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The Association Between IL-2 Gene (RS2069763) Single Nucleotide Polymorphism and Type 2 Diabetes Mellitus in Iraqi Patients

Maysaa Kadhim Al-Malkey
Tropical-Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq

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Abstract

This research attempts to find the association between single nucleotide polymorphism (SNP) of IL_{2+166} gene (rs2069763) and type 2 diabetes mellitus (T2DM) in a sample of Iraqi patients. A total of 44 patients and 55 apparently healthy volunteers were genotyped for the SNP using polymerase chain reaction test. Three genotypes (GG, GT, and TT) corresponding to two alleles (G and T) were found to have SNP. Both study groups' genotypes had a good agreement for the analysis of Hardy-Weinberg Equilibrium. The results revealed increased frequencies between the observed and expected GG and TT genotypes and $IL2_{+166}$ SNP T allele in T2DM patients (40.9 vs. 40.0 %; OR = 1.04; 95% CI, 0.47 - 2.31), whereas the values in the control group were 11.4 vs. 9.1 %; OR = 1.28; 95% CI, 0.35 - 4.68. Nevertheless, both variations did not reach a significant level. In the Iraqi population, the $IL2_{+166}$ SNP was not associated with T2DM and, therefore, no association with its etiopathogensis was found.

Keywords: Diabetes mellitus; Interleukin-2; Single nucleotide polymorphism

دراسة العلاقة بين تعدد اشكال النيكليوتيدة المفردة لجين الإنترلوكين-2 (RS2069763) ومرض السكري من النوع الثاني في المرضى العراقيين

ميساء كاظم المالكي
وحدة الابحاث البايولوجية للمناطق الحارة، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

حاول هذا البحث العثور على ارتباط بين تعدد الأشكال النوكليوتيدة المفردة لجين الأنترلوكين- 2 (rs2069763)وداء السكري النوع الثاني (T2DM) في عينة من المرضى العراقيين. تم التقصي عن الطرز الوراثية في ما مجموعه 44 مريضًا و 55 شخصًا من الاصحاء ظاهرياً كمجموعة سيطرة من العراق لتعدد اشكال النوكليوتيدة المفردة لجين الانترلوكين – 2 باستخدام مقايسة تسلسل تفاعل البلمرة المتسلسل (PCR). تم العثور على ثلاثة أنماط وراثية (GG، GT ، و TT) وتقابل اثنين من الأليلات (G و T) لديها SNP. وأظهرت هذه المورثات توافق جيد مع تحليل هاردي واينبرغ للتوازن للمجموعتين الداخلة في الدراسة. كشفت النتائج عن زيادة في الترددات بين الأنماط الجينية GG و TT المرصودة والمتوقعة والأليل T لجين $IL2_{+166}$في المرضى الذين يعانون من سكري النوع الثاني (40.9 مقابل 40.0 ٪ ؛ OR = 1.04 ؛ 95 ٪ CI، 0.47 – 2.31) بينما كانت في مجموعة السيطرة (11.4 مقابل 9.1 ٪ ؛ أو = 1.28 ؛ 95 ٪ CI،

*Email:maysakadhim@uobaghdad,edu.iq

4.68 – 0.35)، مع ذلك ، لم توجد هناك فروق معنوية ذات دلالات أحصائية في المجتمع العراقي وبالتالي لم يتم العثور على أي ارتباط مع المنشأ المرضي.

Introduction

Type II Diabetes Mellitus (T2DM) is an endocrinological condition resulting from increased secretion of insulin and/or irregular insulin behavior [1, 2]. Reduction of insulin level results in chronic abnormally high blood sugar and resistance to glucose [3]. Currently, diabetes epidemic is related to a gene-environmental affliction. Worldwide prevalence estimates suggest that 85-95 percent of T2DM occurred in the developing countries.

In a 2010 study, the prevalence was reported to be 6.4 percent, affecting 285 million people in the age group of 20-79 years worldwide. It is expected that 438 million people will be diabetic in the developing countries by 2030, reaching 70 percent, along with a rise of 20 percent in developed countries [4]. Due to ongoing insulin resistance development and ß-cell dysfunction, the pancreas is unable to produce enough insulin to overcome insulin resistance. As a result, about 85% of the T2DM population is obese, leading to resistance to insulin [5]. Interleukin-2 (IL2) is the major growth factor for T cells and its binding to its specific receptors on T-helper cells stimulates their proliferation and production of effective cytokines from these cells [6]. This cytokine is under genetic control on chromosome locus 4q27. Expression levels of cytokine genes are often related to SNPs leading to their irregularity, which may modify the disease and thereby may lead to development of various immunological diseases, including T2DM [7]. Correlation of *IL2* genetic variation with systemic lupus erythematous (SLE) [8] and Multiple sclerosis (MS) [9, 10] as well as the IL_{1B} SNP in Rheumatoid arthritis (RA) [11] were investigated to reveal potential impact of genetic variation with the disease etiopathogensis. Therefore, this research focuses on possible association between T2DM and genetic variation of $IL2_{+166}$ G/T in a sample of Iraqi population with T2DM.

Materials and Methods

Subjects

Forty four T2DM patients (19 males and 25 females) were enrolled and their age range was 28-77 years (50.5 ± 15.5 years). The Iraqi Health Ministry Ethical Committee approved the study. During the time from February to March 2019, the patients were referred for diagnosis and treatment to the National Center for Diabetes Care and Research / Al-Mustansiriyah University. The assessment was made by the clinical consultant and was based on the updated diagnostic criteria by the American Diabetes Association (T2D ADA criteria, 2010) [12]. The study also included 55 apparently healthy control volunteers. The control subjects were matched with the patients for the race (Iraqis), gender (27 males and 28 females) and they were aged from 17 to 70 years (31.9 ± 14.9 years).

Gene polymorphism of IL2

The TonkBio Genomic DNA Extraction Kit (New Jersey, USA) was used to extract genomic DNA from EDTA blood samples. Following purity and concentration evaluation, it was subjected to specific sequence primer technique using polymerase chain reaction test (PCR-SSP). Two primers were designed (Forward: 5'-CTGGAGCATTTACTGCTGGATT-3' and Reverse: 5'-ACTCTTTACCTCAGATGAGCTGCTA -3') for the genotyping of $IL2_{+166}$ (G/T) SNP (rs2069763) using the Geneious software, version 10.1.3.

The PCR reaction was conducted at a final volume of 20 µl, including 6.5µl of the master mix (GoTaq green, Promega®- USA), 1 µl of the forward primer (10 µM), 1 µl of the reverse primer (10 µM), 1.5 µl of DNA (50 ng), and 10 µl of nuclease free distilled water. The following steps were applied as PCR reaction conditions: an initial denaturation at 94^{o}C for 5 min, then 35 cycles of denaturation at 94^{o}C for 30 sec, followed by annealing at 63^{o}C for 30 sec and extension at 72^{o}C for 30 sec, and ended by a final extension at 72^{o}C for 10 min. The amplified PCR fragments were then run on agarose gel electrophoresis (1.5% agarose at 5 v/cm^2 for 45 minutes).

Statistical analysis

The age was expressed as mean ± SD, and the normality, randomization and homogeneity were calculated for the age by using IBM SPSS computer program V25.0. Frequencies of allele and genotype were given as percentage. The genotype frequencies were first tested for agreement with Hardy-Weinberg equilibrium (HWE), then Pearson's Chi-square analysis was performed to evaluate the significance of differences between the observed and expected genotype frequencies (https://www.easycalculation.com/health/hardy-weinberg-equilibrium-calculator.php). The relation

between *IL2* SNP and T2DM was addressed in terms of odds ratio (OR) and the exact probability of two-tailed Fisher was evaluated as a significant difference [13]. WINPEPI version 11.65 software for epidemiologists was used (www.brixtonhealth.com) to achieve the latter calculations.

Results and Discussion

IL-2$_{+166}$ gene polymorphism

Genetic variations of *IL2*$_{+166}$ (G/T) (rs2069763) SNP investigated the change of *G* allele to *T* allele at the nucleotide position 15 of the forward DNA strand. This change occurred at chromosome 4:122456327 positions. They were genotyped by PCR with allele specific primer (PCR-ASP) technique [14, 15]. The *IL2* gene PCR amplified products revealed a single band of 301bp on agarose gel electrophoresis (Figure-1).

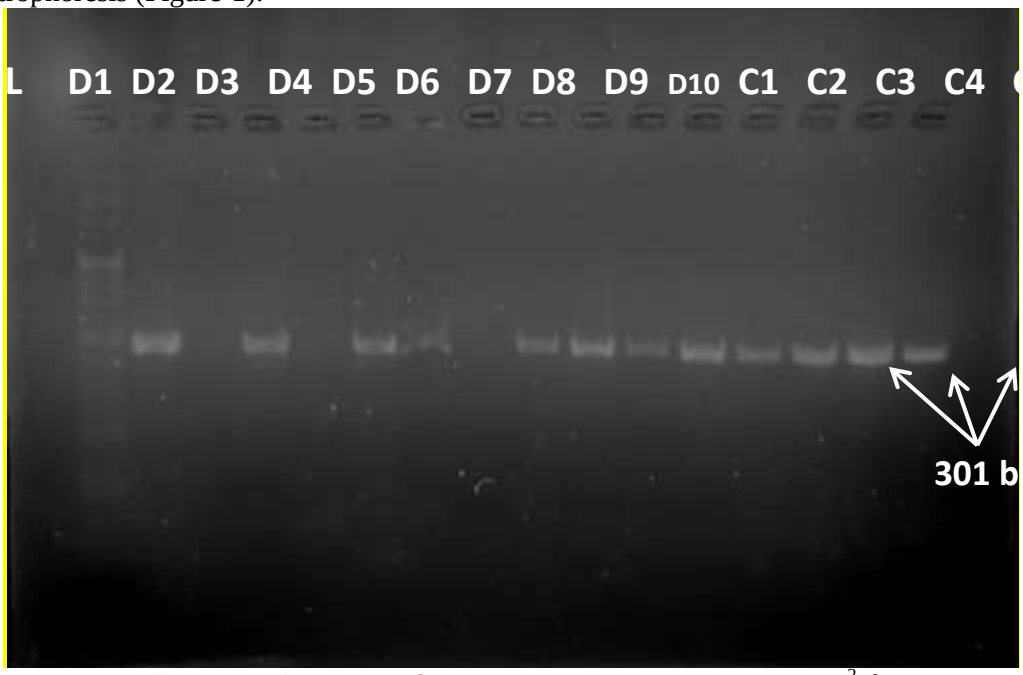

Figure 1- *IL2* SNP (rs2069763) PCR product run on 1.5% agarose at 5 v/cm^2 for 45 min revealing bands of 301 bp molecular size . (Lane L: DNA ladder 1000 bp; Lanes D1-D10 samples of T2DM patients; Lanes C1-C5 samples of controls).

Analysis of HWE indicated that there was significantly no difference between frequencies of *IL2*$_{+166}$ (G/T) (rs2069763) SNP in T2DM patients for both the observed and expected genotype. Therefore; these genotypes revealed a good equilibrium agreement with the HWE analysis in patients and control groups (Table-1).

Table 1-Observed and expected genotype frequencies of *IL2*$_{+166}$ SNP (rs2069763) in type 2 diabetes mellitus and control.

IL2$_{+166}$ SNP Genotype	Patients (N = 44)				Control (N=55)			
	Observed		Expected		Observed		Expected	
	N	%	N	%	N	%	N	%
GG	18	40.9	18.5	42.0	22	40.0	23.6	42.9
GT	21	47.7	20.0	45.5	28	50.9	24.8	45.1
TT	5	11.4	5.5	12.5	5	9.1	6.6	12.0
HWE (*p*-value)	0.761 (NS)				0.351 (NS)			

HWE= Hardy Weinberg equilibrium; N= Absolute number; *p*= probability; NS= Non significant; (*p*-value > 0.05).

The homozygous genotype (GG) showed an increased frequency in patients compared with controls (40.9 vs. 40.0 %; OR = 1.04; 95% CI, 0.47 - 2.31). Similarly, mutant homozygous genotype

(TT) frequency was increased in patients (11.4 vs. 9.1 %; OR = 1.28; 95% CI, 0.35 - 4.68). Nevertheless, there was no significant level of both variations ($p > 0.05$). Also, there was no significant increase in the frequency of mutant allele (T) in patients (35.2 vs. 34.5 %; OR = 0.57 - 1.85; 95% CI, 0.73 - 3.44) as shown in Table-2.

Table 2-frequencies of allele and genotype of $IL2_{+166}$ SNP (rs2069763) in type 2 diabetes mellitus and control.

$IL2_{+166}$ SNP Genotype	Patients (N = 44)		Control (N=55)		OR	95% CI	*p*-value
	N	%	N	%			
GG	18	40.9	22	40.0	1.04	0.47 - 2.31	1.000 (NS)
GT	21	47.7	28	50.9	0.88	0.40 - 1.93	0.840 (NS)
TT	5	11.4	5	9.1	1.28	0.35 - 4.68	0.747 (NS)
G	57	64.8	72	65.5	0.97	0.54 - 1.74	1.000 (NS)
T	31	35.2	38	34.5	1.03	0.57 - 1.85	1.000 (NS)

N= Absolute number; OR= Odds ratio; CI= Confidence interval; *p*= Two-tailed Fisher exact probability; NS= Non significant (*p*-value > 0.05).

Type 2 diabetes mellitus involves multiple metabolic dysfunctions resulting in hyperglycemia from decreased insulin production and increased insulin resistance, in addition to irregular glucagon metabolism and signaling pathways of insulin and beta-cells [16]. It is proposed that several interleukins contribute to T2DM's pathology and have impacts on the signaling pathways of insulin and the function of beta-cell [17]. In the current study, there is a lack of association between *IL2+166* G/T SNP and T2DM in the investigated Iraqi population. A study by Howson *et al*., (2011) in Ulm and the surrounding area, southwest Germany, revealed an increased risk of genetic variation of $IL2_{+116}$ SNPs (rs2096763 and rs2096762). An association between *IL2* (rs2069763) among 786 T2DM cases and 1,484 controls and its predisposition to adult-onset autoimmune diabetes was indicated [18], which contradicts our findings due to variations in sample size and ethnicity.

A relevance association between *IL2* (rs2096763) SNP and type 1 diabetes (T1DM) among white European ancestry was investigated by Howson *et al*., (2012) and revealed that the major allele G confers protection from T1DM, with an average of GG alleles at age of nine years old . *IL2* gene expression is correlated to T1DM pathogenesis, added to that two *IL2* receptor genes ($IL2_{RA}$ and $IL2_{RB}$) are both associated with type 1 diabetes [19]. It is suggested that *IL2* gene variation has a strong correlation to the exacerbation of autoimmune types of diabetes mellitus with increasing age to older than 17 years [19], which was also confirmed by another two previous studies [20, 21].

To the best of our knowledge, $IL2_{+166}$ (rs2069763) genetic variations and its predisposition to T2DM as a risk factor in Iraqi population have not been previously demonstrated. Meanwhile, a study by Hamid and Shani (2018) investigated the association between IL-10 (-592A/C) gene polymorphism with the progression of T2DM in Basrah Province and revealed significant association between CA genotype with the risk of T2DM and that IL-10 (-592A/C) SNP contributes to the development of T2DM [22].

However, the impacts of *IL2* (rs2069763) SNP on several diseases were highlighted, such as systemic lupus erythematous [8], multiple sclerosis [9, 10], tacrlomis gravis [23], susceptibly to different cancer pathologies [24], in addition to its impact on serum level of IL2 among Iraqi Arabs [25].

Conclusions

This study results showed that GG genotype / T allele of $IL2_{+166}$ SNP is not an attributed factor for T2DM in this sample of Iraqi patients. In order to increase the statistical power for further investigations, the sample size is needed to be increased and different clinical courses for the disease could be implemented. Added to that, cytokine gene expression studies will be required with different diabetes mellitus clinical courses and associations to therapy response**.**

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