The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90, Page 88- 92

Evaluation of Interleukin-18 Serum Concentration and Gene Polymorphism (Rs1946518) in A Sample of Type-2 Diabetes Mellitus Patients from Iraq

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ABSTRACT

Background: Type two diabetes (T2DM) is characterized by insufficient insulin production and secretion. Additionally, the body develops insulin resistance which affects 90–95% of diabetics. Complex cytokines, receptors, genetic pathways, and the immune system are involved in T2DM. Interleukin-18 (IL-18) is one of the inflammatory cytokines associated with Type 2 diabetes. Environmental and genetic variables, including genetic polymorphisms, can increase T2DM risk and its consequences. Single nucleotide gene polymorphisms (SNPs) are important risk factors for diabetes that can be used to find the disease early and treat it better.

Objective: This study aimed to determine the levels of IL-18 in the serum of Iraqi patients with Type 2 diabetes mellitus, as well as the effect of IL-18 SNP rs1946518 (-607 G/T) in the etiology of T2DM.

Materials and Methods: This study involved 100 T2DM patients (52 males and 48 females) who visited Al-Karamah Teaching Hospital and Baghdad Teaching Hospital. 52 Iraqi control subjects (26 males and 26 females) were included. A sandwich enzyme-linked immunosorbent assay was used to quantify the IL-18 serum levels of 48 patients and 40 healthy controls. The genotype of IL-18 was determined using Real-time (RT) Taqman PCR.

Results: According to age, the current study revealed a non-significant correlation (p-value > 0.05) among the studied groups. IL-18 levels in the T2DM group were substantially greater than in the healthy control. In addition, the genotyping frequencies revealed that the frequency of TT genotyping was higher in T2DM group than in healthy control (80% versus 66.7%, OR: 2.0), whereas the frequency of GT genotyping was lower in T2DM than in healthy persons (20% versus 33.3%, OR: 0.5).

Conclusion: This Iraqi's novel study indicated that IL-18 and it's SNP(rs1946518) contributes to the pathophysiology of Type 2 diabetes mellitus.

Keywords: T2DM, Interleukin-18, SNP, RT-PCR.

INTRODUCTION

According to the World Health Organization (WHO), diabetes mellitus is a chronic metabolic condition characterized by high blood glucose levels, which overtime causes damage to the heart, blood vessels, eyes, kidneys, and nerves. T2DM, which accounts for 90% of diabetes mellitus cases, is characterized by insufficient insulin secretion by pancreatic islet cells, tissue insulin resistance (IR), and an inadequate insulin secretory compensatory response [(1)]. Since T2D is known as non-insulin-dependent diabetes, T2DM patients do not require insulin therapy to survive [(2)].

The etiology of type 2 diabetes is complicated and involves numerous risk factors included age, genetic inheritance, environmental variables, lifestyle, and infections [(3)]. The risk of developing T2DM is significantly influenced by genetic predisposition, which includes genetic faults of IR and genetic defects in insulin secretion [(4)].

An inflammatory reaction arises due to the immune response to elevated blood glucose levels and the presence of inflammatory mediators generated by adipocytes and macrophages in adipose tissue. This chronic and low-grade inflammation damages pancreatic beta cells, resulting in insufficient insulin production and hyperglycemia (T2D) [(5)].

Patients with T2DM have elevated cytokine expressions and immune cell infiltration of pro-inflammatory macrophages in their pancreatic islets [(6)]. The IL-1 cytokine family, a prominent class of immunoregulatory agents, has key roles in endocrine processes and the regulation of inflammatory stress responses, particularly in T2DM [(7)]. IL-18, a member of the IL-1 family of cytokines, is a proinflammatory cytokine with pleiotropic effects and a molecular weight of 18 kDa and 157 amino acids [(8)].

Interleukin-18 (IL-18) was found as an interferon-gamma (IFN)-inducing factor in the late 20th century. IL-18 also contributes to the pathophysiology of atopic, autoimmune, and chronic inflammatory illnesses, in addition to its role in immunological protection against infectious pathogens [(9)]. Causing inflammation and immune cell infiltration, IL-18 can cause pancreatic islet cell injury/death and dysfunction by inducing inflammation. Then the resulting insulin resistance or insulin sensitivity suppression leads to type 2 diabetes [(10)]. Numerous clinical studies have demonstrated that plasma levels of interleukin-18 (IL-18) are positively linked with the onset and progression of type 2 diabetes [(11)].

Received: 22/6/2022
Accepted: 30/8/2022

IL-18 gene is found on chromosome 11 in humans and chromosome 9 in mice and has seven exons with two promoters [12]. Various genetic and environmental influences, including genetic polymorphisms, can raise the risk of a complicated polygenic metabolic disorders defined by increased blood glucose (T2DM) and accompanying consequences. SNP is one of the primary forms of genetic variation that can impact the expression of genes involved in glucose metabolism [13]**.** Some genetic variants, either regulated non-coding SNPs or incorrect coding SNPs that result in direct amino acid changes within a protein, are associated with type 2 diabetes [14].

SNP is a key element in diabetic susceptibility that can be used for early detection and improved disease treatment, as genes associated with the immune system, nuclear receptors, and insulin signaling pathway contain diabetes-related SNPs [15]. Two IL-18 gene promoter polymorphisms at locations -607 and -137 associated with genotype and serum IL-18 levels. Polymorphisms alter monocyte IL-18 production and gene transcriptional activity [16]**.**

In this research, we evaluated the impact of IL-18 SNP (rs 1946518) [in the promoter region of the IL-18 gene at position -607] in the pathogenesis and development of T2D, as well as the amount of IL-18 in the sera of both study groups (patients and healthy).

MATERIALS AND METHODS

100 Iraqi T2DM patients (52 males and 48 females) ranging in age from 40 to 70 years with mean age of 57.26 ± 1.0 versus 56.23 ± 1.24 years in the control group (52 non-diabetic healthy subjects, 26 males and 26 females) ranged in age from 40 to 70 years. The study was conducted through the period from October 2021 to February 2022. The total number of samples was collected at Al-karamah Teaching Hospital and Baghdad Teaching Hospital.

DNA extraction and gel electrophoresis were performed on one hundred diabetic patients and fifty-two healthy individuals. All participants were processed to extract DNA from 300µl of their whole blood samples by using the FavorPrep TM /Cultured cells Genomic DNA extraction mini kit (FAVORGEN; USA). The purity and concentration of the sample were assessed. The concentration varied between 60 and 95 g/ml, while the purity varied between 1.6 and 2. The complete genomic DNA samples were then electrophoresed at 100 volts for 45 minutes on 0.8% agarose gels stained with red safe and examined at 350 nm with a UV transilluminator.

IL-18 SNP (rs1946518) was determined using real-time Taqman Polymerase chain reaction (RT-PCR) in 50 T2D patients (25 males and 25 females) and 42 healthy persons (20 males and 22 females). For RT-PCR technique, 5 µl of DNA extracted sample was added to PCR Eppendorf tube containing primer and prop, master prop and distilled water. Finally, PCR Eppendorf tube was placed in real time device (Stratagene Mx3005p) for 1.5 hour and the results were printed.

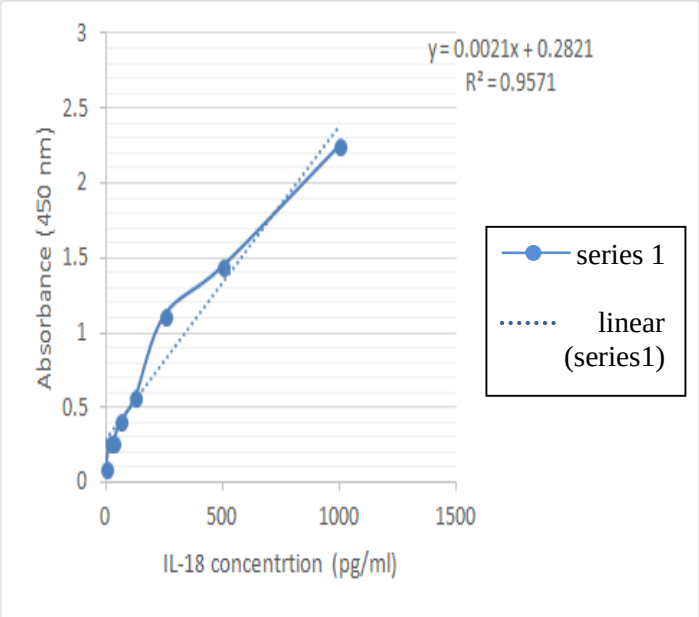

Figure: Standard curve of IL-18 concentration(IU/ml)

The measurement of Interleukin-18 serum levels was performed in the serum of 48 patient volunteers of diabetic group included (25 males and 23 females) and serum of 40 healthy individuals included (19 males and 21 females) and its detection relied on the technique of ELISA according to manufacturer company human Germany by using a commercial sandwich ELISA kit (Al-shkairate institution for medical supply; Jordan).

Ethical approval

The College of Science Research Ethical Committee of Baghdad University approved this project. Every patient gave their informed permission. This research was conducted in accordance with the Declaration of Helsinki, the code of ethics for human research endorsed by the World Medical Association.

Statistical Analysis

IBM SPSS version 28.0 was utilized to calculate the mean and standard error for the parametric data, while the ANOVA table and independent T-test were utilized to evaluate the probability. Pearson chi-square is used to calculate the probability for non-parametric data. When the probability was less than 0.05, it was significant. Utilizing an online Hardy-Weinberg equilibrium calculator, the odd ratio, 95% CI, and Fisher's exact probability are calculated.

RESULTS

According to the age

The results of demographical data, as shown in table (1) revealed that age and gender differences were not statistically significant (P>0.05) across the groups tested.

Table (1): Demographical data of studied groups

Gender	Age mean ± SE (Years)				Probability
	Patients (100)		Control (52)		
Males	52	58.04 ± 1.42	26	5.19 ± 1.73	0.230
Females	48	56.42 ± 1.40	26	57.27 ± 1.79	0.714
Total	100	57.26 ± 1.0	52	56.23 ± 1.24	0.533
Probability		0.420		0.409	

According to the level of IL-18 in the serum

The findings of current study, as viewed in table (2) revealed a significant increase in interleukin-18 level of patients group in comparison with the control group with probability value 0.003. IL-18 level in patients was 36.78 ± 4.58 versus 18.79 ± 3.36 in healthy subjects.

Table (2): Assessment of IL-18 levels among the studied groups

Groups	IL-18 level mean ± SE (pg/ml)	Probability
Patients	36.78 ± 4.58	0.003
Control	18.79 ± 3.36	

According to the genotyping of IL-18 SNP (rs1946518)

The initial report of genotyping results and allele frequencies, demonstrated that the genotypes of both studied groups correspondence with Hardy-Weinberg equilibrium (HWE) as in table (3). Adding, it showed that the TT/T allele (Genotyping /allele) was more prevalent in the T2DM group than in the control group, but the GT/G allele (Genotyping /allele) was less prevalent in T2DM patients than in controls with non significant correlation. In addition, table (4) revealed that the TT/ T (Genotyping /allele) has higher odds ratio (OR) values (2.0), therefore it may be considered a risk factor. Whereas the genotyping and allele GT/G displayed a reduced OR value (0.5), indicating a protective effect as shown in table (4).

Table (3): Genotyping of IL-18 SNP

Genotyping of IL-18 SNP rs1946518	Patients group frequency (%)		Control group frequency (%)	
	Observed	Expected	Observed	Expected
GG	0 (0.0)	0.5 (1.0)	0 (0.0)	1.2 (2.8)
GT	10 (20.0)	9.0 (18.0)	14 (33.3)	11.7 (27.8)
TT	40 (80.0)	40.5 (81.0)	28 (66.7)	29.2 (69.4)
Total	50 (100.0)	50 (100.0)	42 (100.0)	42 (100.0)
P-HWE	0.4321		0.1949	

P-HWE: Hardy-Weinberg equilibrium probability.

Table (4): Results of genotyping and allele frequency in studied volunteers

Genotyping of IL-18 SNP rs1946518	Patients No. (%)	Control No. (%)	OR (95% CI)	EF or PF	*P*
GG	0 (0.0)	0 (0.0)	-	-	-
GT	10 (20.0)	14 (33.3)	0.5 (0.20 – 1.27)	16.7	0.161
TT	40 (80.0)	28 (66.7)	2.0 (0.79 – 5.09)	40.0	0.161
Total	50 (100.0)	42 (100.0)			
Allele frequency					
G	10 (10.0)	14 (17.0)	0.56 (0.23 – 1.32)	7.4	0.195
T	90 (90.0)	70 (83.0)	1.80 (0.76 – 4.27)	40.0	0.195

OR: odds ratio, 95% CI: confidence interval, and P: Fisher's exact probability (two-tailed).

IL-18 serum level and genotyping

Table (5) indicated that both TT and GT genotypes of IL-18 SNP rs1946518 showed significant increase in the level of IL-18 between both studied groups (39.76 ± 5.44 in T2DM patients vs. 22.29 ± 5.04 in control for TT and 25.47 ± 6.64 vs. 12.29 ± 0.85 for GT).

Table (5): Distribution of IL-18 levels among T2D patient genotypes as compared to control genotypes

Genotyping of IL-18 SNP rs1946518	IL-18 level mean ± SE (pg/ml)		Probability
	Patients (50)	Control (42)	
GG	-	-	
GT	25.47 ± 6.64	12.29 ± 0.85	0.029
TT	39.76 ± 5.44	22.29 ± 5.04	0.029

DISCUSSION

The results of current study showed a non-significant difference in age between males and females in the patients compared to those in the control group. This is consistent with the conclusion of a prior study that found no statistically significant difference in age between the two groups [(17)]. There is no statistically significant difference between the genders of the analyzed groups according to an Egyptian study [(18)] and an Iraqi study [(19)].

Our research revealed that the serum concentration of IL-18 was significantly greater in the sick group than in the control group. Numerous clinical research have demonstrated that Interleukin-18 (IL-18) plasma levels are strongly linked with the pathophysiology and progression of Type 2 diabetes (T2DM). One of which suggested that circulating levels of IL-18 have been regularly observed to be raised in individuals with type 2 diabetes mellitus in cross-sectional investigations, and it has also been proposed that microangiopathy, such as nephropathy, contributes to type 2 diabetes [(20)]. In addition to another study refined that T2D patients had a significantly larger serum level of IL-18 than control subjects [(21)].

Polymorphism at positions -607, in Interleukin-18 gene promoter, was analyzed by RT- PCR. In respect to genotyping and alleles frequencies of IL-18 SNP rs1946518(-607 G/T), our study revealed that the TT/ T allele was more prevalent in T2DM group than in the control group. In contrast, GT/T allele was less prevalent in T2DM patients than in controls. Additionally, TT/ T might be considered as a risk factor according to OR ratio (2.0) while the lower OR value (0.5) of GT/T allele meaning a protective characteristic of this genotype and allele. A result of an Egyptian study, about (-607 C/A) SNP, showed a significant high in the frequencies of the mutated AA genotype and A allele of IL-18 -607 SNP that was observed in ischemic stroke (IS) patients than in controls and both genotype and A allele were significantly associated with increased risk of IS in the Egyptian population [(22)]. In addition, a result of other study showed a significant association of IL-18 SNP rs1946518 with pulmonary tuberculosis in the Chinese Han population [(23)]. In other hand, another study findings clarified that there was no relationship between IL-18 (at location -607 C/A) polymorphism and polycystic ovary syndrome (PCOS) in Iraqi females [(24)].

Also, the findings of our study indicated that the TT & GT genotypes of IL-18 SNP rs1946518 showed a significant increase in the level of IL-18 in patients with type 2 diabetes compared to the healthy individuals. This result is consistent with earlier analyses of gene-to-gene interactions. In one of these studies, the rs1946518 of IL-18 was associated with a greater levels of IL-18 in individuals with coronary artery disease (CAD) [(25)]. In addition, another study revealed that low IL-18 levels and the rs1946519 mutation of IL-18 are substantially related with recurrent spontaneous miscarriage (RSM) [(26)].

CONCLUSION

This study showed that the IL-18 -607 TT genotype and T allele are potential risk factors for type 2 diabetes mellitus. In addition, our study that the elevated concentrations of IL-18 in T2D patients contributes to the pathophysiology and progression of Type 2 diabetes mellitus.

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