Association of Interleukin -17A Genotypes with their Serum Levels in Iraqi Type 2 Diabetic Patients

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Abstract— Diabetes mellitus type 2 (T2DM) is one of the most prevalent non-communicable diseases worldwide. Interleukin-17 (IL-17, or IL-17A) is a crucial cytokine that links T cell activation to neutrophil recruitment and activity. In the cytokine storm, up-regulation of T-helper 17 cell cytokine IL-17A, This study aims to investigate the association of polymorphism of IL-17A (rs2275913) gene to their serum levels among Iraqi diabetic type 2 patients. A total of 70 participants, 50 were confirmed previously type 2 diabetic patients, and 20 were apparently healthy. Genetic polymorphism of IL-17AG197A rs (2275913) was carried out using RFLP-PCR. Serum levels of IL-17A were performed using by enzyme-linked immuno-sorbent assay technique (ELISA) using a Human- IL-17A kit. The results of the current study indicated the levels IL-17A were higher in the sera of diabetic patients compared to healthy controls, although there were no significant differences, in patients (243.7233±38.45944), vs. controls (148.8930±11.79280), P= 0.135. . Moreover, T2DM patients with AA and AG genotypes showed higher levels of IL-17A than that with GG genotype: 252.5476±50.775 for AA genotype and253.5817±79.13 for AG genotype compared to163.5100±8.14105, 115.2900±0.0 for each genotype in controls, P> 0.05.

Keywords— T2DM, IL-17A, RFLP-PCR, ELISA

1. Introduction

Diabetes mellitus (DM) is a heterogeneous form of a chronic metabolic disorder characterized by persistent hyperglycemia .T2DM accounts for around 90% of all cases of diabetes. The development of type 2 diabetes is brought on by a confluence of lifestyle choices and inherited variables[1]. Around 1.4 million Iraqis have diabetes. Reported T2DM prevalence in Iraq ranges from 8.5% to 13.9%[2].

Studies using genome-wide association (GWA) techniques have found repeatable relationships between particular single nucleotide polymorphisms (SNPs) and the risk of developing type 2 diabetes[3].Polymorphisms in pro- and anti-inflammatory cytokine genes, as well as their receptors, may exacerbate this cytokine imbalance and thus also the development of T2DM[4]. Based on genotype, there are 'high' and "low cytokine" producers. By identifying specific polymorphisms in each cytokine gene, it is possible to identify high and low cytokine producers by genotype. Therefore, in certain individuals, a genetic predisposition for a hyper-reactive immune response may be responsible for the development of T2DM[5].

Interleukin-17 (IL-17, commonly referred to as IL-17A) is a crucial cytokine that links T cell activation to neutrophil recruitment and activity. IL-17 can therefore promote protective innate immunity against pathogens or contribute to the pathophysiology of inflammatory disorders such as psoriasis and rheumatoid arthritis[6]. According to the findings of research, increased levels of IL-17 were seen in diabetic animal models that were induced with STZ and in non-obese diabetic mice (NOD) that progressed from insulitis to diabetes[7][8]. However, as of yet, there have been very few studies that have been published that investigate the role of IL-17A in type 2 diabetes.

Studies have shown that STZ-induced diabetic animal models and non-obese diabetic (NOD) mice go from insulitis to diabetes with significantly higher levels of the cytokine IL-17A . At present, however, there are only a limited number of published studies exploring the role of IL-17A in type 2 diabetes. In the cytokine storm, up-regulation of T-helper 17 cell cytokine IL-17A, and maybe also IL-17F, is mostly responsible for the immunopathology acute respiratory distress syndrome[7][8] .

This study aims to investigate the association of polymorphism of IL-17A (rs2275913) gene to their serum levels among Iraqi diabetic type 2 patients.

2. Materials and Methods

70 persons, blood samples were conducted in this study (50 confirmed type 2 diabetic patients and 20 controls), with age (40–85 years). They were selected from the local community of Wasit province – Iraq.

(5 M.) of blood were collected from all participants and placed in a tube without anticoagulant and placed in a centrifuge at a speed of 2000 rpm for 10 minutes. After that, the serum was withdrawn into an Eppendorf tube 2ml and preserved after being labeled with deep freezing until further processed.

2.1 Polymerase Chain Reaction –Restriction Fragment Length Polymorphism (PCR-RFLP) for IL-17A G197A Genotyping

IL-17A G197A genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR– RFLP). Two primers were used to detect the polymorphism of SNP of IL-17A gene are shown in table (2-1).

Table (2-1) : The specific primers of gene *IL-17A G197A* gene

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	**5′- AACAAGTAAGAATGAAAAGAGGACATGGT-3′**	**64**	**69.45**	**102 base pair**
Reverse	**5′-CCCCCAATGAGGTCATAGAAGAATC -3′**	**70**	**75.05**	

2.2 Preparation of Primers for IL-17A G197A Gene

The primers were lyophilized, they dissolved in the free ddH2O to give a final concentration of 100 pmol/µl as stock solution and keep stock at -20 to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free ddH2O water to reach a final volume 100 µl. IDT (Integrated DNA Technologies company, Canada).

2.3 Restriction Fragment Length Polymorphism (RFLP) and Genotyping of IL-17A G197A Gene

IL-17A G197A genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR– RFLP). Two primers were used to detect the polymorphism of SNP of the IL-17A gene. Briefly, 0.5 µl of restriction enzyme EcoNI (BioLabs/USA) and 4.5 µl restriction buffers were added to the amplified product for a total volume of 10 µl. The digestion reactions were incubated at 65°C for 20 min. The digested products were visualized on 2 % gel electrophoresis approximately, Amplified PCR products of IL-17A G197A bands sizes were 102. The results of enzyme digestion produced numerous fragments of three unique types as shown in Figure (2-1).

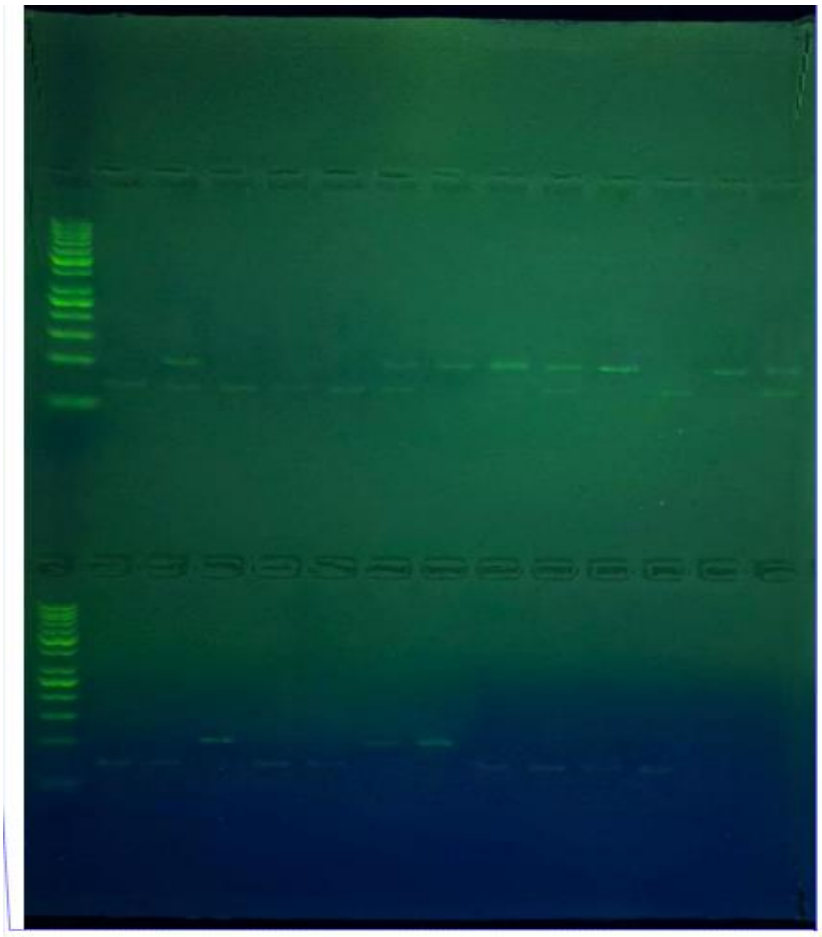

Figure (2-1) : "PCR-RFLP results of IL-17A genotyping,: 2% agarose at (5v\cm) for 1 hour. Amplified PCR products of each of IL-17A G197A bands sizes were 102bp".

3 Results

3.1 Serum Levels of IL-17A According to IL-17A G197A Genotypes

Serum levels of Interleukin-17A according to IL-17A G197A genotypes are shown in Table (3-1). T2DM patients with AA and AG genotypes showed higher levels of IL-17A than that with GG genotype (AA, 25.25476±5.077568, AG25.35817±7.91366 0and GG17.37900±1.655300, $P \leq 0.05$. There is a clear increase in the levels of this interleukin among patients with type 2 diabetes whom have the genotype AG, and this

result mentioned result demonstrated the association of the genotype with the predisposition to disease, as this genotype increases the possibility of developing type 2 diabetes. The patients with the GG genotype showed the lowest level of this interleukin among T2DM patients, as this genotype does not affect the degree of predisposition to the disease.

Table (3-1): Serum levels of IL-17A according to IL-17A G197A genotypes

Parameters / Groups	Pg/ml Mean±SD		
	AA	AG	GG
Control	16.351±0.814105	11.52900 ± 0	14.71850± 1.855770
T2DM patients	25.25476±5.077568	25.35817±7.913660	17.37900±1.6553*
P-value	0.246	0.706	0.433
LSD	1.46	0.369	1.3

NS :Non-significant P> 0.05
SD: Standard deviation
p < 0.05

4 Discussion

IL-17A polymorphisms have been linked to susceptibility to a variety of proinflammatory illnesses including gastric cancer[9][10]. In addition, it was shown that knowing the population structure of this gene was uniquely advantageous for disease prognosis and treatment[11]. The distribution frequencies of IL-17AG197A genotypes and alleles were not statistically significant among T2DM patients and healthy controls, according to the findings of this study. According to[12], there was no statistically significant difference in IL-17A polymorphism between Indian type 2 diabetic patients and controls. In contrast to[13],the present investigation found that "the frequency of the A allele of the IL-17A (rs2275913) gene polymorphism" was considerably greater in diabetic nephropathy patients than in healthy controls. In additional Iraqi research of asthmatic patients, the allele and genotype frequencies of rs2275913 differed significantly between asthmatic patients and healthy controls[14]. The association analysis of IL-17AG197A gene polymorphism with T2DM in the current study displayed that the heterozygous AG genotype of IL-17AG197A showed a risk association among T2DM and controls with OR=1.24 and the G allele was associated with an increased risk ofT2DM. During the clinical phases of other disorders, the rs2275913 polymorphism has been

examined by multiple research in a variety of contexts. Previous research has demonstrated a link between the rs2275913 polymorphism of the IL-17A gene and the inflammatory bowel disease known as ulcerative colitis in both the Korean and Japanese populations[15][16][17]. Showed that the rs2275913 polymorphism of the IL-17A gene was significantly associated with tubulitis, thickening of the arterial hyaline and increased mesenchymal matrix[17].In addition, the IL-17-related polymorphisms in renal disorders, such as diabetic nephropathy, have been the subject of investigation in a few publications. It has been demonstrated that the "Th1/Th2/Th17/Treg paradigm is skewed toward Th1 and Th17" in type 2 diabetic nephropathy patients, which is also in tight association with urine albumin to creatinine ratio as the typical clinical marker in determining the severity of diabetic nephropathy. These alterations may have a role in the increased immune response and inflammation, as well as the subsequent development and progression of type 2 diabetic nephropathy[17]. According to the results of these studies, IL-17A was found to play a substantial part in the pathogenesis of type 2 diabetes. The results of this analysis showed that there is a connection between this polymorphism and the risk of developing type 2 diabetes. IL-17a is one of the cytokines that has been examined concerning the development of diabetes. The levels of this interleukin were elevated in the sera of patients with type 2 diabetes compared to those of healthy controls, although there were no statistically significant changes, which are typically attributable to sample size variations. These findings are consistent with the study of[18] and who studied the level of interleukin-17 in Indian populations with T2DM. The results also are in agreement with[19]who showed an increase in the level of IL-17A in newly diagnosed diabetes than the healthy controls. Based on the results of the current study and previous studies, interleukin 17 may have a role in the inflammatory process of type 2 diabetes, and therefore it also has a significant impact on its pathogenesis. The exact role of IL-17A in the pathogenesis of type 2 DM has not been explored. IL-17A is capable of inducing the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, chemokines, and adhesion molecules, which mediate tissue infiltration and tissue destruction. In addition to stimulating the secretion of other pro-inflammatory cytokines, IL-17 is involved in the induction of potentially harmful mediators of inflammation, such as free radical nitric oxide[20]. Recent studies have described an ongoing process of β-cell destruction by apoptosis in animal models of type 2 diabetes and human T2DM[21].Obesity is regarded as the primary environmental cause of insulin resistance and type 2 diabetes. Asians are obese if their BMI is greater than 25 kg/m2.

Moreover, serum IL-17A levels among T2DM patients and controls, and the asso-ciation between rs2275913 polymorphism and serum IL-17A levels were investigat-ed. T2DM patients with AA and AG genotypes showed higher levels of IL-17A than that with GG genotypes. These results showed a clear agreement with the predisposi-tion to developing type 2 diabetes according to each genotype. These results indicate that the rs2275913 polymorphism may serve as a risk factor for predicting type2 DM risk. To our knowledge, there are no studies that investigated the relationship between IL-17 levels for each genotype in patients with type 2 diabetes. However, Several possibilities should be taken into account to explain the controversy between IL-17A polymorphisms and susceptibility to different types of human diseases. As for the inconsistency

between IL-17A polymorphisms and the risk of different human diseases in diverse cohorts of population, it has been well accepted that genetic polymorphisms usually have distinct effects on different types of human diseases, especially in diverse ethnic groups. Although the results of the present study are promising, several limitations remain to be a consideration. The important one, the relatively small sample size of the present study may result in insufficient statistical power to detect the relationship between IL-17A levels and T2DM risk[22].

5 Conclusion

Polymorphism of the IL-17A (rs2275913) gene can be associated with the serum levels of IL-17A among T2DM patients.

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