Single Nucleotide Polymorphism of Interleukin-6 Gene at Position +565 in Type-1 Diabetic Patients in Baqubah City, Diyala, Iraq

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Abstract

This study aimed to investigate the potential association between the polymorphism of the Interlukin-6 gene (G>C) at position + 565 SNP. and type 1 diabetes (T1D) in Iraqi patients, the study included 39 blood samples which have withdrawn from diabetic patients (12 males and 27 females and an average age of 15.65 ± 1.79 years) who were diagnosed by a medical staff at Baquba General Hospital for the period from October 2015 to March 2016 together with 21 blood samples from apparently healthy people as a control group (7 males and females and an average age of 14.26 ±1.43 years). The polymerase chain reaction-specific sequence primer (PCR-SSP) assay has been used in this study**.** The results revealed that comparing *IL6*$_{+590}$ *genotypes* and *alleles* between T1D patients and controls showed some significant variance. The results of the gel electrophoresis of the IL-6 G / C + 565 gene amplified by PCR-SSP showed that the two genes G and C were present and that there were three genotypes: GG, GC, CC among patients. The results showed that frequency of GG genotype (56.41 vs 28.57%; respectively) and *G* allele (74.36 vs 59.52%; RR =36.7%) was significantly increased in diabetic patients in comparison with the controls (P = 0.058 and 0.102, respectively) and the related RR rates were 39.0% and 36.7%, respectively, while the associated EF values were 3.24 and 1.97, respectively. In contrast, GC genotype (35.89 vs 61.90%; P =0.063; PF =0.34) and *C* allele (25.64 vs 40.48%; P =0.102; PF =0.51) frequencies were significantly decreased in patients. The findings of this study depict the predominant pathogenic role of IL-6$_{+565}$ polymorphism showed associations (positive and negative) with

T1D in the samples of Iraqi patients. Therefore, the functional role *IL6* might have been altered due to the deviations of some genotype and allele frequencies pathogenesis of T1D. This leads to the role of IL-6 as a preventive agent against the disease because it acts as an anti-inflammatory and inflammatory generator at the same time.

Keywords: Type-1 Diabetes disease, *IL-6* polymorphism, Autoimmune disease.

تعدد الاشكال للنيوكليوتيدة المفردة للحركي الخلوي -6 للموقع الجيني +565 لدى مرضى السكري النوع الاول في بعقوبة

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الخلاصة

هدفت الدراسة للتحري عن العلاقة بين التعدد الشكلي للحركي الخلوي -6 لموقع الجين (G>C) IL-6+565 ومرض السكري النوع الاول لدى المصابين في بعقوبة ،حيث شملت الدراسة على 39 مريض تم تشخيص وسحب عينات الدم من المرضى بواسطة كادر طبي متخصص في مستشفى بعقوبة العام للفترة من تشرين الاول للعام 2015م ولغاية اذار 2016م مقارنة 21 من الاصحاء. حيث شملت 39عينة من المرضى(12 من الذكور و27 من الاناث وبمتوسط عمر 15,65±1,79 سنة). مقابل 21 شخصا من الاصحاء (7 ذكور و14 انثى وبمتوسط عمر1,43 ± 14,26 سنة). درس التعدد الشكلي للحركي الخلوي 6 لموقع IL-6+$_{565}$ باستخدام جهاز تقنية التضخيم (PCR-SSP) اظهرت نتائج الترحيل الكهربائي لجين IL-6 G/C+$_{565}$ المتضخم بهذه التقانة الى وجود اليلين هما G و C والى وجود ثلاثة انماط وراثية هي GG,GC,CC ،اذ سجل النمط الجيني GG ارتفاعا ملحوظا لدى المرضى مقارنة بالأصحاء (56.41 *VS*. 28.57%) والاليل G (74.36*vs*. 59.52%; RR =36.7%)وباستخدام احتمالية فشر حيث سجلت P=0.058.وبالاعتماد على النسبة الحرجة (OR) وحدود الثقة (CI) واظهر النمط الجيني GG والاليل G كمسبب (EF) مرتبط مع خطر الاصابة بالمرض بالتعاقب (3.24, 1.97)، بينما سجل النمط الجيني GC والاليل C PF (35.89 *vs*. 61.90%; P =0.063; (0.34= مقارنة بالأصحاء (PF =0.51 (25.64*vs*. 40.48%; P =0.102) كنمط وقائي من خطر الاصابة لمرض السكري النوع الاول حيث سجل اقل نسبة خطر (RR) (%). وهذا يقود الى دور الحركي الخلوي6 كعامل وقائي ضد المرض لانه يعمل كمضاد للالتهابات بنفس الوقت الذي يعمل كمثير او منشئ للالتهابات.

الكلمات المفتاحية : داء السكري النوع الاول ، التعدد الكروموسومي للبين الابيضاضي -6 ، امراض المناعة الذاتية .

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Introduction

Type-1 diabetes disease (T1D) has been classified as autoimmune disease. The International Diabetes Federation (IDF) reported that were approximately 382 million people worldwide infected with diabetes in 2013, and may expected to reach more than 592 million by 2035 (1). India alone has 63.0 million affected individuals and the number is expected to rise to 101 million by 2030 (**1,6)**. Genetic environmental, immunological factors and their interactions contribute to the onset of T1D, and cytokine and cytokine receptor gene polymorphisms are among the genetic markers that have been investigated in both forms of disease, and it is belived that they might be involved in pathogenesis of T1D (**5**). Interleukin-6 is encoded by a gene on the long arm of chromosome 7 (7q14.21). The promoter region of *IL6* gene ($IL6_{-174}$ G/C and $IL6_{+565}$ G/A) has two SNPs location which are the most frequently studied genetic variations of the gene, and previous studies demonstrated that the first SNP is effective in regulating IL-6 production, and $IL\text{-}6_{-174}$ *G* allele was associated with high levels of IL-6 which is produced primarily by mononuclear phagocytes, fibroblasts, and vascular endothelial cells, and some activated T cells can also secrete IL-6 (11). IL-6 is a multi-functional cytokine which plays a role in adaptive immune response, inflammation, hematopoiesis and endocrine system (2,10). Although IL-6 was originally considered to be a pro-inflammatory cytokine, several discoveries prompted a revision of its characteristics and indicated that it also has anti-inflammatory properties. For instance, IL-6 inhibited neutrophil accumulation after lipopolysaccharide (LPS) injection and antagonized the actions of IL-1β and TNF-α via induction of the soluble IL-1Ra and the soluble TNF-α receptor (4). Furthermore, IL-6 was found to be required to control the pro-inflammatory cytokines levels, such as TNF-α, *in vivo* after endotoxic insults, both locally and systemically, and under certain conditions, it has anti-inflammatory characteristics in macrophages (7).Therefore this study aimed to investigate the potential association between the polymorphism of the Interlukin-6 gene (G>C) at position + 565 SNP. and type 1 diabetes (T1D) in Iraqi patients.

Materials and Methods

Subjects

The diagnosis of disease was determined by professional clinical staff. Thirty-nine diabetic patients (12 males and 27 females), aged 15.65 ± 1.79 years, were enrolled in this study together with 21 age-matched (14.26 ± 1.43 years) apparently healthy controls (7 males and 14 females). The patients attended the hospitals in Baqubah for diagnosis and treatment during the period October 2015 – March 2016. According to diagnosis, after 10–12 h blood samples were collected in EDTA from 39 T1D patients and 21 randomly selected healthy controls (HC). The samples stored at 20-C^0 until have been used.

Detection of *IL6* Polymorphism

From EDTA blood the Genomic DNA was extracted using Wizard Genomic DNA Purification Kit (Promega, USA). The polymorphism was detected at IL-6 position of the promoter region ($IL6_{+565}$) by polymerase chain reaction-specific sequence primer (PCR-SSP) assay, followed by electrophoresis on 2% agarose-gel, by using CTS-PCRSSP Tray Kit (Heidelberg-Germany). The thermo cycling conditions were: initial denaturation at 94˚C for 2minutes, followed by denaturation at 94˚C for 15seconds, and then 10 cycles of annealing and extension at 65˚C for 60 seconds. This was followed by denaturation at 94˚C for 15 seconds, and then 20 cyclesof annealing 61˚C at 50 seconds and extension at 72˚Cfor 30 seconds Figure (1).

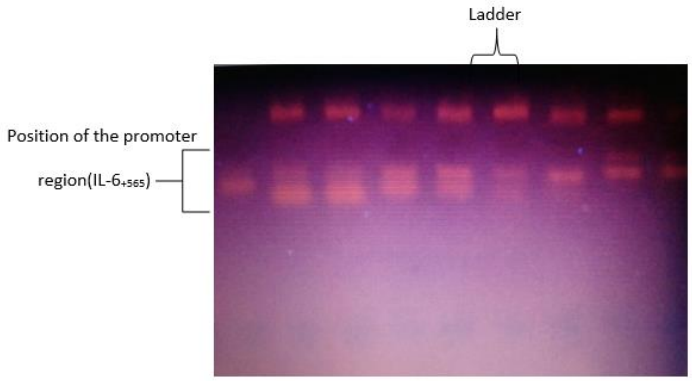

Figure:1 Agarose gel electrophoresis patterens of IL-6_{+565} gene region

Statistical Analysis

Genotypes of *IL6*$_{+565}$ SNP were presented as percentage frequencies, and significant differences between their distributions in IBD patients and controls were assessed by two-tailed Fisher's exact probability (P). In addition, relative risk (RR),etiological fraction (EF) and preventive fraction (PF)were also estimated to define the association between a genotype with the disease. These estimations were calculated by using the WINPEPI computer programs for epidemiologists. The latest version of the WINPEPI package is available free online at http://www.brixtonhealth.com.

Results and Discussion

The SNP of *IL6*$_{+565}$ was presented with three genotypes (GG, GC and CC) that corresponded to two alleles (*G* and *C*). These genotypes were in a good agreement with Hardy-Weinberg equilibrium (HWE) in both groups of patients, and controls. In addition, comparing T1D patients with controls also revealed significant differences in the distribution of *IL6*$_{+565}$ genotypes and alleles. Among patients, frequencies of GG genotype (56.41 *VS*. 28.57%; RR =39.0%) and *G* allele (74.36*vs.* 59.52%; RR =36.7%) were significantly increased in patients compared to controls (P = 0.058 and0.102, respectively). In contrast, GC genotype (35.89 *vs*. 61.90%; P =0.063; PF =0.34) and *C* allele (25.64*vs.* 40.48%; P =0.102; PF =0.51) frequencies were significantly decreased in patients compared to controls; while no significant difference was observed in the frequencies of CC genotype (7.69 *vs*. 9.52 %; P =1.000; PF =0.79) between patients and controls in the distribution of *IL6*$_{+565}$ genotypes and alleles (Tables 1 and 2).

Table 1: Observed numbers and percentage frequencies and Hardy-Weinberg equilibrium (HWE) of *IL6*$_{+565}$ genotypes and alleles in Type-1 diabetic patient and controls

Groups			IL-6$_{+565}$ Genotypes or alleles					H-W X^2 P ≤
			GG	GC	CC	*G*	*C*	
Diabetes type -1 (No. = 39)	Observed	No.	22	14	3	58	20	N.S.
		%	56.41	35.89	7.69	74.36	25.64	
	Expected	No.	21.56	14.87	2.56	Not Estimated		
		%	55.29	38.13	6.57			
Controls (No. = 21)	Observed	No.	6	13	2	25	17	N.S.
		%	28.57	61.90	9.52	59.52	40.48	
	Expected	No.	7.44	10.12	3.44	Not Estimated		
		%	35.43	48.19	16.38			

The present results strongly suggest that *IL6*$_{+565}$ polymorphism is involved in groups of T1D in terms of susceptibility (positive association) and protection (negative association); especially in patients, in whom the RR of GG genotype reached 39.0% , and the protective effect of GC genotype was 0.34 . Therefore, *IL6* allelic changes at position +565 might be associated with increased and decreased risk of T1DM in Iraqi population, and this may also contribute to a better clinic diagnosis. Other studies investigated other polymorphisms in intron and promoter regions of *IL6* gene like Kyi et al.(14) and Elmarakby and Sullivan (13) and the results were almost conflicting due to ethnic variations, but they agreed that *IL-6* is an important cytokine involved in immunity and its polymorphisms play a critical role in T1DM development Such conclusion has also been favored by Javor and co-workers (9) who investigated the prevalence of *IL6*$_{+565}$ polymorphism in 151 Slovak T1DM patients and 140 controls, and reported similar findings. In addition Kristiansen and Poulsen (11) have investigated a further SNP in this region (*IL6*$_{-174}$) in Danish patients and reported that such SNP was positively associated with the disease. In conclusion, SNPs of *IL6* might have a role in etiopathogenesis of T1DM and suggesting that dysregulation of the IL-6 pathway may be a common feature of autoimmunity disease (12).

Table 2: Statistical analysis of associations between *IL6*$_{+565}$ genotypes or alleles betweenType-1 diabetic patients and controls

Type of Comparison	Statistical Evaluation			Fisher's Exact Probability	95% Confidence Intervals
	IL-6$_{+565}$ Genotype or Allele	Relative Risk	Preventive Fraction Etiological		
Diabetic patient Versus Controls	GG	39.0%	3.24	0.058	1.06 - 9.87
	GC	4.6%	0.34	0.063	0.12 - 1.01
	CC	2.0%	0.79	1.000	0.13 - 4.97
	G	36.7%	1.97	0.102	0.89 - 4.35
	C	20.0%	0.51	0.102	0.23 - 1.12

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