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A Single Nucleotide Polymorphism in Interleukin–10 Gene SNP-819 Associated with Type 2 Diabetes Mellitus

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Abstract
Type 2 diabetes is a multifactorial disease caused by a complex interplay of multiple genetic variants and many environmental factors. With the recent genome-wide association (GWA) studies, the number of replicated common genetic variants associated with type 2 diabetes has rapidly increased. In this study, our aim was to determine the role of IL-10 polymorphism among patients with T2DM. A total of 120 Type 2 diabetes mellitus patients (67 males and 53 females) with an age mean ± SD (62.8333± 9.46052), FBS mean ± SD (310.0000± 88.90737), family history (90 positive/ 30 negative to family history, Hypertension (86 positive/ 34 negative) and 120 non-diabetic controls (46 males and 74 females) of Iraqis ethnicity with an age mean ± SD (31.6583± 11.51579). There was significant difference between the groups (T2DM patients and their control) (P = 0.001). And IL-10 gene polymorphism in each group was compared (CC, TT, CT). There is an association between the IL-10 (SNP rs 3021097 (C/T) gene polymorphism among T2DM patients and healthy people.
Keywords: IL-10, Genotype, T2DM, Genetic susceptibility, SNP

Introduction

Diabetes is a non-communicable disease characterized by chronic hyperglycemia and disturbances in carbohydrates, lipids and proteins metabolism due to defects in insulin secretion and its action (Guillausseau et al., 2008). Type 2 diabetes mellitus is the most prevalent type of diabetes and is often caused by decreased insulin production by the pancreas, also known as non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes, (Arababadi et al., 2009; Nosratabadi et al., 2009).T2DM also known as multifactorial disease caused by a complex interplay of multiple genetic variants and many environmental factors. Several genes influence the underline level of glucose tolerance and thereby contribute to overall susceptibility to T2DM (de Lorenzo et al., 2013). Genetic linkage analysis and association studies have identified several candidate genes contributing to T2DM. However, given the ethnic differences in life style, environmental factors as well as in the genetic background, it is important to examine polymorphisms related to T2DM in each ethnic group (Wilson et al., 2007; Yamada et al., 2006).

Interleukin (IL-10) is a pleiotropic Th2 cytokine that is usually considered to have a role in the down regulation of cell mediated and cytotoxic inflammatory responses, thus being a potent anti-inflammatory mediator. It has been suggested that Th2 induced component of anti-β cell immunity is mediated principally by IL-10 (Lee et al., 1996; Lee et al., 1994). The gene encoding IL-10 has been mapped to chromosome 1q. Several polymorphic sites within the promoter region have been described, including two microsatellite polymorphisms and three biallelic polymorphisms at positions: −1082, −819, and −592 from the transcription start site (Eskdale et al., 1998; Turner et al., 1997). IL-10 promoter SNP genotype and haplotype frequencies appear to exhibit different distributions according to ethnicity (Moraes et al., 2003; Pyo et al., 2003; Urcelay et al., 2004). Genetic variants associated with risk of type 2 diabetes could potentially be useful for the prediction, prevention, and early treatment of the disease. The aim of this study was to examine the role of interleukin–10 (IL-10) gene in genetic susceptibility as risk factors for type 2 diabetes mellitus in a sample of Iraqis patient.

Materials and methods

This is a case–control study involving 120 T2DM patients (67 males and 53 females) with an age mean ± SD (62.8333± 9.46052), FBS mean ± SD (310.0000± 88.90737), family history (90 positive/ 30 negative to family history, Hypertension (86 positive/ 34 negative) and 120 non-diabetic controls (46 males and 74 females) of Iraqis ethnicity with an age mean ± SD (31.6583± 11.51579), were enrolled in this study and recruited at Baghdad teaching hospital, medical city, Baghdad, Iraq. T2DM patients, diagnosed according to American Diabetes Association criteria.

The Ethics Committees of participating universities and university hospitals approved the study, and informed consent was obtained from all participants. Blood sampling (one ml of venous blood) was collected in EDTA tubes from each individual (patient or healthy control) and was stored as whole blood at -20oC for subsequent DNA isolation. Genomic DNA was isolated from whole blood according to Sambrook et al 1989 (Shubeita, Sambrook, and McCormick, 1987).

Genotyping of IL-10- SNP -819 gene polymorphism

One SNP rs 3021097 (C/T) in *Il-10* -819 gene was genotyped among the participants groups in this study. The *Il-10* -819 C/T polymorphic region (rs 3021097) was amplified by polymerase chain reaction (PCR) using allele specific PCR technique as shown in Table 1. Three primers (two allele specific primers and common reverse primer) were designed based on the nucleotide sequence of a partial fragment (retrieved from the online dbSNP) of the gene containing the target SNP. The polymorphism was visualized by separating the DNA fragments in a 2% agarose gel that was stained with ethidium bromide and illuminated by UV. To validate the PCR- allele specific results to validate the PCR- allele specific results. All primers used in this study were newly designed using Primer Blast online programme (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

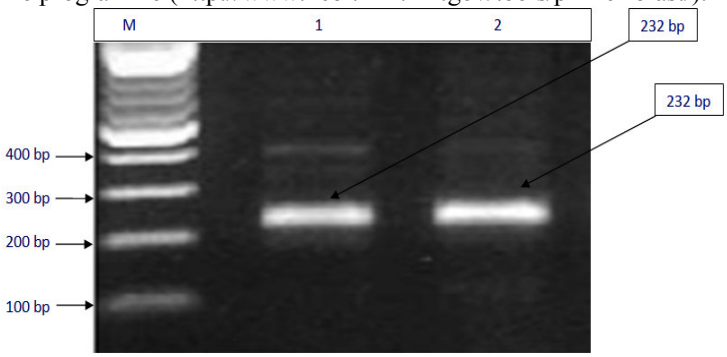

Figure 1: 2% agarose gel electrophoresis for allele specific PCR for IL-10-819 gene (rs 3021097 C/T) M: 100 bp DNA ladder from GeneDireX®. Lanes 1 and 2: PCR products upon using allele specific C primer and allele specific T primer, respectively. Heterozygous genotype will give positive reaction upon using both allele specific primers. However, homozygous genotype will give positive reaction upon using only one of these allele specific primers.

Table 1: Primers sequences, PCR conditions, length of PCR products

SNPs	Primers sequences	PCR Conditions	Size of PCR Products digestion products
Il-10 SNP-819 C/T (rs #3021097)	**T-allele specific primer:** **F1**: 5-CCC TTG TAC AGG TGA TGT AT **C**-3 **C-allele specific primer:** **F2:** 5-CCC TTG TAC AGG TGA TGT AT **T**-3 **Common reverse primer:** 5- GGATGT GTTCCA GGCTCC T-3	**An initial denaturation** at 95ºC for 5 min -Then, **30 cycles** each cycle consisted of denaturation at 94ºC for 60s, annealing at 50 ºC for 30s and extension at 72ºC for 30 s. **-A final extension** at 72ºC for 10min.	**Allele C:** 232 bp **Allele T:** 232 bp

Statistical analysis of data

Statistical analysis of data was done to correlate genotype distribution and allele frequencies were performed by SPSS package version 17. The frequencies of alleles, genotypes in different groups were compared using the Chi-squared test (X2), t-test were used to test the significance of results of quantitative variables. Odds ratio and 95% confidence interval (95% CIs) were calculated for different studied parameters. The confidence interval (CI) at 95% was used to describe the amount of uncertainty associated with the samples (Greenfield et al., 2008; Szumilas, 2010). The significance of the results was taken at the $P < 0.05$ level of significance.

Results and Discussion

Our results approved there was a statistical significant difference between the two groups (P = 0.001) for the *Il-10* SNP-819 C/T (rs #3021097). The genotype CT in cases considers risk factor while TT genotype among control consider protective from the disease as shown in table 2.

Table 2: IL-10 gene polymorphism and allele frequencies among T2DM patients and their control

Gene polymorphism	Cases		Control		Significance	OR (95% CI)
	No.	%	No.	%		
Il-10 SNP-819 C/T (rs #3021097)					$X^2 = 12.053$	
CC	32	20.9	36	30.0	P = 0.001	(1.495 - 3.609)
TT	19	15.8	43	35.9		(0.277 - 0.669)
CT	76	63.3	41	34.1		

X^2: Chi-Square test *significant at P≤0.05

T2DM known as an immune mediated disease leading to defect insulin signaling and selective destruction of insulin producing beta cells in which cytokines play an important role (Pickup, 2004). Interleukin -10 (IL-10) is an important immunoregulatory cytokine that is produced by activated T cells (Del Prete et al., 1993). It plays an important role in activation and suppressing the immune response and functions as an immune response modulator (Chagas et al., 2013; Helminen, Lahdenpohja, and Hurme, 1999; Jin et al., 2013). In recent study showed that meta-analysis provides strong evidence that IL-10 -1082A/G polymorphism associated with risk of T2DM. while, no association of the IL-10 - 592C/A or - 819C/T polymorphism with T2DM risk (Hua et al., 2013). Otherwise no significant found in the A-592C (p=0.088) or T-819C (p=0.160) polymorphism and T2DM, significantly more T2DM subjects carried -592*C (34.28%, p=0.027) and -819*C (32.57%, p<0.001) alleles, which were associated with high levels of IL-10 production. Previous study suggests that IL-10 genetic polymorphisms may play a specific role in determining diabetic susceptibility, but do not seem to be important in the clinical manifestations of diabetes (Chang et al., 2005). On the other hand decreased prevalence of (mutant) –819T allele and –819C/T genotype was seen in diabetes nephropathy patients; neither the –1082G/A nor the –592C/A polymorphism was associated with diabetes nephropathy in Tunisian patients (Ezzidi et al., 2009). Recent studies have documented several polymorphic sites in the IL-10 gene promoter region upstream of the transcription start site, including three polymorphisms at positions -1082 A/G, -819 C/T and -592 A/C (Zhang, Li, and Yang, 2006). Others studies have found an association between IL 10 genetic polymorphism and the risk T2DM (Del Prete et al., 1993; Garcia-Elorriaga et al., 2013; Kolla et al., 2009; Li et al., 2013; Yin et al., 2012). In conclusion, the findings of this present study suggest that the IL10-819 C/T gene polymorphisms increase the risk for type 2 diabetes mellitus in Iraqis population.

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