A Genetic Polymorphism of Interleukin-1*a* and Interleukin 1 Receptor Antagonist Gene with type 1 Diabetes Mellitus of Iraqi Patients

Dr.Ibtesam Badday Hassan **Dr. Maha, Falh, Nazzal**

Department of biology, college of Education
for pure science, University of Diyala.

Abstract

The aim of this study was to evaluate the frequency of polymorphism of interleukin-*1-a* gene (*IL1a*) at position$_{-889}$ and *IL-1* Receptor Antagonist Gene at position$_{-maspl\ 11100}$ of the promoter region (*IL1*-a-$_{889}$ and IL-1R$\alpha_{-maspl\ 11100}$ SNP) in T1DM and in healthy controls subjects in thirty nine of Iraqi patients, (12 males & 27 females;1 5.65 ± 1.79 years) and 21 controls.(7 males & 14females ; 14.66 ± 3.43 years) were enrolled in this study the polymorphism of IL1-a$_{-889}$ and IL1Ra$_{-maspl\ 11100}$ were data waved by polymerase chain reaction-specific sequence primer (PCR-SSP) assay. Results revealed that comparing *IL1*a$_{-889}$ *genotypes* and *alleles* between T1DM patients and controls frequencies of TT genotype and *T allele* (51.28 *vs*. 67.95%; P =0.511 respectively) were significantly high in patients in contrast to controls, (33.33 *vs*. 40.48%; P =0.179) and the related RR rates were 18.4 and 19.8, respectively, and the associated EF values were 1.51 and 1.82 . In contrast CC genotype and *C allele* (15.38 *vs*. 32.05 %, P =0.264 respectively) frequencies were significantly decreased in patients, compared to controls (52.38 *vs*. 59.52%; P =0.179), and associated PF values were 0.73 and 0.66 , respectively. But for IL-1R$\alpha_{-maspl\ 11100}$ polymorphism frequencies of TT genotype and *T allele* (35.89 *vs*. 42.31%; P=0.125) were significantly decreased in patients compared to controls (47.61 *vs*. 61.90% ; p=0.015), and the associated PF values were 0.49 and 0.59, respectively. But for TC genotype no significant differences were found in patients compared to controls. In contrast, CC genotype

and *C allele* (40.51 *vs*. 57.69%, P =0.030 respectively) frequencies were significantly increased in patients, compared to controls (23.81 *vs*. 38.10%; P =0.015), and the associated EF values were 3.29 and 1.58 , respectively. These findings suggest that both *IL1*$_{-889}$ and IL-1R$\alpha_{\text{-maspl 11100}}$ SNP might have a role in the etiopathogenic mechanism of T1DM and IL-1R$\alpha_{\text{-maspl 11100}}$ polymorphism showed associations (positive and negative) with T1D in the samples of Iraqi patients. Therefore, the functional role of such receptor might have been altered due to the deviations of some genotype and allele frequencies.

***Keyword :Polymorphism IL-1a,IL1Ra Diabetes,**

تعدد الاشكال الوراثية للبين ابيضاضي الاول ومستقبل الفا للبين ابيضاضي الاول لدى المرضى العراقيين المصابين بداء السكري/ النوع الاول في بعقوبة

د.ابتسام بداي حسان د. مهى فالح نزال
جامعة ديالى - كلية التربية للعلوم الصرفة - قسم علوم الحياة

المستخلص:
تحرت الدراسة عن العلاقة بين التعدد الشكلي للبين ابيضاضي الاول –الفا لموقع الجين - 889 ومستقبله الفا للموقع الجيني- maspl 11100 لداء السكري النوع الاول لدى المصابين في بعقوبة , حيث شملت 39عينه من المرضى (12 من الذكور و27 من الاناث وبمتوسط عمر 1,79±15,65 سنة). مقابل 21 من الاصحاء (7 ذكور و14 انثي وبمتوسط عمر 3,43 ± 14,66 سنة). تم تسجيلهم في هذه الدراسة باستخدام جهاز تقنية التضخيم (PCR-SSP) حيث اظهرت نتائج الترحيل الكهربائي للبين ابيضاضي لموقع الجين - 889 المتضخم بهذه التقانة الى وجود بعض الاختلافات المعنوية لدى المرضى ,اذ سجل النمط الجيني TT والاليل T 51.28) مقابل ;67.95% وباحتمالية0.511= P مقابل بالتعاقب ارتفاعا ملحوظا لدى المرضى مقارنة بالأصحاء 33.33)مقابل;40.48% وباحتمالية 0.179= P وباستخدام احتمالية فشر حيث بلغت نسبة الخطر(18.4و19.8 بالتعاقب) . واظهر كنمط مسبب (1.51 و 1.82) بالتعاقب لخطر الاصابة بداء السكري النوع الاول. وأبديت ملاحظات مماثلة في النمط الوراثي TC. بالمقابل اظهر النمط الجيني CC والاليل C (15.38مقابل 32.05 %,وباحتمالية 0.264= P) بالتعاقب انخفاضا ملحوظا لدى المرضى مقارنة بالأصحاء

(59.52 مقابل;52.38% باحتمالية P =0.179) ويعتبر كنمط وقائي (0.73و 0.66) من خطر الاصابة بداء السكري النوع الاول . بينما لوحظت فروقا معنوية لمستقبل البين ابيضاضي الاول الفا لموقع الجين- maspl 11100 للنمط الجيني TT والاليل T (35.89 مقابل;42.31% باحتمالية P=0.125) أذ سجل انخفاضا ملحوظا لدى المرضى مقارنة بالأصحاء ,(p=0.015 ; 61.90% *vs*. 47.61) ويعتبر كنمط وقائي ضد خطر الاصابة بالمرض حيث بلغ 0.49 و0.59 بالتعاقب. بينما لم يظهر النمط الجيني TC اختلافا معنويا لدى المرضى مقارنة بالأصحاء. بالمقابل سجل النمط الجيني CC والاليل C (40.51 مقابل 57.69% وباحتمالية P =0.030) ارتفاعا ملحوظا لدى المرضى مقارنة بالأصحاء (23.81 مقابل 38.10% باحتمالية0.015= P) حيث يعتبر كنمط مسبب لخطر الاصابة بالمرض (3.29و 1.58). وهذه الدراسة تقترح ان دور البين ابيضاضي الاول الفا -889 ومستقبله الفا- maspl 11100 كعامل مسبب في احداث مرض السكري النوع الاول وتعدد الاشكال لمستقبل البين ابيضاضي الفا- maspl 11100 لوحظ انه له دور سلبي وايجابي ضد داء السكري النوع الاول في عينات المرضى العراقيين. لذلك الدور الوظيفي لمستقبل البين ابيضاضي الاول الفا.لذلك فان الدور الوظيفي لهذا المستقبل ربما يكون قد تغير بسبب الانحرافات لبعض الطرز الوراثية والترددات الاليليه.

الكلمات المفتاحية: للبين ابيضاضي الاول , مرض السكري.

Introduction

Diabetes is a chronic disease which influences over 3 million people in the UK –about 10% Type 1 diabetes and residual 90% have Type 2 diabetes [18] . T1D is a serious autoimmune disease affecting millions of people worldwide. This information is for adults with T1D and parents of children with this condition [15]. T1D usually starts in childhood, adolescence, or early adulthood, but it may also start later in adult life [1,2].Everyone needs a hormone insulin to keep their blood glucose at a normal level. But with T1D, the pancreatic gland does not synthesis insulin or make very little of it [3]. T1D is an autoimmune disease characterized by the destruction of the insulin-producing islet β cells. 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 [14,5].There is increasing evidence

showing that polymorphisms in cytokine genes may play an important role in modulating the immune response. Numerous cytokines have been shown to participate in the pathogenesis of T1D [4]. 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* and *IL-6* [11,13]. Mediators of inflammation such as *TNF-α, IL-1β*, the *IL-6* family of cytokines, *IL-18,* and certain chemokines have been proposed to be involved in the events result in both forms of diabetes. [12,10,6]. Further supply for inflammation contributing to diabetes comes from researchers who examined the role of inflammatory cytokines in diabetic such as *IL-1* was first implicated in the development of diabetic[14,8]. IL-1 which was first described in 1972 as a lymphocyte-activating factor [19] and later was shown to exert a variety of effects including induction of inflammation [22]. The cytokines *IL-1* and *TNF-a* induce *B*-cell apoptosis in T1D is reported to rise by elevated glucose and is a known powerful stimulus of extra cellular matrix production. Therefore a study found increasing levels of *TNFα* and *IL-1* in both vitreous and serum of diabetic subjects compared to control subjects. A study indicated that an anti inflammatory agent TNF -*α* and *IL-1β*, increased in the sciatic nerves of the diabetic rats [9]. Nowadays, the recombinant non-glycosylated form of IL-1Ra, anakinra, is used for treatment of rheumatoid arthritis and is tested also for IL-1 inhibition during acute gout [20], diabetes mellitus [21] or familial cold auto inflammatory syndrome [19].For all previous information the present study focused on the two types of Interleukins 1-a and 1Ra.

MATERIALS AND METHODS

Subjects

The diagnosis and extent of disease was determined by conventional clinical thirty nine patients ; (12 males & 27 females) attended the hospital in Baquba for diagnosis and treatment during the period October 2015 – June 2016 in addition to twenty one healthy controls (7males and 14females).(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°C. T1D patients, and randomly selected healthy controls (HC). The patients age range was 15.65 ± 1.79 years compared to healthy controls which was 14.26 ± 1.43 years, were enrolled in the study.

Detection of IL1 Polymorphism

Genomic DNA was extracted from EDTA blood using Wizard Genomic DNA Purification Kit (Promega, USA). The polymorphism was detected at $_{-889}$ and IL-1Rα$_{\textbf{-maspl 11100}}$ positions of the promoter region (*IL1a*$_{-889}$ and IL-1Rα$_{\textbf{-maspl 11100}}$) 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 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 20 cycles of annealing at 61˚C for 50 seconds and extension at 72˚C for 30 seconds.

Statistical Analysis

Genotypes of *IL1*$_{-889}$ and IL-1Rα$_{\textbf{-maspl 11100}}$ SNP were presented as percentage frequencies, and significant differences between their distributions in T1DM 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 for free at http://www.brixtonhealth.com.

Rustles:

Genetic polymorphism of *IL1a* gene was determined in the promoter region at position -889 ($IL1a_{-889}$SNP), which was presented with three genotypes (TT, TC and CC) that corresponded to two alleles (*T* and *C*). 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 in some significant differences (Table -1). The frequencies of TT genotype and *T* allele were significantly increased in patients (51.28 and 67.95%, respectively) compared to controls (33.33 and 40.48%, respectively). The relative risks (RRs) of such positive associations were 18.4 and, 19.8 respectively. Similar observations were made in TC genotype (47.34*vs*. 24.28%, P =0.641respectively). In contrast, CC genotype and *C* allele frequencies were significantly decreased in patients (15.38 and 32.05%, respectively) compared to controls (52.38 and 59.52%, respectively). The preventive fractions (PFs) of such negative associations were 0.73 and 0.66 respectively (Table -2).

Table 1: Observed numbers and percentage frequencies and Hardy-Weinberg (H-W) equilibrium of(IL-1α-889 genotypes and alleles) in Diabetes Type 1 patients and controls.

Groups			IL-1α-889 Genotypes or alleles					H-W X^2 P ≤
			TT	TC	CC	*T*	*C*	
Diabetes type -1 (No. = 39)	Observed	No.	20	13	6	53	25	N.S.
		%	51.28	47.34	15.38	67.95	32.05	
	Expected	No.	18.01	16.99	2.40	Not Estimated		
		%	46.17	43.56	7.00			
Controls (No. = 21)	Observed	No.	7	3	11	17	25	0.001
		%	33.33	24.28	52.38	40.48	59.52	
	Expected	No.	3.44	10.12	7.44	Not Estimated		
		%	16.38	48.19	35.43			

Table 2-: Statistical analysis of associations between IL-1α_{-889} genotypes or alleles in Type 1 Diabetes patients and controls.

Type of Comparison	Statistical Evaluation			Fisher's Exact Probability	95% Confidence Intervals
	IL-1α_{-889} Genotype or Allele	Relative Risk	Preventive or Fraction Etiological		
Diabetes Disease Versus Controls	TT	18.4	1.51	0.511	0.31 - 1.72
	TC	8.1	0.75	0.641	0.30 - 1.86
	CC	10.9	0.73	0.264	0.75 - 4.42
	T	19.8	1.82	0.179	0.37 - 1.19
	C	16.1	0.66	0.179	0.84 - 2.73

Genetic polymorphism of *IL1*R*a* gene was determined in the promoter region at position - **maspl 11100** (IL-1Rα$_{-\textbf{maspl 11100}}$), which was presented with three genotypes (TT, TC and CC) that corresponded to two alleles (*T* and *C*). Among T1DM patients, no significant difference was observed between the observed and expected frequencies of the three genotypes (a good agreement with Hardy-Weinberg equilibrium; HWE));

however comparing patients to controls revealed some significant differences (Table -3). The frequencies of CC genotype and *C* allele were significantly increased in patients (40.51and 57.69%, respectively) compared to controls (23.81and 38.10%, respectively). The relative risks (RRs) of such positive associations were 25.6 and, 16.8 respectively, while TC genotype showed no significant differences in patients compared to controls. In contrast, TT genotype and *T* allele frequencies were significantly decreased in patients (35.89 and 42.31%, respectively) compared to controls (47.61 and 61.90%, respectively). The preventive fractions (PFs) of such negative associations were 0.49 and 0.59, respectively (Table 3 -4).

Table 3: Observed numbers and percentage frequencies and Hardy-Weinberg (H-W) equilibrium of (IL-1Rα$_{\text{-maspl 11100}}$ genotypes and alleles) in Type 1 Diabetes patients and controls.

Groups			IL-1Rα$_{\text{-maspl 11100}}$ Genotypes or alleles					H-W X^2 P ≤
			TT	TC	CC	*T*	*C*	
Diabetes type -1 (No. = 39)	Observed	No.	14	17	8	45	33	N.S.
		%	35.89	23.58	40.51	42.31	57.69	
	Expected	No.	12.98	19.04	6.98	Not Estimated		
		%	33.28	48.82	17.90			
Controls (No. = 21)	Observed	No.	10	6	5	26	16	N.S.
		%	47.61	28.57	23.81	61.90	38.10	
	Expected	No.	8.05	9.90	3.05	Not Estimated		
		%	38.32	47.17	14.51			

Table 4: Statistical analysis of associations between IL-1Rα$_{\text{-maspl 11100}}$ genotypes or alleles in Type 1 Diabetes patients and controls.

Type of Comparison	Statistical Evaluation			Fisher's Exact Probability	95% Confidence Intervals
	IL-1Rα$_{\text{-maspl 11100}}$ Genotype or Allele	Relative Risk	Preventive or Fraction Etiological		
Diabetes Disease Versus Controls	TT	3.0	0.49	0.125	0.21 - 1.17
	TC	8.1	0.80	0.663	0.34 - 1.87
	CC	25.6	3.29	0.030	1.17 - 9.23
	T	4.55	0.59	0.015	0.25 - 0.84
	C	16.8	1.58	0.015	0.86 - 2.88

Discussion

According to the presented results, IL1a$_{-889}$ SNP can be highlighted as an important genetic marker in the pathogenesis of T1DM 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 patients, but they were significantly deviated in controls ($P \leq 0.001$).The present study illustrated that IL-a$_{-889}$ is important genetic marker in the pathogenesis of T1DM especially if we consider RR values as 18.4 and 19.8 for it was showed that the frequency of TT genotype and that of *T allele* (51.28*vs*. 67.95%; P =0.511 respectively) were significantly increased in patients contrast to controls, (33.33 *vs*. 40.48%; P =0.179), and the associated EF values were 1.51 and 1.82 , respectively. Similar observations were made in TC genotype. In contrast, CC genotype and *C allele* (15.38 *vs*. 32.05%, P = 0.264 respectively) frequencies were significantly decreased in patients, compared to controls (52.38 *vs*. 59.52%; P =0.179), and the associated PF values were 0.73 and 0.66 respectively.

According to these findings which agree with previous results, it can concluded that *IL1a*$_{-889}$ SNP might have a role in the etiopathogenic mechanism of T1DM, However, other studies investigated other polymorphisms in intron and promoter regions of *IL1* gene and the results were almost conflicting due to ethnic variations, but they agreed that *IL-1* is an important cytokine involved in immunity and its polymorphisms play a critical role in T1DM development [14,8,17]. The present results strongly suggest that *IL-1Rα-*$_{maspl\ 11100}$ polymorphism is involved in T1DM in terms of susceptibility (positive association) and protection (negative association) in the samples of Iraqi patients. due to IL-1Ra binds IL-1R and blocks the activities of IL-1α and IL-1β, so it plays as an anti inflammatory [13]. Interleukin-1 is mainly produced by activated monocytes and macrophages, and acts systemically and locally, while IL-1RA is produced by hepatocytes during the inflammatory acute-phase response, probably to control IL-1 effects[11,12]. Therefore, the functional role of such receptor might have been altered due to the deviations of some genotype and allele frequencies.

References:

1- Ozougwu, J. C.; Obimba, K. C.; Belonwu, C. D.; and Unakalamba, C. B.(2013). The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus**.** *Journal of Physiology and Pathophysiology, Vol. 4(4), pp.* 46-57.

2- Soren, K**.** and Grey**,** M.(2015). Depression, Anxiety, and Eating Disorders Are Common in Teens and Young Adults with Type 1 Diabetes . *Vertical Health, LLC. http://www.endocrineweb.com*.

3- Chisholm, A.O. ; Best, J. ; Frazer, I. ; Pawlak, D. ; Townend, J. and Twigg, S.(2010). Australian Type 1 Diabetes Research Agenda - Partnering science, government and the community. *The Juvenile Diabetes Research Foundation. Sydney, 2nd edition.pp.*1-79.

4- Hata**,Y.;** Nakao**, S.;** Kohno, R.; Oba, K.; Kita,T.; Miura, M.; Sassa, Y.; Schering, A. and Ishibashi, T.(2010). Role of tumour necrosis factor-a (TNFa) in the functional properties of hyalocytes. *Br J Ophthalmol , October 29:pp.*1-5.

5- Gustavsson, C.(2010). Inflammatory mediators in diabetic retinopathy. Published: 01/01. *Citation for published version (APA):PP.*1-133.

6- Ortis, F. ; Miani, M.; Colli, M. L. ; Cunha, D.A. ;Gurzov, E. N. Allagnat, F. ;Chariot, A. and Eizirik, D.L.(2012). Differential usage of NF-jB activating signals by IL-1b and TNF-a in pancreatic beta cells. *586 : Elsevier B.V.*984–989.

7- Hussain, G.S.; Rizvi, A.A. ; Singhal, S.; Zubair, M. and Ahmad, J.(2016). Serum levels of TGF-b1 in patients of diabetic peripheral neuropathy and its correlation with nerve conduction velocity in type 2 diabetes mellitus. *Elsevier B.V.: Clinical Research & Reviews 10S S:*135–S139.

8- Elmarakby, A.A. and Sullivan, J.C.(2012). Relationship between Oxidative Stress and Inflammatory Cytokines in Diabetic Nephropathy. *Blackwell Publishing Ltd. Cardiovascular Therapeutics 30 :*49–59.

9- Omi, M.; Hata, M.; Nakamura,N. ;Miyabe, M.; Kobayashi, Y.; Kamiya, H.; Nakamura, J.; Ozawa, S.; Tanaka, Y.; Takebe, J.; Matsubara, T. and Naruse, K.(2016). Transplantation of dental pulp stem cells suppressed inflammation in sciatic nerves by promoting macrophage polarization towards anti-inflammation phenotypes and ameliorated diabetic polyneuropathy. *J.D.I. Volume 7, Issue 4,July P.* 485–496.

10- Arend,W.P.;Palmer, C. and Gabay, C.(2008).IL-1,IL-18, and IL-23 families of cytokines. *Immunol . Rve.*, 223:20-38.

11- Van de Veerdonk,F. L. and Netea, M. G.(2013).New insights in the Immunobiology of IL-1 family members. *Front. Immunol*. 8(4):167.

12- Ludwiczek,O.;Vannler, E.;Borqqraefe, I.;Kaser, A.;Simqmund, B.;Dinarello, C.A. and Tilq,H. (2004). Imbalance between interleukin-1 agonists; relationship to severity of inflammatory bowel disease. *Clin.Eux.Immunol*.,138(2):323-9.

13- Sainz, J.; Perez, E.; Gomez-Lopera, S. and Jurado, M.(2008). IL1gene cluster polymorphisms and its haplotypes may predict the risk to develop invasive pulmonary aspergillosis and modulate C-reactive protein level. *J. Clin. Immunol*., 28(5): 473-85.

14- Gonzalez, J. F. N. and Fernandez, C. M.(2008). The Role of Inflammatory Cytokines in Diabetic Nephropathy.*J. Am. Soc. Nephrol. 19: 433–442.*

15- Bonifacio, E. (2015).Predicting Type 1 Diabetes Using Biomarkers. *J. Diabetes Care Jun; 38(6):* 989-996.

16- Ma, D.H. ; Xu, Q.Y. ; Liu1, Y.; Zhai Q.Q. and Guo, M.H. (2016).Association between interleukin-10 gene polymorphisms and susceptibility to diabetic nephropathy in a Chinese population. *Genetics and Molecular Research 15 (2): gmr*.15027570.

17- Kyi, M. ; Wentworth, J. M. ; Nankervis, A. J.; Fourlanos, S. and Colman, P. G. (2015).Recent advances in type 1 diabetes. *Med. J. Aust; 203 (7):* 290-293.

18- Ruggenenti, P.; Cravedi, P. and Remuzzi, G. (2010).The RAAS in the pathogenesis and treatment of diabetic nephropathy. *Nature Reviews, vol. 6, no. 6, pp.* 319–330.

19- Metyas, S. K. and Hoffman, H. M.(2006). Anakinra prevents symptoms of familial cold auto inflammatory syndrome and Reynaud's disease. *J. Rheumatol. 33*: 2085-2087.

20-So A.; De Smed, T. T. ; Revaz, S. and Tschopp, J.(2007). A pilot study of IL-1 inhibition by anakinra in acute gout. *Arthritis Res. Ther.* 9: R28.

21- Tellez, N.; Montolio, M.; Biarnes, M. ;Castano, E. ;Soler, J. and Montany,A. E.(2005). Adenoviral overexpression of interleukin-1 receptor antagonist protein increases beta-cell replication in rat pancreatic islets. *Gene Ther* 12:120-128.

22- Vicenova, B.; Vopalensky, V.; Burysek, L. and Pospisek, M.(2009). Emerging Role of Interleukin-1 in ardiovascular Diseases**.** *Physiol. Res.* 58: 481-498.