TGF-β1 Gene Polymorphism in Codon 10 +869*C/T and Codon 25 +915*G/C Positions in Iraqi Patients with Type 2 Diabetes Mellitus

التعدد الشكلي لجين *TGF-β1* في الموقعين Codon 10 +869*C/T وCodon 25 +915*G/C في المرضى العراقيين المصابين بمرض السكري-النوع الثاني

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Abstract

This study included 50 blood samples that were collected from patients with age ranged between 35-65 years. Thirty samples were collected from patients with Type 2 Diabetes Mellitus (T2DM), while 20 blood samples were collected from healthy individuals as a control sample. The polymorphism results of *TGF-β1* gene in codon 10: +869*C/T position by using amplification refractory mutation system (ARMS-PCR) showed that the *T* allele was suggested to have a protective effect, while *C* allele was associated with an increased risk of T2DM. The TT and CT were suggested to have a protective effect, while CC genotype was associated with an increased risk of T2DM. The polymorphism results of *TGF-β1* gene in codon 25: +915*G/C position in samples showed that the *G* allele was suggested to have a protective effect, while *C* allele was associated with an increased risk of T2DM. The GC genotype was suggested to have a protective effect, while GG and CC genotypes were associated with an increased risk of T2DM.

Keywords: *TGFβ1* gene, Type 2 Diabetes Mellitus, codon 10: +869*C/T, codon 25: +915*G/C

الملخص

تضمنت هذه الدراسة 50 عينة دم جمعت من اشخاص تراوحت اعمارهم بين 35-65 سنة. ثلاثون عينة دم جمعت من مرضى السكري- النوع الثاني و20 عينة دم جمعت من اشخاص اصحاء والتي اعتبرت عينة قياسية. اظهرت نتائج التعدد الشكلي لجين *TGF-β1* في الموقع Codon 10: +869*C/T باستعمال تقانة نظام الممانعة للتضخيم Amplification refractory mutation system (ARMS-PCR) بأن الأليل *T* قد ظهر كأليل وقائي من المرض Preventive faction (PF)، بينما ظهر الأليل *C* كأليل مسبباً للمرض Etiological faction (EF). ظهرا النمطين الوراثيين TT وCT كنمطين وقائين من المرض، بينما النمط الوراثي CC ظهر كنمط وراثي مسبباً للمرض. اظهرت نتائج التعدد الشكلي لجين *TGF-β1* في الموقع Codon 25: +915*G/C بأن الأليل *G* قد ظهر كأليل وقائي من المرض، بينما ظهر الأليل *C* كأليل مسبباً ومرتبطاً بالمرض. ظهر النمط GC كنمط وراثي وقائي من المرض، بينما ظهرا النمطين الوراثين GG وCC كنمطين مرتبطين ومسببين لمرض السكري- النوع الثاني.

الكلمات الدالة: جين *TGFβ1* ، مرض السكري- النوع الثاني، Codon 10: +869*C/T ، Codon 25: +915*G/C

Introduction

The type 2 diabetes mellitus (T2DM) is a common, chronic, complex disorder of rapidly growing global importance. It accounts for 95% of diabetes worldwide is characterized by concomitant defects in insulin secretion (from the β-cells in the pancreatic islets) and insulin action (in fat, muscle, liver, and elsewhere); the latter being typically associated with obesity [1].

The maximal capacity of cytokine production in individuals has a major genetic component [2]. A potential mechanism was described involving polymorphisms within the coding regions or signal sequences of cytokine genes. These genetic polymorphisms were shown to affect the overall expression and secretion of cytokines *in vivo* [3]. TGF-β1 is an anti-inflammatory immune mediator, which inhibits or reverses the activation of macrophages by interfering with their activation pathways [4]. The highly polymorphic human *TGF-β1* gene is located on chromosome 19q 13.1–13.3 [5]. There have been some known *TGF-β1* gene polymorphisms such as 2988C/A, 2800G/A, 2509C/T and +869T/C; however, previous studies concerning association between 2509C/T polymorphism and risk of T2DM are limited and their results are rather conflicting [6-8]. The polymorphisms at codons 10 and 25 may be associated with higher or lower *TGF-β1* synthesis *in vitro* [9]. Increases or decreases in the production of TGF-β1 have been linked to numerous diseases including atherosclerosis, and fibrotic diseases of the kidney, liver, and lung [10]. This work aimed to explore association between T2DM and *TGF-β1* gene polymorphisms at +869 T>C and +915 G>C positions.

Materials and Methods

Subjects

Thirty T2DM patients and 20 control (healthy) persons were enrolled in the study. All samples were collected from Abu-Graeeb and Al-Shaheed Saif Saad hospitals in Baghdad-Iraq. The disease was diagnosed international criteria, by the consultant medical staff at both hospitals. All cases and controls were in the age range 35-65 years, and they were from the same geographical and socioeconomics conditions. The peripheral blood was collected at the time of diagnosis in anticoagulants EDTA tubes for DNA isolation.

Genotyping

DNA from venous blood was isolated using ReliaPrep™ Blood gDNA Miniprep System kit (Promega) and used for the genetic analysis. The *TGF-β1*gene at positions codon 10 +869*C/T and codon 25 +915*G/C were genotyped using the amplification refractory mutation system (ARMS-PCR) approach. Primers for these positions were used depending on [11,12], and synthesized in Alpha DNA company (Canada). Primer sequences of the *TGF-β1*gene at position codon 10 +869*C/T was 5′- TCCGTGGGATACTGAGACAC-3′ as generic primer and 5′- AGCAGCGGTAGCAGCAGCA-3′ for specific T primer and 5′- GCAGCGGTAGCAGCAGCG-3′ for specific C primers. Primer sequences of the *TGF-β1*gene at positions codon 25 +915*G/C was 5′- GGCTCCGGTTCTGCACTC-3′ as generic primer and 5′- GTGCTGACGCCTGGCCG-3′ for specific G primer and 5′- GTGCTGACGCCTGGCCC-3′ for specific C primers. *TGF-β1*gene forward internal control primer was 5′- GCCTTCCCAACCATTCCCTTA- 3′ and the reverse primer was 5′- TCACGGATTTCTGTTGTGTTTC-3′.

ARMS-PCR

ARMS-PCR approach was used for detecting polymorphisms at *TGF-β1* gene positions. AccuPower® PCR PreMix (Bionner, Korea) was prepared depending on [11,12] with some modifications. Twenty micoroleter of PCR reaction was used: 12.5 µl master mix (1x), 1 µl of each primer (1 µM) and 2 µl of DNA template (100 ng). Total volume of reaction was completed with nuclease-Free water. The spcific T and generic primers were used for detecting *T* allele and the spcific *C* and generic primers were used for detecting *C* allele for the *TGF-β1* gene at position codon 10 +869*C/T. The specific *G* and generic primers were used for detecting *G* allele and the specific *C* and generic primers were used for detecting *C* allele for the *TGF-β1* gene position codon 25 +915*G/C. The reaction mixers were placed in Thermo cycler (Esco, Singapore). PCR conditions for the all reaction mixers was: template denature at 95°C for 1 min, first initial denaturation at 95°C for 15 sec, first annealing at 65°C for 15 sec and first extension at 72°C for 40 sec. The last three steps were 10 cycles. The second initial denaturation is at 95°C for 15 sec, second annealing at 56°C for 20 sec and second extension at 72°C for 50 sec. The last three steps were 20 cycles and the final extension at 72°C for 7 min. The PCR products were resolved on 2% agarose gels prepared in 1x TBE buffer (Bioner, Korea). The DNA ladder (2000 bp) with intervals 100 bp was also loaded on the agarose gel (Bioner, Korea). Three microliter of Bromophenol blue dye was loaded with the all reaction mixers. The gel electrophoresis was done by using 75V for 3-4 hrs in 1x Tris-borate buffer (TBE). The gel was stained with ethidium bromide (Promega, USA) for 20 min and documented with gel documentation system (Biocom, USA).

Statistical analysis

Data were given as percentage frequencies and significant differences between patients and controls were by Fisher's exact test. The Odds ratio (OR) and Confidence Intervals (CI) were analyzed by using Compare 2 Ver.3.04 program designed by J. H. Abramson/2003-2013. Deviations from Hardy–Weinberg were tested using an exact test available at http://www.had2know.com/academics.

Results and Discussion

The polymorphism results of *TGF-β1* gene in codon 10: +869*C/T position by using ARMS-PCR showed the presence of *T* and *C* alleles and three genotypes (TT, CT and CC) by using the specific *T*, specific *C* and generic primers. Presence of one band in *T* lane and absence this band in *C* lane refers to the genotype TT. In contrast, presence of one band in *C* lane and absence of this band in *T* lane refer to the genotype CC. Presence of two bands in both lanes refer to the genotype is CT Figure (1).

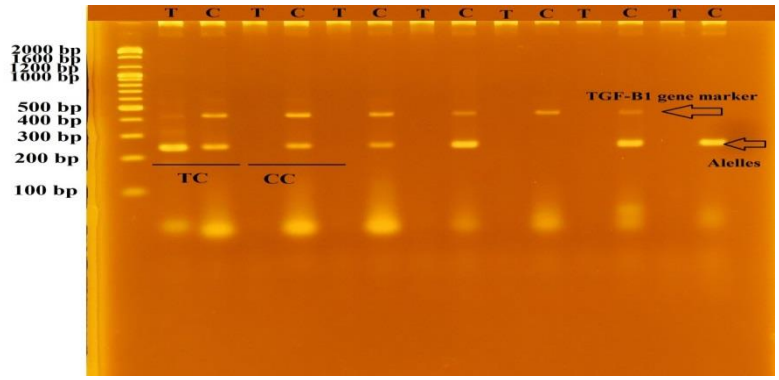

Fig. (1): Gel electrophoresis for the *TGF-β1* gene codon 10 +869*C/T showing the *T* and *C* alleles in some T2DM patients (Gel electrophoresis was done by using 1.5%agarose gel concentration, 75 Volt for 2 hours)

The frequency of *T* allele in control sample was higher in comparison with the same allele in patients (57.5% vs. 33.3%). In contrast, the *C* allele in patient sample (66.7%) was higher as compared with control sample (42.5%) Table (1). The odds ratio (OR) for the *T* and *C* alleles were 0.37 and 2.71, respectively; therefore the *T* allele was suggested to have a protective effect, while *C* allele was associated with an increased risk of T2DM Table (1). The genotypes of *TGF-β1* gene codon 10 +869*C\T position are shown in Table (2). The frequencies of TT and CT genotypes in control sample were higher in comparison with the same genotypes in patients and the frequencies were 35% and 45% for the TT and CT genotypes, respectively in control and 23.3% and 20% for the TT and CT genotypes, respectively in patients. In contrast, the CC genotype in patient sample (56.7%) was higher as compared with control sample (20%). The odds ratio for the TT, CT and CC genotypes were 0.57, 0.31 and 5.23, respectively. The TT and CT were suggested to have a protective effect, while CC genotype was associated with an increased risk of T2DM.

Table (1): Allele frequencies of *TGF-β1* codon 10 +869*C/T and Codon 25 +915*G/C genotypes in patient and control samples

Gene position	Allele	T2DM No. (%)	Control No. (%)	OR(95%CI)	P value
TGF-β1 codon 10 +869*C/T	T	20 (33.3)	23 (57.5)	0.37(0.16-0.84)	#0.014
	C	40 (66.7)	17 (42.5)	2.71(1.20-6.12)	
TGF-β1 codon 25 +915*G/C	G	42 (70.0)	29 (72.5)	0.89(0.37-2.13)	0.485
	C	18 (30.0)	11 (27.5)	1.13(0.47-2.72)	

OR=Odds ratio, CI=Confidence Intervals, #=Significant differences at P<0.05 level by using Fisher's test.

Table (2): Frequency distribution of the *TGF-β1* codon 10 +869*C/T and codon 25 +915*G/C genotypes in patient and control samples

Gene	Genotype	T1DM No. (%)	Control No. (%)	OR(95%CI)	P value
*TGF-β1*gene codon 10 +869*C/T	TT	7(23.3)	7(35.0)	0.57(0.17-1.91)	0.280
	CT	6(20.0)	9(45.0)	0.31(0.09-1.04)	#0.058
	CC	17(56.7)	4(20.0)	5.23(1.45-18.86)	#0.010
*TGF-β1*gene codon 25 +915*G/C	GG	19(63.3)	12(60.0)	1.5(0.37-3.59)	0.522
	GC	4(13.4)	5(25.0)	0.46(0.11-1.93)	0.247
	CC	7(23.3)	3(15.0)	1.72(0.04-7.40)	0.365

OR=Odds ratio, CI=Confidence Intervals, #=Significant differences at P<0.05 level by using Fisher's test.

The polymorphism results of *TGF-β1* gene in codon 25: +915*G/C position in samples by using ARMS-PCR showed the presence of *G* and *C* alleles and three genotypes (GG, GC and CC) by using the specific *G*, specific *C* and generic primers. Presence one band in *G* lane and absence this band in *C* lane refers to the genotype GG. In contrast, presence one band in *C* lane and absence of this band in *G* lane refer to the genotype GG. Presence two bands in both lanes refer to the genotype GG Figure (2).

The frequency of *G* allele in control sample was higher in comparison with the same allele in patients (72.5% and 70%). In contrast, the *C* allele in patient sample (30%) was higher as compared with control sample

(27.5%) Table (1). The odds ratio (OR) for the *G* and *C* alleles were 0.89 and 1.13, respectively; therefore the *G* allele was suggested to have a protective effect, while *C* allele was associated with an increased risk of T2DM Table (1). The genotypes of *TGF-β1* gene codon 25 +915*G\C position are showed in Table (2). The frequencies of GC genotype in control sample were higher in comparison with the same genotypes in patients and the frequencies were 25% and 13.4%, respectively. In contrast, the GG and CC genotypes in patient sample (63.3% and 23.3%, respectively) were higher as compared with control sample (60%) and (15%), respectively. The odds ratio for the GG, GC and CC genotypes are 1.5, 0.46 and 1.72, respectively. The GC genotype was suggested to have a protective effect, while GG and CC genotypes were associated with an increased risk of T2DM.

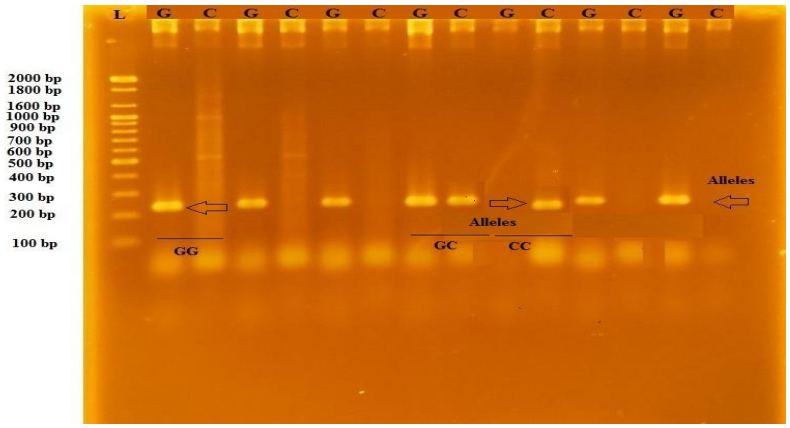

Fig. (2) Gel electrophoresis for the *TGF-β1* gene codon 25 +915*G/C showing the G and C alleles in some T2DM patients
(Gel electrophoresis was done by using 1.5%agarose gel concentration, 75 Volt for 2 hours)

Type 2 diabetes (T2D) is the most common form of diabetes and an increasingly prevalent metabolic disease. It is associated with microvascular and macrovascular complications and is considered one of the major causes of morbidity and mortality [13]. There is mounting evidence that the ability of an individual to produce high or low levels of TGF-β1 may be genetically predetermined [10,14]. Polymorphisms at codons 10 and 25 have been linked with several diseases including autoimmune diseases, such as type 1 diabetes, rheumatoid arthritis, systemic lupus erythematous, infectious, and fibrotic diseases [15]. Respectively, [16,7] studied *TGF-β1* codon 10 polymorphism on Polish and Chinese populations. They reported different distribution of alleles, and genotypes compared with those in Egyptians. The differences between the two studies could be due to differences in the population genetic background. Because of the variation in cytokine allele frequency in different populations, it is not surprising that the results of this study of *TGF-β1* genetic polymorphisms associated with T2D are conflicting in different ethnic groups. However, the small sample size is a limiting factor to have conclusive results, which suggested that *TGF-β1* might play an important role in the etiology in T1D and T2D [17]. Association between diabetic control and change in genotypes of *TGF-β1* codons 10 and 25 was performed to demonstrate if the *TGF-β1* codons 10/25 genotypes had any relation in control of diabetes. In codon 10, most of T2D patients with TT genotype had moderate grade of control where the bad grade was associated with the TC. The same results were obtained for *TGF-β1* codon 25 [18]. Also, [19] found same relationship between the gene and the disease and also, found significant differences in TT genotype frequencies of *TGF-β1* gene codon 10, which related with the disease. In contrast, the same researchers found significant differences in GG genotype frequencies of *TGF-β*1 gene codon 25, which related with this disease. Role of the transforming growth factor-β1 (*TGF-β1*) gene polymorphisms located at codons 10 and 25 in the genetic predisposition to type 2 diabetes (T2D) and in diabetic nephropathy (DN) in Egyptian patients was investigated by [11]. They found that *TGF-β1* (T869C) C allele, TC and TC+CC genotypes were significantly higher in patients; the T allele and TT genotype were significantly higher in controls (Pc<0.001). They also found non-significant differences were detected between T2D patients and controls in the frequencies of TGF-b1 (G915C) alleles and genotypes. The results of this study agreed with the other results especially in codon 10, which done by other researchers [7,11,20] and in codon 25, which done by [7,20] and disagreed with the study, which done by [11].

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