University of Thi-Qar Journal of Science (UTsci)
The 4th Scientific Conference of Science College/ University of Thi_Qar
Volume (7), No.2

Single Nucleotide Polymorphism of IL-2 Gene at Position +166 in Type 1 Diabetes of Iraqi Patients

Ibtesam, B. Hassan*
Maha F. Nazzal[1]
Ibtesam K. Alwan
Department of Biology, College of Education for Pure Science, University of Diyala. Iraq
Email: ibtesambh67@gmail.com

Marah S. Hameed
Department of Physiology Biochemistry and Pharmacology , College of Veterinary Medicin and Surgery, University of Diyala, Iraq

Ali, A. Aabud
Technical Institute of Diyala, Middle Technical University, Iraq

Abstract **: The present study aimed at investigating of frequency of polymorphism of interleukin-2 gene (IL2) at position$_{+166}$ SNP) in Type 1 Diabetes and in healthy controls subjects 39 of Iraqi patients, (12 males and 27 females;1 5.65 ± 1.79 years) and 21 controls.(7 mail & 14 female ; 14.66 ± 3.43 years) attended the hospital in Baghdad for diagnosis and treatment during the period February 2015 – January 2016 were enrolled in this study, the polymorphism of IL2$_{+166}$ was data waved by polymerase chain reaction-specific sequence primer (PCR-SSP) assay. The results showed a comparison *IL2*$_{+166}$ *genotypes* and *alleles* between T1D patients andcontrols frequencies of TT genotype and *T allele* (71.69*vs*. 68.95%;P =0.589 respectively) There was a significant increase in patients contrast to controls, (51.38*vs*. 48.08%; P =0.312) and associated RR rates were 19.2% & 27.0%, respectively. the related EF values were 1.56 and 1.60 .Similar observations were made in GG genotype. In contrast TG genotype and *G allele* (23.93 *vs*. 28.55%,P =1.000 respectively) Low frequency was observed in patients compared to healthy subjects (43.28 *vs*. 43.22%; P =0.312), and the related PF values were 0.56 and 0.62 , respectively. The results of the study indicate that IL2$_{+166}$ SNP may have a role in the mechanism of etiopathogenic of T1D in the samples of Iraqi patients**

***Key words: Polymorphism, IL-2, T1D,**

I. Introduction

Type 1 Diabetes is considered the most common severe chronic autoimmune diseases worldwide. In Europe, it incidence attributed to its occurrence in children, with a predicted 70% rise in disease over the next 15 years. More than 20 million people in Europe suffer from Type 1 diabetes T1D (Todd *et al.*, 2016). T1D is the second most common chronic disease of children, and adults with T1D parents with this condition (Bonifacio, 2015). It usually appears in childhood, adolescence, or early adulthood, but sometimes it appears later in adult life (Ozougwu *et al.*, 2013**; Soren** and **Grey**, 2015). Healthy people have a normal level of insulin hormone But with T1D patients, the pancreatic gland not synthesis insulin or make very little of it (Chisholm *et al.*, 2010; Hartemann and Bourron, 2012). As T1D is an autoimmune disease the immune system attacks and destroys insulin-producing islet *β* cells. Cytokines play role as pleiotropic polypeptides To regulate inflammatory immune response through its effect on cells. They occur important signals in the pathophysiology of a range of diseases, as in T1D (Elmarakby and Sullivan, 2012; Kyi *et al.*, 2015). There is ample evidence to suggest that polymorphisms in cytokines may play an important role in modifying the immune response. Many cytokines have been found to have an effect in causing pathogenesis of T1D (Chisholm *et al.*, 2010), as at TGF-B1, IFN-γ and IL-1 (Van de Veerdonk and Netea, 2013; Gonzalez and Fernandez, 2008). Mediators of inflammation such as TNF-α, IL-6, IL-2 the IL-10 family of cytokines, IL-18, IL-4 and found that certain chemokine's work on the occurrence of both types of diabetes (Vincenz *et al.*, 2011; Arend and Gabay, 2008; Oo *et al.*, 2012). The contribution of inflammation to diabetes comes from studies by researchers studying the role of inflammatory cytokines in diabetes. One of these cytokines is interleukin-2 (IL-2), which was discovered in 1975 as a growth-promoting activity for bone marrow-derived T lymphocytes (Fallahzadeh *et al.*, 2011). *IL2* is encoded by a gene located on the long arm of chromosome 4 (4q26). Two (SNPs) in *IL2* gene (-330 T/G & +166 G/T) have been shown to influence *IL-2* levels (Gao *et al.*, 2009).It is produced and released from active T cells and plays a major role in the cellular immune response. IL-2 has been found to increase lymphocyte secretion of T, B and NK cells and has many immune effects (Wrenshall *et al.*, 2014). Moreover, it is a strong T cell growth factor that asserts that lymphocytes are amplified in vivo (Fallahzadeh *et al.*, 2011).In humans, allelic variation of the *IL-2* receptor gene, *IL2RA*, encoding

the α subunit (CD25) was identified as a susceptibility determinant for T1D (Francisco *et al.*, 2004). Functionally, it initiates a pro-apoptotic pathway by improving the expression of FasL on activated T cells, and since T cells also activated Fas/CD95, this events leads to apoptosis of activated T lymphocytes. Also, it elevates production of NK-derived cytokines such as tumor necrosis factor alpha (TNF-α), interferon-γ (IFN-γ) and granulocyte monocyte-colony stimulating factor (GM-CSF), and can be synergistically represented with IL-12 to enhance NK cytotoxic activity. (Oo *et al.*, 2012), (Hulme *et al.*, 2012). For all previous information present study focused on Interleukins 2.

II. Materials & Methods

Subjects

The diagnosis and detected of disease was determined by clinical specimens thirty nine patients; (12 males & 27 females) attended the hospital in Baghdad for diagnosis and treatment during the period February 2015 – January 2016 in addition to twenty one healthy controls (7males and 14females).According to diagnosis after an overnight fasting on food about 10–12 h for all investigations. Blood samples were collected in EDTA. The specimens were stored in deep freeze at -20°C. T1D patients and randomly Collected healthy controls (HC). The patients age range was 15.65 ± 1.79 years compared subject of health's controls was 14.26 ± 1.43 years, were record in the study.

Detection of IL2 Polymorphism

Genomic DNA was extracted from EDTA blood using Wizard Genomic DNA Purification Kit (Promega, USA). followed by electrophoresis on 2% agarose-gel by CTS-PCRSSP Tray Kit (Heidelberg, Germany) .

Statistical Analysis

Genotypes of *IL2*$_{+166}$ SNP were presented as percentage frequencies, 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.

III. Rustles

SNP of *IL2*gene was determined in the promoter region at position $_{+166}$ (*IL2*$_{+166}$SNP), it was presented with three genotypes (TT, TG and GG) that correlate with two alleles (*T* and *G*).Among T1D patients, no significant difference was observed between the observed and expected frequencies of the three genotypes (a good agreement with Hardy-Weinberg equilibrium; HWE), while in controls, a departure from HWE was observed (i.e. a significant difference between the observed and expected genotype frequencies they were significantly deviated in controls ($P \leq 0.001$).) however comparing patients to controls results some significant differences (Table -1).The frequencies of TT genotype and *T* allele were significantly increased in patients (71.69 & 68.95%, respectively) compared to controls (51.38 & 48.08%, respectively). The relative risks (RRs) of such positive associations were 19.2% and, 27.0% respectively .Similar observations were made in GG genotype(40.51*vs*. 33.33%, R =11.8% respectively). In contrast, TG genotype & *G* allele frequencies were significantly decreased in patients (23.93 & 28.55%, respectively) compared to controls (43.28 & 38.98%, respectively).The preventing fractions (PFs) of such negative associations were 0.56 & 0.62, respectively. (Table -2).

I. TABLE 2-:STATISTICAL ANALYSIS OF ASSOCIATIONS BETWEEN IL+2-166 GENOTYPES OR ALLELES IN DIABETES TYPE 1 PATIENTS AND CONTROLS

Type of Comparison	Statistical Evaluation.				
	IL-2$_{+166}$ Genotype or Allele	Relative Risk.	Preventive or Fraction Etiological.	Fisher's Exact Probability	95% Confidence Intervals
Diabetes Disease Versus Controls.	TT	19.2%	1.56	0.589	0.55 - 4.43
	TG	16.7%	0.56	0.381	0.18 - 1.71
	GG	11.8%	1.10	1.000	0.30 - 4.07
	T	27.0%	1.60	0.312	0.74 -3.48
	G	14.5%	0.62	0.312	0.29 -1.35

IV. DISCUSSION

According to the presented results,IL2$_{+1166}$ SNP" can be highlighted as an important genetic marker in the pathogenesis of T1D was presented with three genotypes (TT, TG and GG) that corresponded to two alleles (T and G). These genotypes were in a good agreement with Hardy-Weinberg equilibrium (HWE) in patients, but they were significantly deviated in controls ($P \leq 0.001$). present study illustrated that IL-2$_{+166}$ important genetic marker in the "pathogenesis of T1D especially if we consider RR values was19.2% & 27.0% it was showed that frequency of TT genotype and T allele (71.69 vs. 68.95%; P =0.589 respectively) were significantly rise in patients contrast to controls, (51.38vs. 48.08%; P =0.312), and the associated EF values were 1.56 & 1.60, respectively. Similar observations were made in GG genotype(40.51vs. 33.33%; R =11.8%). In contrast, TG genotype and G allele (23.93vs. 28.55%, P = 0.381 respectively) frequencies were significantly decreased in patients, compared to controls (43.28vs. 38.98%; P =0.312), and the associated PF values" were 0.56 and0.62 respectively. The presented results strongly suggest that IL2$_{+166}$ polymorphism is involved in T1D in terms of susceptibility (positive association) and protection (negative association); especially in whom the RR of GG genotype. (Table 1and 2). Therefore, IL2 allelic changes at position +166 might be associated with increased and decreased risk of T1D in Iraqi population, and this may also contribute to a better clinic diagnosis. Such conclusion has also been favored by Dogan, and co-workers (2006) who investigated the prevalence of IL2+166 polymorphism in 27 Turkish T1D patients and 25 controls, and reported similar findings.. According to these finding which agrees with previous results, can concluded that IL2$_{+166}$ SNP might have a role in the etiopathogenic mechanism of T1D, multiple studies have been conducted testing the ability of

IL-2 supplementation to prevent and reverse type 1 diabetes in the NOD mouse. Overall, these studies support the notion that exogenous IL-2 treatment can protect NOD mice from diabetes development, (Francisco *et al.*, 2004; Oo *et al.*, 2012; Chentoufi *et al.*, 2011). Results of a study conducted in Egypt showed significant differences in genotype GT in patients with T1D (*EL-MOHAMADY et al.*, 2009). However, other studies investigated Type 1 diabetes subjects are reported to exhibit reduced IL-2 production and subsequent Treg dysfunction (Hulme *et al.*, 2012) These cells depend on IL-2 for proliferating and controlling the T effector cells (Teff) reaction, but they do not have the capacity to produce IL-2. In type 1 diabetes (T1D), a hypothesis is that a lack of IL-2 in pancreas could prevent Tregs action and lead to beta cells destruction (Hartemann and Bourron, 2012; Connor *et al.*, 2016) . These interleukins IL-2, IL-1, IFN-Y and TNF stimulate T-cytotoxic cells and are effective in destroying insulin-producing beta cells in patients with T1D(22). SNPs of IL2 might have a role in etiopathogenesis of T1D.

v. Conclusion

1- That frequency of TT genotype and *T allele* were significantly rise in patients contrast to controls associated with a risk of developing T1D while G *allele* and TG genotype showed association with to prevention of the risk of the disease.
2- IL2 allelic changes at position +166 might be associated with increased and decreased risk of T1D in Iraqi population, and this may also contribute to a better clinic diagnosis.
3- SNPs of *IL2* might have a role in etiopathogenesis of T1D.
4- There are no local studies on IL-2 in T1D of Iraqi patients. this is the first study.

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