Original Research Article

The IFIH1 gene polymorphism (Ala946Thr) association with the type 2 diabetes mellitus and hypertension among Iraqi patients

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ABSTRACT

Numerous genes with Single Nucleotide Polymorphisms (SNPs) are implicated in the Type 2 Diabetes Mellitus (T2DM) development. This work conducted as a case-control to assess the association of IFIH1 gene polymorphism (Ala946Thr) to the T2DM and hypertension among Iraqi patients. A total of 100 T2DM patients were enrolled, 50 of them had hypertension. Moreover, 100 subjects were healthy that used as control group. All participants were genotyped using Real Time Polymerase Chain Reaction (RT-PCR). The G allele was more frequent in T2DM+ Hypertension (HTN) and T2DM without HTN (0.51 % and 0.52 %, respectively), while were less frequent (0.425 %) in the control group. The AA genotype was a significant risk factor for T2DM -HTN development. The GA genotype was also significantly ($p < 0.01$ and OR: 5.8-8.3 associated, while the combination of the genotypes GA and AA depicted a significant P value<0.03 and OR of 4.42. The GA genotype was significantly related to the T2DM without hypertension.

***Keywords*:** Type 2 diabetes, IFIH1, polymorphism, hypertension, Iraq

INTRODUCTION

The national public health goal is to regulate blood glucose and blood pressure because the hypertension is a silent killer as the hyperglycemia plays as a risk factor for Cardiovascular Disease (CVD) with increasing mortality rate between 1990 and 2010 [1]. Diabetes and hypertension usually coexist, and the both conditions affect each other. Accordingly, the primary risk factors for CVD include diabetes and hypertension,

and their co-existence lead to more serious atherosclerosis [2].

The Type 2 Diabetes Mellitus (T2DM) is a chronic condition characterized by insulin resistance caused by the inefficient or impaired insulin secretion. There is an initial compensatory rise in insulin levels, but β-cells deficiency leads to reduced insulin levels at a later stage [3]. Genetic and environmental factors influence the epidemiology of the T2DM. The exposure to an obesogenic environment characterized by sedentary activity and excessive consumption of sugar and fat lead to more prone to the T2DM [4] and obesity [5]. For one parent having diabetes, the incidence of T2DM increases about 30-40 %, while both parents with diabetes raises the subject risk up to twice [6]. There are several genes acting as regulatory agents participating in the T2DM development. The susceptibility of these genes differs depending on their position in different chromosomal loci. The identification of the triggering genetic factor associated with T2DM is difficult due to the interaction of environmental factors with these genes that lead to the disorder initiation [7] Hundreds of genetic variants are associated with the T2DM, in particular related to insulin secretion. However, the evidence for their single or combined impact on β-cells function depends largely on the genetic correlation between variants or genetic risk [8]. In defining loci contributing to T2DM susceptibility, Genome-Wide Association Studies (GWAS) of common variants, identified by Minor Allele Frequency (MAF) around 5 %, have been effective. GWAS defined positions are usually represented by a lead Single Nucleotide Polymorphism (SNP) with the region's strongest association signal [6].

An association between T2DM variants and transcriptional enhancer activity has been seen in many studies, especially in human pancreatic islets, liver cells, adipose tissue and muscle [9,10]. More than 400 signals are associated with the risk of T2DM identified by the GWAS. The biology behind GWAS signals may be unlocked by combining genetic, epigenetic and cellular data improvements in human β-cell models combined with technology for genome editing that gives a new possibilities for modeling T2DM pathogenesis [11,12]. There is controversy about the contribution of unusual genetic variation in the prevalence of T2DM. The contribution of unusual variants to the heritability of T2DM is likely to be minimal in a recent analysis [12]. Since a family has one identical twin with T2DM, the probability of this happening in other family members, illness is more than 90 %, while the risk is reduced to 25-50 % among non-

identical [13]. To date, in diverse communities worldwide, more than 50 candidate genes have been studied for T2DM [14]. This represents about ten percent of the likelihood of T2DM occurring. β-cell activity is contributory in most of these genes [13]. The increased need for β-cell secretion in the presence of an early β-cell deficiency caused by genetic and environmental factors contributes to relative hyperinsulinemia and the progression of dysglycaemia and eventually T2DM, overstimulation of β-cells, which can be inherited or due to environmental factors, contributes to hyperinsulinemia. When β-cell exhaustion happens, the T2DM eventually evolves [15].

In exon 15 of the interferon-encoding gene induced helicase C domain 1 (*IFIH1*, 2q24.3), a non-synonymous single nucleotide polymorphism (SNP, rs1990760) results in adenine (A)/guanine (G) nucleotide and alanine [Ala] 946 threonine [Thr] amino acid change [16]. This SNP was most related marker in *IFIH1* used a logistic regression analysis [17]. Recent findings regarding the *IFIH1* sequence has been linked with multiple autoimmune disorders such as Systemic Lupus Erythematous (SLE), type 1 diabetes, psoriasis, and polymorphism of vitiligo found in the GWASs [18].

In view of the observational and clinical correlations between viral infection and type 1 diabetes susceptibility, it is considered a candidate for a causal disease locus because of its position as a receptor for double-stranded DNA from viral infections [19]. Advancement to diabetes was linked with polymorphisms within the *IFIH1* gene, but not really the progress of autoimmunity. The results were consistent with the alteration of the reaction to environmental features that influence the transition from autoimmunity to T2DM that is related through the *IFIH1* gene [20].

MATERIALS AND METHODS

Study Design and Population

A case–control study including 200 subjects were enrolled herein. Two main groups of this study, one main group of 100 patients divided into two subgroups (one group was 50 patients of T2DM with hypertention, and another group of 50 patients of T2DM without hypertention), and second group of 100 healthy indiviuals used as control. For both age and gender, the patients and control groups were matched. There were 51 females and 49 male patients and also 59 female and 41 males in the control group. All patients groups were randomly selected from Diabetic Center in AL-Sadder Teaching Hospital in An Najaf governorate during August 2020 to January 2021. The study was carried out in

the department of biochemistry in college of pharmacy/ university of Kufa. It was conducted to assess the association of IFIH1 gene rs1990760 SNP with the T2DM with and without CVD or hypertension.

Collecting samples

Two milliliters of overnight fasting blood samples were obtained from all T2DM as well as healthy subjects by vein puncture and collecting in the EDTA containing tube and kept at -20˚C.

DNA Extraction

The genomic DNA (gDNA) was extracted from whole blood according instruction of corporation (Promega; USA). Typically, its concentration and purity were the first thing one need to hear about a DNA harvested. Both were measured by the calculation of ultraviolet light absorption. Dependent on the wavelength, DNA more or less intensely absorbs UV. A Quantus spectrophotometer (Promega; USA) was used, which takes 260/280 nm wavelength measurements.

Real time –polymerase chain reaction (RT-PCR) Assay

In the application of the standard Real-Time PCR, all the extracted DNA samples were subjected to the quantitative real time PCR (RT-qPCR). The polymorphism of *IFIH1* rs1990760 (G/A) was genotyped by TaqMan method using forward, revers primers and specific unique probes to this SNP, (Assays-By-Design Service; Life Technologies, Foster City, CA) 5'-ACC-ATT-TAT-TTG-ATA-GTC-GGC-ACA-CT-3'(forward); 5' CTC-CAT-GAT-GAT-TCT-TTC-CCT-TTG-ATA-CTT-3' (reverse); 5'-AAG-AGA-AAA-CAA-AGC-ACT-GC-3' (VIC; specific to the G allele) and 5'-AAG-AGA-AAA-CAA-AAC-ACT-GC-3' (FAM, specific to the A allele). Reactions were performed in 0.2 μL wells, with a total volume of 25 μL using 2 ng of template DNA and a qPCR Master Mix GoTaq® Probe (Promega, USA). Then the wells were located in a thermal cycler (Rotor Gene Q 6PLEX; QIAGEN, USA), where the thermal cycling included 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 63 °C for 1 min. With a calculated error rate based on PCR duplicates of less than 1 percent, the genotyping success rate was higher than 95 %.

Statistical Analysis

The presented data was analyzed using SPSS software v.20.0 (PASW Statistics, Journal Pre-Proof Journal Pre-proof 8 SPSS Inc., Chicago, IL, USA). All clinical parameters and demographic data was defined as mean ±SD and analyzed using t-test and Chi-square ($\chi 2$) test. Hardy-Weinberg equilibrium was done by SNP-Analyser version 1.15 ga easy analysis ($p>0.05$) using the frequencies of intended gene for healthy individuals. Genotyping calculations between healthy

subjects and patients was performed using odds ratio (OR) and 95 % confidence intervals (95 %). P value less than 0.05 was considered a significant finding.

RESULTS

Clinical Population and Data

This study included a total of 200 randomly unrelated Iraqi subjects; 50 patients with T2DM and 50 T2DM patients with hypertension and 100 controls. The average age [(±Standard Deviation (SD)] was (45.77±5.42; 46.03±5.62), 47.78 ± 7.43 years respectively.

Genotyping and gene polymorphism

Subsequent to PCR, the genotyping of *IFIH1* gene SNPs was conducted using the RT-qPCR. The rate of alleles has been depicted in the Table 1.

In the Table1, the predominant heterozygote included the GA SNP. The Table 2 exhibits the *IFIH1* rs1990760 (Ala946Thr) SNP analysis using the Chi-square ($\chi 2$) test. Considering the Table 2 results, the GA SNP was significantly associated with the T2DM without hypertension ($p<0.05$). The comparison between genotypes both allele and genotypes of the *IFIH1* Ala946Thr (rs1990670) SNP were not significantly related to the T2DM +HTN. Table 3 represents the genotypes of rs199760 (G/A) SNP in control and T2DM-HTN groups.

Table1. Frequencies of *IFIH1* gene SNPs among T2DM+HTN, T2DM patients without hypertension (T2DM-HTN) and healthy subjects

Genotype	**Patients**			**Healthy control=100**
	T2DM+HTN NO=50	T2DM-HTN NO=50	Total NO=100	
GG	11	3	14	18
GA	29	46	75	49
AA	10	1	11	33
Total	50	50	100	100

Table 2. The *IFIH1* rs1990760 (Ala946Thr) SNP analysis

Control samples					
Genotype of Control	Observed	Expected	Difference	X^2	p value
GG Reference	18	18.06	0.06	$6.5*10^{-4}$	0.98
GA Heterozygote	49	48.88	0.12		
AA Recessive	33	33.1	0.1		
Genotype of DM & Hypertension					
GG Reference	11	13.005	2.005	1.3	0.26
GA Heterozygote	29	24.9	4.1		
AA Recessive	10	12.005	2.005		
Genotype of DM without hypertension					
GG Reference	3	13.5	10.5	35,53	<0.05
GA Heterozygote	46	24.96	21.04		
AA Recessive	1	11.52	10.52		

The genotype GA in codominant and in overdominant showed significant (p=0.0085) with OR of 5.6 and in over dominant (p < 0.0001 and OR of 12), while the AA genotype was significant (p=0.002 with OR of 24.1) in T2DM+HTN. Hence, the AA genotype was a significant risk factor in the T2DM-HTN group. Table 4 demonstrates the genotypes of rs199760 (G/A) SNP in T2DM+HTN and T2DM-HTN groups. In the Table 4, the GA genotype in co-dominant and over-dominant groups showed significant (p<0.01 with OR is 5.8 and p=0.0004 with the OR of 8.3, respectively), while the combination of the GA+ AA genotypes in the dominant group showed significant (p=0.03 with OR of 4.42) association to the T2DM.

Table 3. Genotypes of SNP rs199760 (G/A) in control and patients of T2DM-HTN groups

SNP rs1990760 (G/A)	**Control No=100**	**T2DM-HTN No=50**	**OR (CI 95%)**	**p value**
Codominant				
GG (Wild type)	18	3	1.00	0.0085
GA	49	46	5.6 1.5555 to 20.3970	
AA	33	1	0.2 0.0176 to 1.8780	>0.05
Dominant				
GA+AA	82	47	3.4 0.9621 to 12.2924	>0.05
Over dominant				
GG+AA	51	4	1.00	< 0.0001
GA	49	46	12 4.0068 to 35.7560	
Recessive				
GG+GA (Wild type)	67	49	1.00	0.002
AA	33	1	24.1 3.1910 to 182.5341	
Additive				
2AA+GA	115	48	2.5 0.7048 to 8.8983	>0.05

Table 4. Genotypes of rs199760 (G/A) SNP in the T2DM+HTN and T2DM-HTN groups

SNP rs1990760 (G/A)	**T2DM+HTN No=50**	**T2DM-HTN No=50**	**OR (CI 95%)**	**p value**
Codominant				
GG (Wild type)	11	3	1.00	<0.01
GA	29	46	5.8 1.4949 to 22.6286	
AA	10	1	0.37 0.0326 to 4.1227	>0.05
Dominant				
GA+AA	39	47	4.42 1.1509 to 16.9664	0.03
Over dominant				
GG+AA	21	4	1.00	0.0004
GA	29	46	8.3 2.5952 to 26.7216	
Recessive				
GG+GA (Wild type)	40	49	1.00	<0.01
AA	10	1	0.08 0.0100 to 0.6651	
Additive				
2AA+GA	49	48	3.6 0.9431 to 13.6795	>0.05

DISCUSSION

The T2DM and HTN, the two main groups of the international overburden diseases are generally identified as coexisting. The T2DM and HTN conditions increase the risk of CVD (2~4-folds), end-stage kidney disease and death compared to healthy subjects [21]. Hypertension is normal in patients with diabetes, among other factors, depending on the form and period of diabetes, age, gender, race/ethnicity, body mass index (BMI) and history of glycemic control [22]. *IFIH1* gene encodes the gene-

I-like(RIG-I-like) receptor which is a member of retinoic acid-inducible gene-I-like receptors (RLRs) family identifying viral structures and triggering innate immune responses, specifically type I interferon's, to inhibit the viral infection in most tissues [23]. Polymorphism in the gene contributes to differences between persons in *IFIH1* expression and could affect the study of the causes of disease with rise risk of multiple autoimmune diseases, like the diabetes [24]. The aim of this study was to estimate how the predominance of T2DM in the Iraqi population affected by genotypes and allele distribution of polymorphism in this gene.

In the present study, various parameters were assessed in order to verify how polymorphism influences T2DM development. Therefore, the genotype and allele distributions in patients and control groups were compared. No statistically relevant findings have been found for this polymorphism ($p > 0.05$) related to T2DM of Iraqi population previously. Our results indicated that the *IFIH1* rsSNP rs1990760 (Ala946Thr) was not an important susceptibility locus for T2DM in the registry-based Iraqi population. A study by Hatmal, *et al*, [25] had similar findings regarding the genetic polymorphism and lipid profile in T2DM. The GA genotype was more frequent than other genotypes in patients and control group but the AA was more frequent in the control group. The A allele was significantly more frequent in healthy controls (57.5 %) than T2DM patients which was in agreement with Jameel *et al* [26] who had the results of the genotyping of rs1990760 (G>A) by 65 % of the patient population carrying the AA homozygous genotype. Our study was in contrast to Bouças *et al* [27] findings in which the G/G genotype was associated with the *IFIH1* gene expression (2 fold-increase) in mononuclear cells.

CONCLUSION

In our study the mutant of AA genotype occurred in control group was more frequent significantly than the GA in the T2DM patients without HTN. Moreover, the G allele was more frequent in T2DM+ HTN and T2DM without HTN by (0.51 % and 0.52 %, respectively), while being less frequent (0.425 %) in control group. The AA genotype was a significant risk factor for T2DM -HTN development. The GA genotype was also significantly associated, while the combination of the genotypes GA and AA depicted a significant association. The GA genotype was significantly related to the T2DM without HTN.

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