Iraqi Journal of Biotechnology, 2023, Vol. 22, No. 1, 134-139

Role of IFIH1 Gene Polymorphism rs3747517 and IL-17A with its Receptor in Detection and Therapeutic approach for Type 1 Diabetes Patients

Baneen S. Mahdi, Wisal S. Abd Almohsen

Biotechnology Department, College of Sciences, University of Baghdad

Received: January 2, 2023/ Accepted: March 1, 2023/Published: June 4, 2023

Abstract: Type 1 diabetes (T1D) is multifactorial autoimmune disease, overlapping factors including genetic and environmental factors contribute to the appearance of T1D in children mostly and adults. The aim of the study was to find the correlation of T1D with the chosen immune parameters and with *IFIH1C* polymorphisms as genetic factor for the disease and study the role of interleukin-17 in the exacerbation and evolution of T1D. This study at pediatric Teaching Hospital in the medical city between November 2021 and January 2022.The study included 100 Iraqi children divided into two groups 50 T1D patients and 50 controls. Two SNPs (rs3747517 and rs1990760) from the *IFIH1* gene were carefully chosen to examine their relationship with T1D. Human IL-17A and IL-17AR enzyme linked immunosorbent assay (ELISA) kits were used to estimate the levels of interleukins mentioned above. The recognition of SNPs was achieved by using HRM (high resolution melting) real-time PCR. A Rotor gene was employed to perform qPCR-HRM, followed by an HRM analysis with ramping by 0.2 °C from 55 to 95 °C. In both selected parameters there was significant differences with average (IL-17A=260.0 , IL-17RA=238.4) of patients and an average (IL-17A=93.0, IL-17RA=90.9) in control group the *p* -value (0.001) , data of *IFIH1* gene polymorphism rs3747517 presented as shown in wild TT, hetero TC and mutant CC genotype ,comparison between IL-17A and IL-17RA and TT,TC,CC genotype in patients group result no significant value for the IL-17A(p =0.106) but there was a significant consequence between IL-17RA and SNP data (p =0.018). The IL-17A and IL-17R levels is a key indicator of damaged β cell function and progression to explicit T1D with it is receptor. It was concluded that IL-17A involvement in T1D increasing and a possible therapeutic approach for T1D directing IL-17A with IL-17RA besides highlight on status of IFIH1 as significant genetic factor and indicator to T1D.

Keywords: T1D**,** IFIH1C**,** single nucleotide polymorphism**,** genotype**,** IL-17A**,** IL17R.

Corresponding author: (Email: baneensalman97@gmail.com)

Introduction

Type 1 diabetes juvenile, childhood-onset diabetes is described by lack of insulin production. Individuals with T1D required take insulin repeatedly to regulate the glucose in their blood for survival (1). Interferon induced with helicase 1(*IFIH1*) gene which express viral pattern-recognition receptor, melanoma differentiation associated protein 5 (MDA5), which have critical role in innate immune system, identifying RNA viruses like picornaviruses. This family holds the enteroviruses too, and it has been proposed that at high *IFIH1* gene levels can incite anti-viral immune responses that give rise to autoimmune system (2). Many immune diseases were triggered by a virus such as multiple sclerosis with EBV either in male (3) or female (4). Enteroviruses (EV), in specific and respiratory tract viruses playing an essential role in the pathogenicity of T1DM (5). IL-17A is a proinflammatory cytokine produced by T-helper (Th17) cells, has been associated with autoimmune diseases, IL-17 family of cytokines has 6 basically linked cytokines, IL-17A to

IL-17F (6). Numerous types of cells secrete IL-17 like (CD8+)T cells, group innate lymphoid cells (ILC3) , natural killer cells(NK) and T-helper 17 (Th17).Th17 cells have succeeded prominence for their important role in psoriasis (7), Systemic lupus erythromatosus (8) in human T1D (9), and many autoimmune diseases. Other interleukins may contribute with T1D (10). Like IL-17A the IL-17 receptor family is also composed of members from IL17RA to IL-17RE. IL-17RA is ubiquitous and is a mutual co-receptor subunit for other interleukins members of the IL-17 family (11). The purpose of the resent study is to find the correlation of T1D with the chosen immune parameters and with *IFIH1C* polymorphisms as genetic factor for the disease, study the role of IL-17A in the exacerbation and progression of T1D to link the hole between environmental causes and the enlargement of the T1D in patients with genetic predispositions.

Materials and methods

Population studied

This study has been achieved at pediatric Teaching Hospital in the medical city between November 2021 and January 2022. The study included 100 Iraqi children divided into two groups 50 T1D patients and 50 controls. The medical history from all T1D patients has been taken, supported by an T1D Patients Questionnaire Survey information recorded, the age range for patients is between new born - 15 years, Complete information has been gained from all T1D patients by direct interview. Detailed clinical checks with a particular questionnaire formula occupied for each patient contains; name, age, gender, The area or the province of residence in Iraq, the age of diagnoses, Symptoms, injury, inflammation or type of infection that led to the occurrence of diabetes, the Level of RBS for last test, how can the diabetes have controlled, patient's family history, surgical and social history, and other complications associated. T1D. Patients already have been diagnosed by pediatrician and asserted according to hemoglobin A1C (HbA1c) test. Ethics Committee approval was obtained from the college of science research ethics committee of University of Baghdad in November 1, 2021.

Measurements

Blood Specimens have been collected, by taking 4ml of venous blood from each T1D patient and healthy child, blood have been placed in serum-separating tube; serum was separated and isolated from this 4 ml of blood by centrifuging for 10 minutes at 3000 round per minutes (rpm), The serum was preserved in 1.5 ml Eppendorf tubes and kept in the freezing temperature -20ºC until all the serum tests measured. The tube was preserved at -20ºC until all sample serum levels were determined. Human IL-17A and IL-17AR ELISA kits (Elabscience, USA) were used to estimate the levels of interleukins mentioned above. The principle behind this EḶISA kit is that it uses the ELISA Sandwich; the microplates in the package were coated previously with a human IL-17A-specific antibody. Samples were then applied in combination with unique Ab to the EḶISA microplate wells. Standards and protocols were also applied in conjunction with unique Ab on micro EḶISA platforms. Each microplate form was well filled with a special biotinylated detection Ab for human IL-17A and HRP conjugates, a substrate was used on each micro plat form well afterward. Avidin (HRP) conjugate was changed to blue color for biotinylated Ab detections. The reaction was ended

by adding the stop solution; color simply turned to orange. A spectrophotometer measured the optical density (OD) at 450 nm. The OD value is associated with Human IL-17A concentrations. The recognition of SNPs was achieved by using HRM (high resolution melting) real-time PCR. A Rotor gene (Qiagen, Germany) was employed to perform qPCR-HRM, followed by an HRM analysis with ramping by 0.2 °C from 55 to 95 °C.

Statistical analysis

The data for IL-17A and its receptor were examined using the software, Microsoft excel, Minitab v17, and IBM SPSS V26. The outcomes testified in this study which expressed as mean ± SD., Statistical analyses of the two SNPs (*rs3747517* and *rs1990760*) were performed in which allele and genotype frequencies were presented in number and percentage. When the data were estimated, the continuous variables were expressed as mean ± standard deviation and the frequency was given as the number (%). Hardy-Weinberg equilibrium (HWE) test was used for statistical analysis. Probability values less than 0.05 were considered significantly different.

Results and discussion

The total of 100 Iraqi child including 50 T1D patients with an age mean of 9.28± 3.25 including 28 (56.00%) males and 22 (44.00%) females. Other 50 healthy controls with an age mean of 5.41 ±2.80 including 26 (52.00%) males and 24 (48.00%) females were registered in this research. Pathogenicity of T1D is a magnitude of complex events, responded by a set of genetic predispositions, essentially unclear environment factors and random actions (12).The strongest link was located with *rs1990760* polymorphism by (13). *IFIH1* is supposed to contribute to innate immune reactions by secreting interferon-gamma (IFN) and provokes apoptosis cell death of many infected cells (14). Two main immune parameters are selected and were associated with T1D inflammation , the data from (15) show that although there is an increase in Th17 cell activity associated with T1D progression in the lymphoid compartment, with an increase in IL-17A , especially at initial period of the infection the Remaining IL-17-secreting cells induce the other pro-inflammatory cells, such as neutrophils and dendritic cells, into site of inflammation in pancreas (16). Serum levels of IL-17A and IL-17RA showed significant variation in T1D patients compared to healthy controls, The results in table 1 revealed a significant *p* -value (0.001) In both selected parameters with average (IL-17A=260.0, IL-17RA=238.4) of patients and an average (IL-17A=93.0, IL-17RA=90.9) in control group. this result was expected, since the selected parameter is proinflammatory cytokine that improved release of TNF-α, IFN-γ, IL-1. Furthermore, induced nitric oxide synthase and induced expression of the inflammatory factors (17). This result approved with (18) which indicated clear elevation in IL-17 releasing in the pancreatic islets of newly diagnosed T1D patients as well as proposed that IL-17A expands inflammatory response and oxidative tension in beta cells through IL-17RA role and thus augments cell apoptosis and insulin deficiency that are specific for T1D in Akita mice, While (19) result was disapproved with the resent result study where suggested that serum IL-17 and IL23 levels were not affected in diabetic patients.

Table (1): Mean serum levels of parameters in patients and control groups.

Parameter	Study groups								P-value ¥
	Patients				Control				
	N	Mean	±	SD	N	Mean	±	SD	
IL-17A	48	260.0	±	114.0	41	93.0	±	10.3	0.001**
IL-17RA	48	238.4	±	136.4	40	90.9	±	11.3	0.001**

Data presented as Mean ± SD, ¥: Independent t-test (chi-square)

IFIH1, also known as MDA5 (melanoma differentiation-associated protein 5), is a 1025– amino acid cytoplasmic protein that recognizes genome of picornaviruses and initiate immune system stimulation (20). The *IFIH1* role in the activation of immune response against viruses might be a vital factor linking the genetic and environmental factors, and could be complicated in the pathogenicity of a number of autoimmune diseases, its association with multiple sclerosis (MS), diabetes and Graves' disease (GD) by (21). Single nucleotide polymorphisms (SNPs) are the smallest genetic polymorphism unit and they are the form of SNP that is most prevalent in humans (22). According to our research released which indicated high significant differences for the two SNPS especially rs3747517 polymorphism (23), In table 2 the data of *IFIH1* gene polymorphism rs3747517 presented as shown in wild TT, hetero TC and mutant CC genotype ,comparison between IL-17A and IL-17RA and TT,TC,CC genotype in patients group result no significant value for the IL-17A(p =0.106) but there was a significant consequence between IL-17RA and SNP data (p =0.018) with mean(TT=322.6 ,TC=266.2,CC=181.9) of IL-17A and (TT=371.7, TC=208.7, CC=161.1) of IL17RA.in the other hand control group showed no significant value for both parameters (IL-17A p =0.147, IL-17RA p = 0.199). In secondary analysis, Comparison of immune parameters with rs1990760 genotype as wild CC, hetero CT and mutant TT result non-significant value of patients IL-17A p= 0.632, IL-17RA p= 0.867 As well as control group IL-17A p= 0.899, IL-17RA p= 0.589, IFIH1C or MDA5 receptor protein is over expressed in the beta cells of the pancreas which is protein recognition receptor that senses viral nucleic acids and leads to the induction of type I IFN which stimulate dendritic cell DC , natural killer cell NK and activate the function of both B and T cell to produce proinflammatory cytokines. any mutation in this gene lead to abnormal expression of MDA5 receptor which initiate autoimmunity (24).

Table (2): Comparison of immune parameters with the genotype Frequencies of *IFIH1* gene polymorphism (rs3747517T>C) among patients' group and Control group.

Patient group (n=50)	*IFIH1* polymorphism rs3747517 T>C			P-Value
	TT (n=5)	TC (n=38)	CC (n=7)	
IL-17A	322.6 (166.0 -479.0)	266.2 (227.3 -305.0)	181.9 (55.25 -308.5)	0.106 N.S
IL-17RA	371.7 (252.6 -490.6)	208.7 (160.6 -256.7)	161.1 (90.0 -232.1)	0.018 *
Control group (n=50)	***IFIH1* polymorphism rs3747517 T>C**			**P-Value**
	TT (n=35)	**TC (n=14)**	**CC (n=1)**	
IL-17A	91.07 (87.17-94.97)	97.80 (91.37-104.22)	87.7 (87-7.87.7)	0.147 N.S
IL-17RA	89.88 (85.58-94.18)	91.80 (84.21-99.40)	110.40 (110.4-110.4)	0.199 N.S

***IFIH1*: interferon induced with helicase 1 c domain; *P* value: Two- tailed fisher's exact probability.**

Table (3): Comparison of immune parameters with the genotype Frequencies of *IFIH1* gene polymorphism (rs1990760 C>T among Patients group and control group.

Patient group (n=50)	*IFIH1* polymorphism rs1990760 C >T			P-Value
	CC (n=27)	CT (n=19)	TT (n=4)	
IL-17A	244.8 (202.9 – 286.6)	277.7 (204.7 - 351.8)	278.7 (6.43 – 559.3)	0.632 N.S
IL-17RA	211.00 (152.8 – 269.9)	222.2 (157.7 – 288.1)	249.3 (110.7 – 636.3)	0.867 N.S
Control group (n=50)	***IFIH1* polymorphism rs1990760 C >T**			**P-Value**
	TT (n=41)	**CT (n=8)**	**TT (n=1)**	
IL-17A	93.06 (91.37-104.22)	93.18 (76.82-99.25)	88.15 (88.15-88.15)	0.899 N.S
IL-17RA	89.99 (85.55-94.43)	94.36 (89.47-99.25)	96.50 (96.50-96.50)	0.589 N.S

***IFIH1*: interferon induced with helicase 1 c domain; *P* value: Two- tailed fisher's exact probability**

Conclusion

In summary, IL-17A and IL-17R levels is a key indicator of damaged β cell function and progression to explicit T1D with it is receptor. This study offers precisely suggestion for IL-17A involvement in T1D enlargement and a possible therapeutic approach for T1D directing IL-17A with IL-17RA.besides highlight on status of *IFIH1* as significant genetic factor and indicator to T1D.

References

1. World Health Organisation (WHO).(2016). global report on diabetes. Switzerland : World Health Organisation (WHO): 88-100.
2. Jermendy, Á.; Szatmári, I.; Körner, A.; Szabó, A. J.; Tóth-Heyn, P. and Hermann, R. (2018). Association between interferon-induced helicase (IFIH1) rs1990760 polymorphism and seasonal variation in the onset of type 1 diabetes mellitus. Pediatric Diabetes, 19(2):300–304.
3. Abd, W. S. and Abd Al Kareem, R.M. (2020). Impact of EBV on multiple sclerosis in some of the Iraqi males: Immunological and molecular study. AIP Conference Proceedings, 2290: 20023-20029.
4. Kareem, RM Abd Al and Abd, W. S. (2020) .Impact of EBV on Multiple in a Sample of

Iraqi Females.Immunological and Molecular Study., Iraqi Journal of Science, 30: 1008-1015.

5. Karaoglan, M. and Eksi, F. (2018). The Coincidence of Newly Diagnosed Type 1 Diabetes Mellitus with IgM Antibody Positivity to Enteroviruses and Respiratory Tract Viruses. Journal of Diabetes Research, 2018, 8475341: 1-7.
6. McGeachy, M. J.; Cua, D. J. and Gaffen, S. L. (2019). The IL-17 Family of Cytokines in Health and Disease. Immunity, 50(4): 892–906.
7. kareem, Y.a. and A.N. Al-Thwani. (2021). Evaluation of IL-17, IL-18 and IL-22 as vital indicators of Iraqi Patients with Psoriasis. Iraqi Journal of Biotechnology, **20**(1): 103-108.
8. Peliçari, K.deO.; Postal, M.; Sinicato, N. A.; Peres, F. A.; Fernandes, P. T.; Marini, R., et al. (2015). Serum interleukin-17 levels are associated with nephritis in childhood-onset systemic lupus erythematosus. Clinics Sao Paulo, Brazil, 70(5): 313–317.
9. Honkanen, J.; Nieminen, J. K.; Gao, R.; Luopajarvi, K.; Salo, H. M.; Ilonen, J., et al. (2010). IL-17 immunity in human type 1 diabetes. Journal of Immunology (Baltimore, Md.: 1950), 185(3): 1959–1967.
10. Al-Shehmany, A.S., et al. (2014) . Genetic Association between Interleukin IL-18-137G/C and IL-18-607 C/A polymorphisms and Type 1 Diabetes in Egyptian population. Iraqi Journal of Biotechnology, 13(2): 47-54
11. Kuestner, R. E.; Taft, D. W.; Haran, A.; Brandt, C. S.; Brender, T.; Lum, K., et al. (2007). Identification of the IL-17 receptor related molecule IL-17RC as the receptor for IL-17F. Journal of Immunology (Baltimore, Md.: 1950), 179(8): 5462–5473.
12. Bluestone, J. A.; Herold, K. and Eisenbarth, G. (2010). Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature, 464(7293): 1293–1300.
13. Smyth, D. J.; Cooper, J. D.; Bailey, R.; Field, S.; Burren, O.; Smink, L. J., et al. (2006). A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. Nature Genetics, 38(6): 617–619.
14. Mehers, K. L. and Gillespie, K. M. (2008). The genetic basis for type 1 diabetes. British Medical Bulletin, 88(1): 115–129.
15. Li, C. R.; Mueller, E. E. and Bradley, L. M. (2014). Islet antigen-specific Th17 cells can induce TNF-α-dependent autoimmune diabetes. Journal of Immunology (Baltimore, Md.: 1950), 192(4): 1425–1432.
16. Pelletier, M.; Maggi, L.; Micheletti, A.; Lazzeri, E.; Tamassia, N.; Costantini, C., et al. (2010). Evidence for a cross-talk between human neutrophils and Th17 cells. Blood, 115(2): 335–343.
17. Qiu, A. W.; Cao, X.; Zhang, W. W. and Liu, Q. H. (2021). IL-17A is involved in diabetic inflammatory pathogenesis by its receptor IL-17RA. Experimental Biology and Medicine (Maywood, N.J.), 246(1): 57–65.
18. Arif, S.; Moore, F.; Marks, K.; Bouckenooghe, T.; Dayan, C. M.; Planas, R., et al. (2011). Peripheral and islet interleukin-17 pathway activation characterizes human autoimmune diabetes and promotes cytokine-mediated β-cell death. Diabetes, 60(8): 2112–2119.
19. Roohi, A.; Tabrizi, M.; Abbasi, F.; Ataie-Jafari, A.; Nikbin, B.; Larijani, B., et al. (2014). Serum IL-17, IL-23, and TGF-β levels in type 1 and type 2 diabetic patients and age-matched healthy controls. BioMed Research International, 2014, 718946.
20. Kato, H.; Takeuchi, O.; Sato, S.; Yoneyama, M.; Yamamoto, M.; Matsui, K., et al. (2006). Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature, 441(7089): 101–105.
21. Wawrusiewicz-Kurylonek, N.; Gościk, J.; Chorąży, M.; Siewko, K.; Posmyk, R.; Zajkowska, A., et al. (2020). The interferon-induced helicase C domain-containing protein 1 gene variant (rs1990760) as an autoimmune-based pathology susceptibility factor. Immunobiology, 225(1): 151864
22. Nuhiar, R.S., A.N. Salman, and H.R. AL-Rekaby. (2019) . Association of Transforming Growth Factor Beta1 Gene Polymorphism with Diabetes MellitusRisk in Iraq Patients. Iraqi Journal of Biotechnology. 18 (2): 49-56.
23. Mahdi, B.S. and Abd, Wisal, S. **(**2022). The Correlation Between Type 1 Diabetes (T1D) and interferon induced with helicase (IFIH1C) gene polymorphism rs3747517 and rs1990760.Malaysian Journal of Microbiology.(in press).
24. Borysewicz-Sańczyk, H.; Sawicka, B.; Wawrusiewicz-Kurylonek, N.; Głowińska-Olszewska, B.; Kadłubiska, A.; Gościk, J., et al. (2020). Genetic Association Study of IL2RA, IFIH1, and CTLA-4 Polymorphisms with Autoimmune Thyroid Diseases and Type 1 Diabetes. Frontiers in Pediatrics, 8, 481-488.