequency percentages between T2DM patients of normal weight and obese T2DM patients with regard to the TG and TG +GG genotypes in obese patients.However, normal-weight T2DM patients had a significantly higher frequency of the GG genotype than obese T2DM patients (20% versus 8%, respectively, X2= 0.0463, OR = 1.17, p0.05).In addition, T2DM patients of normal-weight had a significantly higher percentage of the G allele than obese T2DM patients (0.44 versus 0.34, respectively, X2 = 0.0497, OR = 1.039, p0.05).As depicted in table 3.

Table (3). Genotypes and alleles frequency of rs17173608 SNP at *chemerin* gene in apparently healthy subjects *versus* obese type 2 diabetes mellitus patients.				
Rs17173608 1	Frequency, n (%)		X2	OR (CI)
	Control2	T2DMO3		
Co-dominant				
TT	18 (36%)	20 (40%)	--	1.00 (reference)
TG	28 (56%)	26 (52%)	0.492 NS	0.831 (0.26 -1.44)
GG	4 (8%)	4 (8%)	1.00 NS	0.066 (0.02 – 0.079)
Dominant				
TT	18 (36%)	20 (40%)	--	1.00 (reference)
TG + GG	32 (64%)	30 (60%)	0.1 NS	0.066 (0.02-0.079)
Recessive				
TT + TG	46 (92%)	46 (92%)		1.00 (reference)
GG	4 (8%)	4 (8%)	1.00 NS	1.0 (0.2-4.2)
Allele				
T	0.64	0.66	--	1.00 (reference)
G	0.36	0.34	0.492 NS	0.831 (0.26-1.44)
[1] SNP in chemerin gene; [2] apparently healthy subjects; [3] type 2 diabetes mellitus (obese weight) ; X^2 :chi square; OR: odd ratio; CI: confidence interval. N=50 for each group.				

The findings of Dahpy et al. are supported by these outcomes (2020), which was linked to the rs17173608 SNP at the chemerin gene, suggested that the GG genotype and G allele may reduce the risk of T2DM. Chemerin levels in the serum of the G allele carrier were significantly higher, according to Abdelhamid and Zaafan (2019).This variant polymorphism may have an impact on both the expression of chemerin and its serum level because the polymorphism of chemerin was found to be correlated with its concentrations in the blood. Hashemi et al. (2012) showed that the G allele had a metabolic syndrome danger. Mehanna et al. (2016) found higher fasting glucose in rs17173608 G allele carriers. Hasanvand et al. (2018), as related to the chemerin gene polymorphism rs17173608, the genotypes and alleles were equally distributed between the gestational diabetes mellitus group and the control group, so there was no distinction in the risk of gestational diabetes mellitus associated with this variant. Olt et al. (2019) learned that G allele frequency was higher in the metabolic syndrome group.

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