Biosc.Biotech.Res.Comm. Special Issue Vol 14 No 07 (2021) Pp-01-

The Significance of Genetic Polymorphism for IL-17Agene (rs 3819024) A/G in Iraqi Arab Patients with Type II Diabetes Mellitus (T2DM)

Ali Saad Kadhim[1], Hamzia Ali Ajah[2] and Ekhlass N. Ali[2]
[1]*College of Basic Education, University of Waist, Kut, Iraq, Department of Science, Al-Kut city. Iraq*
[2]*Department of Biology, College of Science, Mustansiryiah University, Baghdad, Iraq*
Corresponding author email: alisaad@uowasit.edu.iq

ABSTRACT
Diabetes mellitus type II is the most common type of diabetes mellitus in the Iraqi population and is affected via immunogenic factors. The ILA gene polymorphism may be impact significant on the development of disease. The current study investigates the impact of rs(3819024) on the serum level of IL17A.The study enrolled 75 patients (32 male and 43 female) and 75 healthy as control (42 male and 33 female); their age means± SD. were (40.2 ± 13.4)years. Tetra –ARMS-PCR,ELIZA are techniques used in this study.The results revealed that IL17A level showed no-gender-associated variation in patients groups, whereas it was significantly increased in both genders of patients compared healthy. The genotype of SNP showed polymorphic frequencies, and GGgenotype recorded ahigh significant (28%)withthe impact of the SNP genotype on cytokines levels. Only AG genotype impactsthe levels with particular in females more than males. In conclusion, a positive influence of rs3819024 on IL17A leveland gender played a role in influence.

KEY WORDS: POLYMORPHISM; IL-17AGENE;TYPE II DIABETES MELLITUS.

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a global epidemic on a worldwide scale. Elevated blood glucose levels characterize this metabolic disorderdue to the pancreas producing inadequate insulin (Al-Rifai, et al 2017). Inflammation occurs due to the immunological response to elevated blood glucose levels and the presence of inflammatory mediators produced by adipocytes and macrophages in adipose tissue. (Cho, et al 2018). Slightly elevated inflammation damages pancreatic beta cells, resulting in inadequate insulin production and hyperglycemia (Abbas et al 2020).

Biosc Biotech Res Comm P-ISSN: 0974-6455 E-ISSN: 2321-4007

Identifiers and Pagination
Year: 2021 Vol: 14 No (6) Special Issue
Pages: 201-204

Article Information
Received: 11th May 2021
Accepted after revision: 19th July 2021

DOI: *http://dx.doi.org/10.21786/bbrc/14.7.47*

Cytokines are substantial glycoprotein molecules with a low molecular weight generated by various cells concerningvarious immunological triggers. They are involved in the regulation of the majority of components of innate and adaptive immune responses, including inflammation and activation, cell migration and proliferation, as well as apoptosis and hematopoiesis (Kany, et al 2019).Their actions may be synergistic or antagonistic, and they may be autocrine, paracrine, or endocrine. Cytokines stimulate cell signaling by binding to their associated receptors on specific cells, resulting in subsequent biochemical changes that result in the expression ofcytokine genes suppression and transcription factors(Tabarkiewicz, et al 2015).

Interleukins-17A (IL-17A) is a form of cytokine released by Th 17 cells that stimulate inflammation and causes organ injury (Akbulut, et al 2017). It also stimulates the production of pro-inflammatory cytokines by resident cells, which results in increased neutrophil penetration into the infected organ, causing inflammation and harm (Zhao, et al 2018 and Summers, et al 2014). Interleukin-

17A genes are located on chromosome 6p12.3-q13 and consist of three exons and two introns. Transcript occurs in the same manner as gene replication, with the same regulatory elements utilized by cytokine genes resulting together (Shirazian, et al 2017). These variants include homodimers and heterodimers in humans, with approximately half of the sequence similarity between IL17A and IL17F(Angkasekwinai and Dong, 2011). The cytokine gene can influence on cytokine product. Polymorphisms by transcription effect of this gene Single nucleotide polymorphism (SNPs) may also be used to detect causes that lead to inflammatory diseases due to SNP occurring in regulatory regions of cytokine genes (Mohsen, et al).The current study describes The genetic association between alleles of IL-17A (rs3819024) A/G and susceptibility to diabetic Mellitus in Iraqi Arab patients.

Subjects and Methods

Subjects: The study was authorized by the Iraqi ministry's ethics commission in which a sample of 75 patients (32 males and 43 females) and 75 (42 males and 33 females) healthy as control (were recruited during the period July 2020-April). There is an age mean ± SD. in patients (male: 40.2±13.4 and female: 43.1 ± 14.7) and in healthy (male: 43.1±13.8 and female: 46.0 ± 13.2). Depending on ethnicity, the samples were collected and carefully inspected, and Arabic was the native language of all of them.Furthermore, they were not married to the mixed-race men and women in their families.The participants were gathered at Baghdad's central bank). healthy groupwas notsmoking or under therapyand had no chronic disease.

Blood sampling: All each participant, 5 ml blood was collected and distributed in two tubes: 2 ml in gel tube for sera collection and 3 ml blood in EDTA to extractDNA. The serum and DNA were stored in 20 Co.

Serum level of assessment: Sandwich enzyme-linked immunosorbentassay (ELZA) was used to deter the concentration of level, using communicably kit (Bio-sources kit, Korea).

Genotype: DNA was extracted from EDTA blood by gDNA extraction kits (Geneaid, Korea), .and IL17A genotype was done via Tetra allele refractory mutation system-polymerase chain (TETRA-ARMS) (12).The reaction was carried out in a 20 L volume that included DNA (4 L), outer primers (1 L), inner primers (2 L), and D.W (10 L).The primers were in the sequence (outer primer: forward: AGGGTTGGAACATGCCTTTAAC, revers primer: GCTGCTATGCTATGGGTCAATATC) and in the ratio (1:2, Frw: Rev). The inner primers dependent on the detected SNP are as follows (inner forward primer:GGCCAAGGAATCTGTGATGA, revers primer:). TTGATTTTCCATTTGATCTTTCTGTC).and then followed the PCR program, which included one step of pre-denaturation at 95 degrees Celsius for 5 minutes, followed by cycling steps in a total of 30 cycles:Denaturation at 95 C0 for 30 seconds, annealing at 53 Co for 40 seconds, extension at 72 C0 for 40 seconds, and final extension at 72C0 for 5 minutes. The amplicon of PCR included 380 pbs total gene, allele G 246 pbs, and allele A 180.

Statistical analysis: To perform statistical analysis on our data, we used the SPSS program. However, the LSD test and chi-square tried to foster class sympathies (spss, 2020). The odds ratios (OR) and 95% confidence intervals (CI) were calculated using MedCalc for Windows, version 18.10. (13,14).

RESULTS AND DISCUSSION

The current study showed that female patients were higher (57.3%) than male patients (42.7). Moreover, compared with the healthy as a control,the females of patients were higher than in control(Figure:1). The mean of aged for the females of the patient (43.1 ± 14.7) compared with control (46.0 ± 13.2), which were nonsignificant relative in the table (1).

Table 1. Distribution patents and healthy according on age

Groups	Healthy Mean ±SD	Patients	
		Mean ±SD	P-value
Male	43.1±13.8	40.2±13.4	0.374
Female	46.0 ± 13.2	43.1 ± 14.7	0.368

Table 2. Serum levels of IL17A for among Iraqi Arabs of (patients and controls)

Groups	Healthy Mean ±SD	Patients	
		Mean ±SD	P-value
male	22.0±7.4	99.7±16.3	0.022
Female	27.5±8.6	107.4 ± 12.3	0.005
p-value	0.864	0.758	

Cytokines serum levels: The result of table (2) showed the IL17 A level was non-significant increased (107.4 ± 12.3pg/ml) in femalepatientscompared with the male patients (99.7±16.3pg/ml).In contrast, the level of IL17A of female patients showed a significantly increased level (107.4 ± 12.3 pg /ml) compared to healthy females (27.5±8.6pg/ml).

HWE,genotype,and allele frequencies: HWE test carries out for SNP of IL17A .there were no significant variations between the control groups. Therefore a good agreement with the hardy test was observed.Table (3) showed that all alleles showed frequencies of polymorphic and G alleleshoweda significantly highfrequency(44%). In total, samples showedGG.Genotype high significance ($p<0.001$) in patients compared with healthy under the co-dominant genetic model. The distribution of genotypes according to gender was significant variation between females and males. And significant gender-associated between males and females in different groups(Table 4).

Impact of SNP on cytokines serum level: The rs (3819024) significantly influencedthe serum level of IL17A .as well, the significant – gender variation on serum levels of IL17A.Furthermore,AG genotype was high significant (P<0.001) influence females in (117.8±2.7) more than males (95.2±4.7) which reported in (table 5).

Table 3. Genotype and allele frequency of IL17ASNP (3819024 A>G) among patients and healthy under different genetic models.

Models rs: 3819024 HWE:0.483	Genotype	Patients	Control	OR(95%CI)	P-value
Co-dominant	AA	30(40%)	36(48%)	0.72 (0.37to 1.37)	0.324
	AG	24(32%)	30(40%)	0.70(0.36 to 1.32)	0.308
	GG	21(28%)	9(12%)	2.85(1.21-6.73)	0.016
Dominant	AA	30(40%)	36(48%)	1.38((0.72 to 2.64)	0.322
	GG+AG	45(30%)	39(26%)		
Recessive	AG+AA	54(36%)	66(44%)	0.35(0.14-0.82)	0.016
	GG	21(28%)	9(12%)		
Allele	A	84(56%)	102(68%)	0.59(0.37-.095)	0.033
	G	66(44%)	48(32%)		

Table 4. Genotype of IL17ASNP (3819024 A>G) in among patients and healthy according on gender.

Genotype	patients		control	%	OR	CI 95%	P-Value
	Male	%	Male				
AA	17	53.1	4	9.5	10.7	3.10-37.29	0.0002*
AG	0	0	29	69.1	NA	NA	NA
GG	15	46.9	9	21.4	3.23	1.17 to 8.90	0.023*
Genotype	Female		Female				
AA	13	30.3	32	96.6	0.013	0. 011 to 0.40	0.0001*
AG	24	55.8	1	3.4	40.4	5.05 to 48.3	0.0005*
GG	6	13.9	0	0	NA	NA	NA

Table 5. Relation of IL17A genotype on level of serum in among Iraq Arabs.

Genotype	Levels (Pg/ml)		p-value
	Male	Female	
AA	103.6±3.2	114.0±2.7	0.24
AG	95.2±4.7	117.8±2.7	<0.001
GG	NA	101± 2.2	NA

Type 2 diabetes (T2D) is a complex genetic diseaseglobally and is the most prevalent type of diabetes.It is complex, with genetic and environmental factors both contributing to its development.In the disease population, It is widely established that cytokines play critical roles in immune responses and that changes in their serum levels may result in abnormal or ineffective immune responses. There were no published studies about the associated rs(3819024) and T2DM.Therefore, our study is first. The present study is the first to indicate IL17A gene rs 3819024 on the serum level of IL17A in the Iraqi Arab population. Studies in this context have not been well arranged, and some result was scattered in the patients and healthy as control groups.The serum level of IL17A was highly significant in patients, similar to our result. Moreover, we have not agreed with Mina et al. that they indicated an associatedautoimmune disease.

In addition, we also found that the GG.The genotype of rs 3819024 was associated with an increase with T2DM., which was different from that reported gastric cardia-adenocarcinoma in a Chinese population study was found that allele A was associated with reduced risk and that not agree with our result.A similar finding was that the GG.Genotype was high significant (p= 0.02) compared with a nonsmoker. A finding on consistent with the present study.In contrast, with Slattery et al, the rs 3819024 G allele to be marginally associated with decreased (GC. risk) and high risk with autoimmune disease.In the present study, we showed significantly highest serum levels of T2DM patients in females than males .that similar finding was serum level of patients highest than control.However, In a further study, The patient withT2DM was significantly higher than the control.

As shown, there were disparities between studies regarding the cytokine influence of SNP genotypes on their phenotypic expression in sera of healthy people, which could be attributed to differences in genetic and non-genetic variables among ethnic groups. Additionally, each community faces unique environmental challenges, which may affect cytokine production. This issue was addressed in research conducted as part of the Human Functional Genomics Project, which examined thevariability of host genetic or non-genetic.Therefore, future studies in this field should develop and concern this SNP and interest in D.M. studies.

CONCLUSION

Our current study was the first study in Iraq investigating IL17A gene rs 383819024 with T2DM in the Iraqi Arab population. A positive impact of IL17Ars383819024 genotype on the serum level of IL17A is suggested. Moreover, we found that females are more exposedto infection with disease, Specially, carry heterozygote AG.compared with males in the same groups. The serum level of IL17A was a significant influence onthe AGgenotype. Moreover, concern the G allele was etiological fraction or risk allele with the disease. The result should be more clear if genotypes of SNP werecorrelated with cytokines gene expression.

REFERENCES

Abbas, K.M., Alaaraji, S.F. and Alâ, R.S., 2020. A study of the association between IL-17 and HOMA-IR in Iraqi type 2 diabetic patients. Iraqi Journal of Science, pp.491-498.

Akbulut, U.E., Çebi, A.H., Sag, E., Akbal, M. and Çakır, M., 2017. Interleukin-6 and interleukin-17 gene polymorphism association with celiac disease in children. Turk J Gastroenterol, 28(6), pp.471-5.

Al-Rifai, R.H. and Aziz, F., 2018. Prevalence of type 2 diabetes, prediabetes, and gestational diabetes mellitus in women of childbearing age in Middle East and North Africa, 2000–2017: protocol for two systematic reviews and meta-analyses. Systematic reviews, 7(1), pp.1-7.

Alzamil, H., 2020. Elevated serum TNF-α is related to obesity in type 2 diabetes mellitus and is associated with glycemic control and insulin resistance. Journal of obesity, 2020.

Anderson, O.D., 2008. BatchPrimer3: a high throughput web application for PCR and

Statistical analysis system, User's guide. Statistical. Version 9. SAS. Inst. Inc. USA.

Angkasekwinai, P. and Dong, C., 2011. T H 17 Cytokines: Characteristics, Regulation, and Biological Function. In TH17 cells in health and disease (pp. 27-40). Springer, New York, NY. sequencing primer design. BMC bioinformatics, 9(1), pp.1-13.

A., Wang, Z. and Li, M., 2018. IL-6/STAT3 pathway induced deficiency of RFX1 contributes to Th17-dependent autoimmune diseases via epigenetic regulation. Nature communications, 9(1), pp.1-14.Cary, N., 2012. Cho, N., Shaw, J.E., Karuranga, S., Huang, Y.D., da Rocha Fernandes, J.D., Ohlrogge, A.W. and Malanda, B., 2018. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes research and clinical practice, 138, pp.271-281.

Hidalgo, M.A.R., Cirelli, T., da Silva, B.R., Nicchio, I.G., Nepomuceno, R., Orrico, S.R., Cirelli, J.A., Theodoro, L.H., Barros, S.P. and Scarel-Caminaga, R.M., 2021. Polymorphisms and haplotypes in the Interleukin 17 Alfa gene: potential effect of smoking habits in the association with periodontitis and type 2 diabetes mellitus. Molecular Biology Reports, 48(2), pp.1103-1114.

Kany, S., Vollrath, J.T. and Relja, B., 2019. Cytokines in inflammatory disease. International journal of molecular sciences, 20(23), p.6008.

Kuwabara, T., Ishikawa, F., Kondo, M. and Kakiuchi, T., 2017. The role of IL-17 and related cytokines in inflammatory autoimmune diseases. Mediators of inflammation, 2017.

Ren, Z., Li, M., Liu, R., Wang, Y. and Gu, H., 2014. Interleukin 17A rs3819024 A> G polymorphism is associated with an increased risk of gastric cardia adenocarcinoma in a Chinese population. Biomarkers, 19(5), pp.411-416.

S.M., Saleh, M.A.D. and Farhan, A.A., ROLE OF IL-17A GENE POLYMORPHISMS IN IRAQI RENAL FAILURE PATIENTS.

Shirazian, S., Aghahosseini, F., Salehi, E., Vatanpour, M., Banijamali, S.N. and Pourshahidi, S., 2017. Comparison of interleukin 17 and 22 in saliva of oral lichen planus patients with healthy people. Bioscience Biotechnology Research Communications, 10, pp.587-591.

Summers, S.A., Odobasic, D., Khouri, M.B., Steinmetz, O.M., Yang, Y., Holdsworth, S.R. and Kitching, A.R., 2014. Endogenous interleukin (IL)-17 A promotes pristane-induced systemic autoimmunity and lupus nephritis induced by pristane. Clinical & Experimental Immunology, 176(3), pp.341-350.

Tabarkiewicz, J., Pogoda, K., Karczmarczyk, A., Pozarowski, P. and Giannopoulos, K., 2015. The role of IL-17 and Th17 lymphocytes in autoimmune diseases. Archivum immunologiae et therapiae experimentalis, 63(6), pp.435-449.

Thijail BSc, H.A. and Mousa PhD, H.M., 2020. Comparative Study Of Some Immunological Aspects Between Type I And Type II Diabetic Mellitus In Iraqi Patients Of Thi-Qar Province. European Journal of Molecular & Clinical Medicine, 7(1), pp.3537-3546.