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The association of IL-10 (-592A/C) gene polymorphism with progression of Type 2 Diabetes Mellitus in Basrah Province-Iraq

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Abstract

The study was aimed to investigate the association between IL-10 (-592A/C) gene polymorphism with the progression of type 2 diabeteis mellitus in Basrah Province. This study included (100) subjects (30) person as a control group and (70) patients with T2DM. The patients were distributed as two groups according to Their gender and duration of the disease: group for short duration (≤ 5 years) and group for long duration(>5 years)**.** Lipid profile and glucose concentrations were measured by COBAS analyzer while IL-10 (-592A/C) gene polymorphism was genotyped by using (RFLP-PCR) technique. CC genotype frequency showed a significant decrease while CA genotype revealed significant increase ($p \leq 0.05$) in T2DM compared with controls .No significant differences were observed in the allelic frequencies between both groups. Gender and duration of diabetes didn't show any significant differences. There is significant association between CA genotype with the risk of T2DM (OR=1.50, 95% CI=1.035-2.173). We concluded that IL-10 (-592A/C) gene polymorphism contribute in development of T2DM.

Keywords : IL-10 -592A/C, gene polymorphism,T2DM.

علاقة تعدد الاشكال الجيني ل (592A/C-) IL-10 بتقدم مرض السكري النوع الثاني في محافظة البصرة-العراق

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الخلاصة

هدفت دراستنا الى معرفة العلاقة بين تطور مرض السكري النوع الثاني و متعدد الاشكال المشفر للانترلوكين- ١٠(592A/C-) في محافظة البصرة . تضمنت الدراسة ١٠٠ شخص منها ٣٠ كعينات سيطرة و٧٠ من مرضى السكري النوع الثاني . ووزع المرضى الى مجموعتين بالاعتماد على الجنس وفترة المرض الى مجموعة ذات فترة اقل من خمس سنوات وفترة اكثر من خمسة سنوات .صورة الدهون والكلوكوز تم قياسها بجهاز COBAS بينما IL-10 (592A/C-) بطريقة (RFLP-PCR) .اظهرت النتائج ان النمط الجيني CC انخفاضا معنويا بينما اظهر النمط الجيني CA زيادة معنوية لدى مرضى السكري النوع الثاني . اما الجنس وفترة المرض لم تظهر اي اختلاف معنوي ملحوظ بين المجاميع. ان هناك ارتباط كبير بين النمط الجيني CA وخطر الإصابة بمرض السكري النوع الثاني (95% CI=1.035-2.173, OR=1.50). نستنتج من الدراسه الحاليه ان انترلوكين-١٠ (592A/C) كمحدد جزيئي وراثي مهم في تقدم المرض للمصابين بمرض السكري النوع الثاني.

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Introduction

Diabetes mellitus (DM) is a set of metabolic disorders known by hyperglycemia caused by fault in insulin secretion or action or both [1]. Diabetes mellitus caused many complications such as macrovascular and microvascular [2]. International Diabetes Federation (IDF) (2013) has shown that 371 million people whom suffer from diabetes worldwide, and it may increase up to 552 million by 2030, meaning that, there are three new cases every second [3 ,4].

Diabetes mellitus had been classified into three major types: Type 1 diabetes mellitus (T1DM) which is characterized by beta cell destruction, usually leading to absolute insulin deficiency , type 2 diabetes mellitus (T2DM), which is the most predominant type and is characterized by (insulin resistance) and gestational diabetes mellitus (GDM). Type 2 diabetes is an international health problem featured by a defect in insulin secretion and or a decrease in sensitivity to insulin, also termed insulin resistance [5]. Interleukin-10 (IL-10) is also an anti-inflammatory T helper2 cells (TH2) mediated cytokine that suppress the inflammatory responses of pro-inflammatory cytokines [6]. It is an immune regulatory cytokine, which regulates T cells and monocytes/ macrophages[7].It is mostly produced by monocytes and TH2 , mast cells, regulatory T cells, and in a certain subset of activated T and B cells [8] .In humans, IL-10 is encoded by the IL10 gene, which is located on chromosome 1(q31– 1q32) and consists of five exons [9]. There are three common single nucleotide polymorphisms(SNPs) have been identified at the transcriptional start site in the 5' flanking region of IL-10, which is a strong determinant for IL-10 production: (C/A) at position -592 , (C/T) at position -819 and (G/A) at -1082 [10]. Interindividual differences in cytokine production also appeared to be related to such allelic polymorphisms [11]. Several studies reported that there is a positive correlation between serum level of IL-10 and whole-body insulin sensitivity and have been revealed that low IL-10 production was associated with hyperglycemia [12,13]. Chang *et al*. [14] have been recommended that the gene polymorphism of IL-10 has a specific role in defining diabetic susceptibility . So, recent work aimed concern to study the association between T2DM progression with IL-10 -592A/C gene polymorphism in patients of Basrah Province.

Materials and Methods:

Subjects: The study was involved (100) subjects including(30) person apparently healthy individuals as a control group through using inclusion cretaria which included as [1]Fasting plasma glucose < 100mg/dl; [2] No any past medical history for type 2 diabetes; [3] No family history of diabetes in first-degree relations on the other hand 70 patients selected according to the WHO guidelines [15] any participant with a symptoms of diabetes whose fasting glucose level >120 mg/dl with T2DM and no taking insulin or have T1DM and pregnancy . All patient have divided as two groups according to their gender and duration of the disease: group for short duration (≤ 5 years) and group for long duration(>5 years) . The patients were (35 males and 35 females) their ages were Ranged between 40-70 years were selected from Al-Mawani Hospital Specialized Center for Diabetes and Endocrinology in Basrah City during the period from February 2016 - February 2017.

Blood sampling : Five milliliters of venous blood were drawn by disposable syringe. each blood sample was divided into 3 ml placed in an sterile plane tube and allowed to clot, then serum was separated by centrifugation at 4000 rpm for 15 minutes. Serum was stored at -20 C˚. these sera were used for estimating lipid profile. The remaining 2 ml of blood were put directly in EDTA tube for estimating HbA1c and DNA extraction.

Methods: lipid profile and HbA1c were measured by COBAS automated analyzer Integra 400 Plus (Roch, Germaney).

DNA Extraction and Genotyping

DNA was extracted from blood samples by (gSYNCTM DNA Extraction Kit). Whole Blood Protocol, Geneaid, Korea) according to the manufacturer's instructions. Primers used for both genes are shown in Table-1. PCR conditions for IL-10 gene were an initial denaturation for 5 min at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 64°C for 45s and extension at 72°C for 1 min, and final extension at 72°C for 10 min. The amplification products were separated by electrophoresis through 1% agarose gel stained with and visualized ethidium bromide with positive band at 412 bp [16].

Table1- primers sequences used for amplification of *IL-10 gene*

Primers	Primer sequences
Forward	5'- CCTAGGTCACAGTGACGTGG -3'
Reverse	5'- GTGAGCACTACCTGACTAGC -3'

Restriction Fragment Length Polymorphism- Polymerase Chain Reaction (RFLP - PCR)

IL10-592 A/C single nucleotide polymorphisms(SNPs) were genotyped by restriction fragment length polymorphism (RFLP) according to the method of [17].RFLP assays were done in (20 µL) reaction volume having 10 µl of the PCR products for the *IL- 10 gene* were mixed with 10 unit/1 µl of Rsa1 restriction enzyme (provided by Promega catalog number R6371) with 6.5 µl nuclease-free water , 2 of 10X Buffer and 0.5µl of BSA. The mixture was incubated for at 37 ºC for 4 hours then 10µl of the products was loaded into a 1% agarose gel containing ethidium bromide for electrophoresis. The undigested band was 412 bp and digestion products were 236 bp and 176 bp

Statistical analysis

Data were processed and analyzed using the Statistical Package of Social Science (IBM SPSS , version 19). Quantitative variables were expressed as (mean ± SD) and compared using student t-test. On the other hand, The genotyping and allelic frequencies were compared using the Chi-squared test for comparing between the groups. Odds ratio (OR) and 95% confidence intervals (CI) were used to determine the association between the IL-10 genes and risk of T2MD disease [18].

Results:

Recorded data represented that there was no significant differences between study groups related to gender age and duration of disease as in Table-1.The concentrations of fasting blood glucose (FBG) and HbA1c were significantly increasing ($p \leq 0.05$) in T2DM patients compared with the controls also the results of the lipid profile (total cholesterol, triglyceride and LDL) displayed a significant increasing ($p \leq 0.05$) in T2DM patients as compared with controls. Whereas the mean of HDL were significantly lower ($p \leq 0.05$) in T2DM patients than controls as Table-2.

Table 2-Distribution of the studied groups according to gender , age and duration of disease

Parameters	Controls N=30	T2DM N=70		P-value
Gender (n)% Male Female	 (15) 50% (15) 50%	 (35) 50% (35) 50%		1
Age	29.714 ±6.019	54.991±8.182		0.999
Duration of disease	___	Less than 5 years	More than 5 years	1
	_____	35	35	
		50%	50%	

Table 3- Glucose and lipid profile in T2DM patients and control group

Parameters	Controls (Mean ± SD)	Patients (Mean ± SD)	P-value
HbA1c %	5.573± 0.238	9.598± 2.190	0.00*
FBG (mg/dl)	98.811± 11.064	229.342±97.18	0.00**
Total cholesterol (mg/dl)	160.534± 26.168	186.841±60.239	0.05*
Triglyceride (mg/dl)	110.318± 23.387	188.977±111.871	0.028*
HDL (mg/dl)	47.796± 6.336	37.730± 11.077	0.00*
LDL (mg/dl)	91.131 ± 31.583	151.044± 50.152	0.00*

* = significant difference at p≤0.05
**= significant difference at p≤0.01

The Results were confirmed by (1%) agarose gel electrophoresis analysis which showed that IL-10(-592 A/C) amplification product was obtained to have a size of 412 bp as shown in Figure-1 .Interleukin-10 gene promoter polymorphism (-592A/C) of PCR product was digested by restriction enzyme Rsa1. The products of digestion were analyzed by (1%) agarose gel electrophoresis. The samples which have (CC) genotype showed one band at size (412) bp. Those which have heterozyote (CA) genotype showed three bands with sizes of 412bp, 176bp and 236 bp as shown in Figure-2. The current results revealed a significant difference in the frequencies of the CC and CA genotypes of IL-10 -592C/A between diabetic patients and control subjects ($\chi 2 = 4.889$, $p = 0.027$). Findings exhibited significantly (p≤0.05) lower frequency of CC genotype (34%) in T2DM patients compared with the controls (56%) while CA genotype frequency showed a significant increase(p≤0.05) (66%) in T2DM compared with (44%)controls .Furthermore, A and C allele didn't show any significant differences between the groups. In addition, there is a significant association between CA genotype with the risk of T2DM (OR=1.50, 95% CI=1.035-2.173) when as seen in Table-3.

Table 3-Comparison of the genotype Frequencies and Alleles within the −592 Region of the *IL-10 gene* between T2DM and controls

	T2DM (n) %	**Controls (n) %**	**OR (95% CI)**	**p value χ^2**
Genotype Frequency (n) %				
CC	(17) 34%	(28) 56%	0.607(0.384-0.960)	**p=0.٠٢7** χ^2=4.889
CA	(33) 66 %	(22) 44%	1.50(1.035-2.173)	
Total	(٥٠) 100%	(50) 100 %		
Allele Frequency (n) %				
C allele	(67) 67%	(78) 78%	0.859(0.723-1.021)	p= 0.082 χ^2=3.034
A allele	(33) 33%	(22) 22%	1.500(0.944-2.383)	
Total	(100)100%	100)100%		

According to the duration of the disease and gender allelic and genotypic frequency did not exhibit any significant differences among T2DM as in Tables-(4,5).

Table 4-Comparison of the genotype frequency and Alleles within the −592 Region of the *IL-10 gene* among T2DM according to the duration of disease

	Less than 5 years (n) %	**More than 5 years (n) %**	**OR (95% CI)**	**p value χ^2**
Genotype Frequency (n) %				
CC	(10) 40%	(8) 32%	1.250(0.593-2.637)	p=0.347 χ^2=0.347
CA	(15) 60 %	(17) 68%	0.882(0.581-1.340)	
Total	(٥٠) 100%	(50) 100 %		
Allele Frequency (n) %				
C allele	(35) 51.5%	(33) 53.1%	1.098(0.711-1.697)	p=0.668 χ^2=0.184
A allele	(15) 48.5%	(17) 46.9%	0.913(0.608-1.373)	
Total	(50)100%	(50)100%		

Table 5-Comparison of the Frequency of Genotypes and Alleles within the −592 Region of the *IL-10 gene* among T2DM according to the gender

	Female n (%)	**Male n (%)**	**OR (95% CI)**	**p value χ^2**
Genotype Frequency (n) %				
CC	(10) 33.3%	(12) 60%	0.556(0.299-1.033)	0.063 χ^2=3.463
CA	(20) 66.7%	(8) 40 %	1.667(0.921-3.017)	
Total	(30) 100%	(20)100 %		
Allele Frequency (n) %				
C allele	(40) 66.7%	(32) 80%	0.833(0.658-1.056)	0.146 χ^2=2.116
A allele	(20) 33.3%	(8) 20%	1.667(0.815-3.409)	
Total	(60)100%	(40)100%		

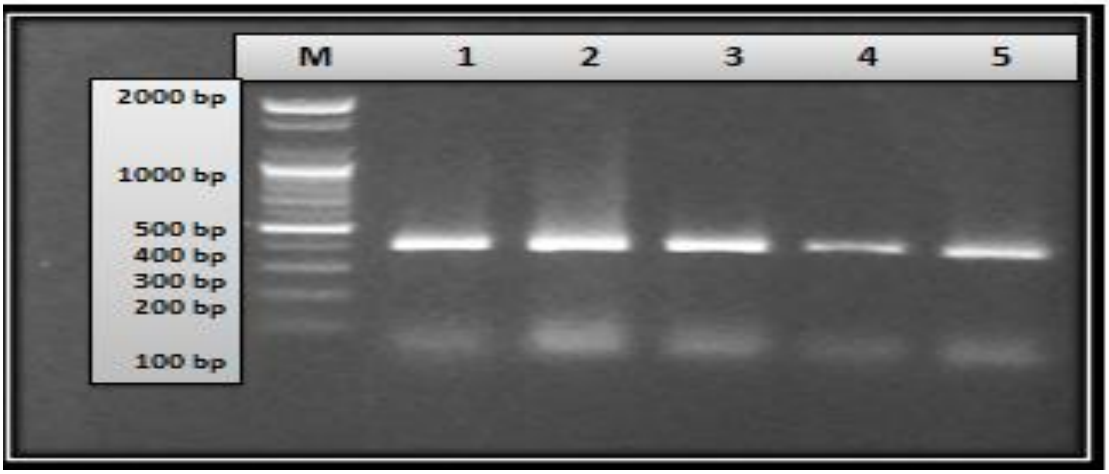

Figure 1- PCR product of *IL-10 gene* polymorphism at 412 bp were confirmed by (1%) Agarose gel electrophoresis . **Lane 1** :shows 100 bp ladder, **Lane [2, 3, 4, 5**]: show the amplification product of *IL-10 gene* at 412 bp.

Figure 2- Digestion products of *IL-10 gene* were applied on (1%) agarose gel electrophoresis. Lane(1) represent 100 bp ladder, Lane (2,3) represent CC homozygote at 412 bp, Lane (4,5) represent CA heterozygote at (412-236-176) bp.

Discussion

Interleukin-10 has a vital role by stimulating and suppressing of the immune response, and functions as an immune response modulator [14]. The genetic polymorphisms of interleukins have a an important role in their activity which could influence on them by alteration cytokine function and dysregulation of their expression. Therefore, the genetic differences among the individual may be thoroughly linked to the development of T2DM [6]. In our study, we found a significant($p \leq 0.05$) decrease in the frequency of CC genotype in T2DM than controls and this genotype is more prominent in the controls. Thus we can conclude that genotype 'CC' has a protective influence with a negative association with T2DM. Recorded results concluded that the IL-10-(592) CC genotype related with an elevated risk of T2DM .So recent data agreement with Bakheet *et al.*[19] study who found that the IL-10-(592) CC genotype and the C allele were related with an elevated risk of type 2 Diabetes Mellitus . As well as Arabadi *et al.*[20] study from Iran noticed that the IL10 gene polymorphism(-592 A/C) was correlated with type 2 diabetes mellitus with and without nephropathy. In addition to Kung *et al*. [21] also recorded a significant correlation between the -592A/C polymorphism in IL-10 and T2DM with or without nephropathy, via altered IL-10 production.

In contrary to this study Tsiavou *et al.* [22]and Hua *et al.*[23] were recorded no significant correlation between the -1082G/A and -592A/C variants and the risk of type 2 diabetes mellitus. In addition to Mahmoud *et al.* [24] study in Egypt demonstrated in their study that IL-10 levels was elevated in IL-10 (−592C/C) genotype compared to IL-10-(592A/A) and IL-10 (-592A/C) genotypes in diabetic nephropathy patients while such variance was not found in T2DM patients without nephropathy.

The reason for the differences between the current study and the others defined belong to the unique genetic features and many factors such as etiological and environmental factors may be contributed in the occurrence of type 2 diabetes [25]. It is clear that the secreted levels of IL-10 can determine by the genetic composition of the IL-10 promoter which had a specific role in the diabetic susceptibility [14]. Recorded results revealed that the total cholesterol, triglyceride and LDL are increased significantly ($p \leq 0.05$) in the T2DM patients while HDL is significantly ($p \leq 0.05$) decreased when compared with the controls. These results agreed with Agrawal *et al*. [26] and Huang *et al.* [27] .The probable reason of the elevating concentrations of serum cholesterol may be referred to many factors such as obesity, increase calorie intake and lack of muscular exercise or suppression of cholesterol catabolism [28].

Conclusion

We concluded that there is a significant association betweenIL-10 -592 gene polymorphism and T2DM incidence in Basrah Province.

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