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A Genetic Polymorphism of Interleukin-4 Gene at Position-590 in Type-1 Diabetes of Iraqi Patients

Ibtesam B.Hassan[1]*; Mahaa F. Nazzal[1]; Hind T.Qadir[1]; Ashwaq T. Hammadi[1]

[1]Department of biology, college of Education for pure science, University of Diyala, Iraq.

Abstract : The aim of this study was to investigate a potential association ofinterleukin-*4*polymorphisms with the susceptibility of type 1 diabetes (T1D) in Iraqi patients and between a single nucleotide polymorphism of interleukin-*4* gene (*IL4*) at position$_{-590}$SNP. (T1D) was determined in 39 Iraqi patients, (12 males and 27 females;1 5.65 ± 1.79 years) as well as 21 controls.(7 mail and 14femal ; 14.66 ± 3.43 years) by polymerase chain reaction-specific sequence primer (PCR-SSP) assay. The results revealed that comparing *IL4*$_{-590}$*genotypes* and *alleles* between T1D patients and controls show some significant variance. Among patients, it was showed that frequency of TT genotype and *T allele* (51.28*vs*. 70.51%; P =0.056respectively) were significantly rise in patients contrast to controls, (23.31*vs*. 47.82%; P =0.018) and the related RR rates were 36.1 and43.7, respectively .and the associated EF values were 3.37 and 2.63, respectively. In contrast, CC genotype and *C allele* (10.25*vs*. 29.49 %, P =0.143 respectively) frequencies were significantly decreased in patients, compared to controls (28.57*vs*. 52.38 %; P =0.018), and the associated PF values were 0.29 and 0.38, respectively. So as in the frequencies of TC genotype (38.46*vs*. 47.61 %; RR =14.9; P = 0.587) was high significant in controls compared to patients. It's the associated PF values were (0.69**)** related withT1D**.**These findings suggest that*IL4*$_{-590}$SNP might have an important role in protection against type 1 diabetes.

Key words : Polymorphism IL-4, Diabetes.

Introduction

Diabetes is one of the fastest growing diseases. World health organization estimates that approximately 340 million people have type 1 diabetes and this number increases by 3-5% each year so the type 1 diabetes population reached 25 million by 2010. Type 1 diabetes is an autoimmune disease that is caused as a result of destruction of pancreatic β-cells. Several factors may contribute to the pathogenesis of type 1 diabetes. Genetic susceptibility of type 1diabetes is determined by polymorphisms/mutations in multiple genes in both human and animal models[20,21]. T1D usually starts in childhood, adolescence, or early adulthood, but it may also start later in adult life[1,2].Everyone needs a hormone an insulin to keep their blood glucose at a healthy level. But withT1D, humane bodies release very little amount of insulin or non[15].Cytokines act as pleiotropic polypeptides regulating inflammatory and immune responses through actions on cells. They provide important signals in the pathophysiology of a range of diseases, including T1D[5,17].There is increasing evidence showing that polymorphisms in cytokine genes may play an important role in modulate the immune response. Numerous cytokines have been shown to participate in the pathogenesis of T1D [12,9]. As gene polymorphisms can influence in cytokine production or function, they may potentially contributed to genetic predisposition to

the disease, as at*TGF-B1*,*TNF-a, IL-18*,IFN-y and *IL-6*[6,4, 7,13,14]. Mediators of inflammation such as *IL-10, IL-12*, the *IL-4* family of cytokines, and certain chemokines have proposed to be involved in the events result in both forms of diabetes.[19,16, 10].Interleukin 4 (IL-4) was originally discovered as a low molecular weight in 1982, and secreted by activated T cells and basophils as a mature 129 amino acid glycoprotein. It is a pleiotropic cytokine that acts on T and B lymphocytes, monocytes, polymorphonuclear cells, fibroblasts and endothelial cells,which is encoded by the IL-4 gene on chromosome 5q23.31[26]. It is secreted by helper T cells (CD4) type 2 (Th2) lymphocytes, and natural killer (NK) T cells, and by cells of the innate immune system, [3,11].This occurs by the modulation of the homing of autoreactive cells to inflammatory sites and the stabilization of a protective Th2-mediated environment in the thymus, spleen, and pancreatic islets. Thus, IL-4 treatment favors the expansion of regulatory CD4+ Th2 cells in vivo and prevents the onset of insulitis and IDDM mediated by autoreactive Th1 cells[24]. IL-4 is suggested to protect human islets from cytotoxic damage induced by proinflammatory and Th1 cytokines. [8,18].The local expression of IL-4 in the pancreatic islets of NOD mice (ins-IL-4 mice) restricted the activation of autoreactive T-cells and promoted complete protection against spontaneous diabetes[14].

Materials and Methods

Subjects

The diagnosis and extent of disease was determined by conventional clinical The patients attended the hospitals in Baquba for diagnosis and treatment during the period October 2015 – June2016 which was based on clinical. According to diagnosis, after an overnight fasting of 10–12 h in fasting state for all investigations. Blood samples were collected in EDTA .The samples were stored frozen at 20 -8C or below in vials for storagefrom39 T1D patients, and 21 randomly selected healthy controls (HC). The patients were: 39 cases and their age mean ± S.E. was (12 males and 27 females;15.65 ± 1.79 years).For the purpose of a comparison, 21 apparently healthy controls of blood donors (7 males and 14 females) matched patients for age (14.26 ± 1.43 years) and ethnicity (Iraqi Arabs) were also enrolled in the study.

Detection of IL4Polymorphism

Genomic DNA was extracted from EDTA blood using Wizard Genomic DNA Purification Kit (Promega, USA). The polymorphism was detected at positions of the promoter region ($IL4_{-590}$) by polymerase chain reaction-specific sequence primer (PCR-SSP) assay, followed by electrophoresis on 2% agarose-gel, by using CTS-PCR-SSP Tray Kit (Heidelberg, Germany). The thermocycling conditions were: initial denaturation at 94˚C for 2 minutes, followed by denaturation at 94˚C for 15 seconds, 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 20cycles of annealing 61˚C at 50 seconds and extension at 72˚C for 30seconds.

Statistical Analysis

Genotypes of $IL4_{-590}$ SNP were presented as percentage frequencies, and significant differences between their distributions in T1D 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 agenotype 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.

Table 1: Observed numbers and percentage frequencies and Hardy-Weinberg (H-W) equilibrium of IL-4-$_{590}$ genotypes and alleles in Type -1Diabetes) patients and controls.

Groups			IL-4-$_{590}$ Genotypes or alleles					H-W X^2 P ≤
			TT	TC	CC	*T*	*C*	
Diabetes type -1(No. = 39)	Observed	No.	20	15	4	55	23	N.S.
		%	51.28	38.46	10.25	70.51	29.49	
	Expected	No.	18.40	13.20	2.40	Not Estimated		
		%	54.10	38.90	7.00			
Controls (No. = 21)	Observed	No.	5	10	6	20	22	N.S.
		%	23.31	47.61	28.57	47.82	52.38	
	Expected	No.	20.30	16.26	4.20	Not Estimated		
		%	50.58	23.67	9.90			

Table-2: Statistical analysis of associations between IL-4-$_{590}$ genotypes or alleles and (Type -1 Diabetes disease Versus Controls).

Type of Comparison	Statistical Evaluation			Fisher's Exact Probability	95% Confidence Intervals
	IL-4-$_{590}$ Genotype or Allele	Relative Risk	Preventive Fraction Etiological		
Diabetes Disease Versus Controls	TT	36.1%	3.37	0.056	1.06 -10.74
	TC	14.9%	0.69	0.587	0.24 - 1.97
	CC	20.4%	0.29	0.143	0.07 -1.13
	T	43.7%	2.63	0.018	1.22 - 5.68
	C	32.5%	0.38	0.018	0.18 -0.82

Results and Discussion

According to the findings of the present study, The SNP of *IL4*-$_{590}$ was presented with three genotypes (TT, TC and CC) that corresponded to two alleles (*T* and *C*). These genotypes were in a good agreement with Hardy-Weinberg equilibrium (HWE) in (tables1and 2).In addition *IL4*-$_{590}$ SNP can be highlighted as an important genetic marker in the pathogenesis of T1D especially if we consider RR values of 36.1 and43.7 for it was showed that frequency of TT genotype and *T allele* (51.28*vs*. 70.51%; P =0.056respectively) were significantly rise in patients contrast to controls, (23.31*vs*. 47.82%; P =0.018), and the associated EF values were 3.37 and 2.63, respectively. In contrast, CC genotype and *C allele* (10.25 *vs*. 29.49 %, P =0.143 respectively) frequencies were significantly decreased in patients, compared to controls (28.57 *vs*. 52.38 %; P =0.018), and the associated PF values were 0.29 and 0.38, respectively. So as in the frequencies TC genotype (38.46 *vs*. 47.61 %; RR =14.9; P = 0.587) was high significant in controls compared to patients. It's the associated PF values were (0.69**)** related withT1D**.**So These findings suggest that *IL4*-$_{590}$ SNP might have an important role in protection againstT1Din terms of susceptibility (positive association) especially inpatients, of CC and C allel estimated by PF values (PF range:0.29 – 0.38), and(negative association); especially inpatients, in whom the RR of TT genotypereached36.1**.**Therefore, *IL4* allelic changes at position -590 might be associated with increased and decreased risk of T1D in Iraqi population, and this may also contribute to a better clinic diagnosis ofT1D.However other studies investigated other polymorphisms in intron and promoter regions of *IL4*gene and the results were almost conflicting due to ethnic variations, but they agreed that *IL-4* is an important cytokine involved in immunity and its polymorphisms play a critical role to prevent fromT1D development [8,13,19].This is because of its role in immune regulation and a potent regulator of inflammation as it regulates inflammatory response by increasing the expression of IL1RN and blocks TNF B and IFN Gammais also termed anti-inflammatory because of its ability to suppress TNF-α, IL-1, IL-6, and prostaglandin E2 (PGE2) production by activated monocytes[25,27]. But Reimsnider and co-workers (2000) who investigated the prevalence of *IL4*-$_{590}$ polymorphism in USA they believed that the *IL4* promoter region is unlikely a major genetic factor in Type 1 diabetes, even though variations in this region may be related to Type 1 diabetes in a

very small number of patients[22].Another study demonstrated that there were no significant differences in the IL-4 polymorphisms between patients with type 1 DM and healthy controls[23].In conclusion, that *IL4*$_{-590}$ SNP might have an important role in protection against type 1 diabetes **.**

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