Immunopathologia Persa
http www.immunopathol.com
DOI:10.34172/ipp.2022.29320

IL-17A rs2275913 gene polymorphism in patients with diabetic nephropathy

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Received 1 Nov. 2021
Accepted 28 Dec. 2021
Published online 24 Apr. 2022

Keywords: Diabetes type 2, Gene polymorphism, Interleukin-17 A gene, Kidney disease, Diabetic nephropathy

Abstract

Introduction: Diabetic nephropathy (DN) is a major microvascular complication of diabetes, leading to end-stage renal disease (ESRD).
Objectives: The aim of this study was to investigate the association between the rs2275913 polymorphism of interleukin-17 (IL-17) gene and the incidence of kidney disease in patients with type 2 diabetes (T2D).
Patients and Methods: Blood samples were collected from 113 T2D patients including 56 patients with nephropathy and 57 patients without nephropathy. In addition, 150 healthy individuals were included in this study to compare the results. Gene study was conducted by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) method.
Results: The frequency of A allele of IL-17A (rs2275913) gene polymorphism was significantly higher in patients with DN compared to healthy controls (P=0.043). In addition, serum creatinine levels were significantly higher in DN patients regardless of their genotypes (*P*<0.001).
Conclusion: Our results showed that diabetic patients who carry at least one A allele of IL-17A (rs2275913) gene polymorphism may develop DN.

Citation: Nazarian A, Hejazian SM, Ahmadian E, Zununi Vahed S, Haghi M, Mobasseri M, Ardalan M. IL-17A rs2275913 gene polymorphism in patients with diabetic nephropathy. Immunopathol Persa. 2022;x(x):e29320. DOI:10.34172/ipp.2022.29320.

Introduction

Diabetic nephropathy (DN) is a main microvascular complication of diabetic patients. It is estimated that up to 30% of patients with diabetes (type 1 and type 2) can develop the DN (1,2). It has been evidenced that chronic inflammation is participated in the pathogenesis and the progression of the DN, where the essential role of the cytokine network in the modulation of immune responses has been identified. Several factors, including infectious agents, hormonal conditions and cytokine genetic polymorphism regulate the expression of cytokine-related genes (3,4). The interleukin-17 (IL-17) is a pro-inflammatory factor that plays a significant role in the pathogenesis of the DN by mediating mesangial expansion, podocyte damage, and kidney fibrosis; highlighting the complexity of immune mechanisms in the DN (5). However, studies exert a paradoxical role for IL-17 in the progression of the DN (6).

The IL-17A, a pleiotropic pro-inflammatory cytokine, is participated in the disease processes by stimulating the expression of cytokines and matrix metalloproteases. This cytokine is not only produced by T helper 17 cells (Th17) but also secreted by other immune cells. Through the IL-17A receptors (IL-17RC/IL-17RA), numerous intracellular signals are activated. The IL-17A signaling triggers the nuclear factor-kappa B (NF-kappa B) cascade, resulting in transcription of pro-fibrotic [fibronectin and transforming growth factor beta (TGF-β)], chemokines [CXCL2 (C-X-C motif chemokine ligand 2) and C–C motif chemokine ligand 2 (CCL2)] and the pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6) genes (7). Beyond plasma/urinary levels of IL-17 and T follicular helper

Key point

Diabetic nephropathy is a major microvascular complication of diabetes, leading to end-stage renal disease. Interleukin-17 plays a significant role in the pathogenesis of the diabetic nephropathy. This study indicated that diabetic patients who carry A allele of rs2275913 gene polymorphism (homozygous AA genotype) had kidney complication.

$CD4^+$ $PD\text{-}1^+$ (programmed cell death ligand 1) $CXCR5^+$ (C-X-C chemokine receptor type 5) cells, in patients with the DN (8), renal infiltrated immune cells play central roles. Several kidney resident cells express IL-17A receptors and can respond to the increased IL-17 level that is locally produced by infiltrated Th17 cells in the diabetic kidney (9).

Objectives

Under diabetic conditions, kidney-produced pro-inflammatory mediators are contributed to constant inflammation and progress to kidney injury, resulting in tubulointerstitial fibrosis. Further, the IL-17A can trigger other mechanisms, such as redox processes and protein kinases (10,11), as the mechanisms of which have not been revealed in renal damage. Moreover, IL-17 increases the expression of its receptor on mesangial cells leading to kidney leukocyte recruitment and expansion of the mesangial matrix (12,13). In this context, several experimental and human studies have confirmed the involvement of IL-17 signaling in the progression of the DN. The current study evaluated the rs2275913 polymorphism of the IL-17A gene in patients with DN.

Patients and Methods

Subjects

In this cross-sectional study, 113 patients with type 2 diabetes including 56 patients with DN and 57 patients without DN along with 150 healthy individuals were enrolled. Patients with the age range of 20-60 years old with proteinuria and hypoalbuminemia were included. Healthy urinary tract (no history of urinary obstruction, no active urinary tract infection, urinary catheter and ureteral catheter) and no history of other chronic diseases were the inclusion criteria in this study. Accordingly, patients with cardiovascular diseases, any inflammation, or any kind of malignancy were excluded from this study. Clinical and laboratory data of all patients including serum creatinine and hemoglobin A1c (HbA1c) levels were collected during the study.

DNA extraction and amplification

Two milliliters of blood sample was collected and DNA extraction was conducted using magnetic nanoparticles manufactured by ZiAViZ Company (Tabriz, Iran). In brief, 200 µL of blood was completely centrifuged with 1 ml of lysis buffer at 1000 rpm for two minutes and then, 500 µL of binding buffer was added to the sediment and incubated at 60˚C for dissolving. After that, nanoparticles (200 µL) were added to the samples and placed in a magnetic rack to collect the nanoparticles containing DNA and the sediment was separately washed twice with 500 µl wash one buffer and 500 µL wash two buffers and then dried in magnetic rack. Then, the samples were incubated at 60˚C for five minutes with 100 µL of Elution buffer. The prepared DNA solution was placed at -20˚C until use. DNA samples were amplified in a 25-µL PCR reaction solution employing specific primers that were designed using OLIGO7 software. The allele G forward primer was 5′-CTTGGCATTTTCCCTCAGAAGG-3′, allele A reverse primer was 5′-ATGCCCACGGTCCAGAAATAC-3′ and common reverse primer was 5′-CTTGGCATTTTCCCTCAGAAGA-3′. Polymerase chain reaction (PCR) reaction was conducted at a temperature program of 94˚C for two minutes and then 30 cycles in 94˚C for 30 seconds, 57˚C for 30 seconds and 72˚C for 40 seconds. Finally, the samples were incubated for 5 minutes at 72˚C.

Data analysis

SPSS software version 23 was utilized to analysis of data. The Shapiro-Wilk test was applied to check the normality of data. The studied quantitative variables were reported as mean ± standard deviation (SD). Independent *t* test was applied for analyzing data. In the analysis of the results, *P* values less than 0.05 were considered as statistically significant.

Results

During this study, 113 diabetic patients (56 with nephropathy and 57 without nephropathy) with mean age of 64.27 ± 7.52 years old were included; 41.6% of them were men (n=47). Serum creatinine (Cr) and HbA1c levels were 0.97 ± 0.25 mg/dL and 7.84 ± 1.69%, respectively. In addition, 150 healthy individuals were enrolled as normal controls with the mean age of 59.48 ± 5.97 years and the HbA1c level of 5.07 ± 0.139%. The mean age of DN and non-nephropathy diabetes mellitus (DM) patients were 64.02 and 64.51 years, respectively. There was no statistically significant differences in serum levels of HbA1c, Cr and age between the two nephropathy and non-nephropathy diabetic groups (Table 1).

The allele and genotype frequencies of the IL-17A rs2275913 polymorphism were compared between the studied groups with Fisher's exact test. A statistically significant difference between the presence of nephropathy and IL-17A rs2275913 gene polymorphism in comparison to healthy individuals was detected ($P=0.014$). The frequency of the A allele of the rs2275913 polymorphism was 21.3%, 18.42% and 13% in DN, DM and also healthy individuals, respectively ($P=0.043$). Moreover, the rs2275913 AA genotype frequency was higher in the DN group when compared to the non-nephropathy diabetic (11.1 versus 5.3, $P=0.45$) and healthy control (11.1 versus 4.7, $P=0.538$) groups. The observed results indicated that AG and AA genotype frequencies were not statistically significant neither between the DN and non-nephropathy diabetic groups ($P=1$) nor DN and healthy controls ($P=0.091$). No differences were observed in frequency of the three genotypes (GG, AG and AA) among DN and non-nephropathy diabetic patients (Figure 1).

Serum levels of the HbA1c were compared with different

Table 1. Demographic information of the studied groups

Parameters (Mean± SD)	DN patients (n= 56)	DM patients (n= 57)	*P* value*	HC (n= 150)	*P* value**
Male/Female	22/34	25/32	0.626	39/111	0.156
Age (years)	64.02±7.15	64.51±7.93	0.73	59.48±5.97	0.001
Serum Cr (mg/dL)	1.01±0.32	0.94±0.149	0.114	0.81±0.15	<0.001
HbA1c (%)	8.15±1.9	7.54±1.41	0.055	5.07±0.139	<0.001

SD: Standard deviation; Cr: Creatinine; DN: diabetic nephropathy; DM: diabetes mellitus; HbA1c: Hemoglobin A1c; HC: healthy control.
Variables were assessed via independent *t* test and $P < 0.05$ was considered as a significant result.
* DN versus DM patients.
** DN patients versus HC.

rs2275913 genotypes, the difference was not statistically significant ($P=0.306$). In evaluating diabetic patients with AA or AG genotype, DN patients has higher levels of serum Cr compared to non-nephropathy diabetic patients ($P<0.001$). However, no statistically difference was found between serum Cr levels of DN patients with the different genotype distribution of IL-17A rs2275913 ($P=0.535$; Table 2).

Discussion

According to the results of the current study, the frequencies of the allele A and homozygous genotype AA of the IL-17A rs2275913 gene polymorphism were higher in diabetic patients with nephropathy than healthy individuals. These values were not significant in later comparison between the DN and DM groups.

Different studies have investigated the rs2275913 polymorphism during the clinical courses of other diseases. Previous studies have shown a positive correlation between the rs2275913 polymorphism of the IL-17A gene and ulcerative colitis in Korean and Japanese populations (14, 15). Domanski et al showed that the rs2275913 polymorphism of the IL-17A gene was significantly associated with tubulitis, thickening of the arterial hyaline and increased mesenchymal matrix (16). In addition, some studies have investigated the IL-17-related polymorphisms in renal diseases including DN. It has been shown that the Th1/Th2/Th17/Treg paradigm skews to Th1 and Th17 in T2DN patients which is additionally in close correlation with urine albumin: creatinine ratio as the common clinical marker in assessing the severity of DN. These changes might contribute to the increased immune response and inflammation and consequent development and progression of type 2 DN (T2DN) (16). Coto et al showed an association between rs4819554 polymorphism in the IL 17 receptor A (IL17RA) promoter region and renal dysfunction. Therefore, it can be concluded that rs4819554 polymorphism can be employed as an early marker of kidney disease (17). Kim et al suggested that genetic alterations in IL-17E and IL-17R were associated with an increased risk of end-stage renal disease (ESRD). Thus, the inflammatory cascade may play a major role in the pathogenesis of the ESRD. However, the presence of rs4819554 polymorphism of the IL17RA gene did not play a statistically significant role in increasing the risk of ESRD (18). Examination of rs2275913 polymorphism in DN disease showed delayed graft function and significant long-term impairment of renal allograft function in cases with rs2275913 IL-17A GG genotype. Higher risk of allograft function loss and return to post-transplantation dialysis may be related to GA genotype rs11465553 polymorphism of IL-17F gene and with rs763780 polymorphism of IL-17F gene (19,20). In addition, a correlation between the rs763780 polymorphism of the IL-17F gene and the progression of DM after transplantation was shown. This finding demonstrates the prominent role of IL-17A in the pathogenesis of DM and the modulation of the inflammatory system (21). Another study found that polymorphisms in IL-17A and IL-17F genes play a significant role in rheumatoid arthritis (22). In a recent

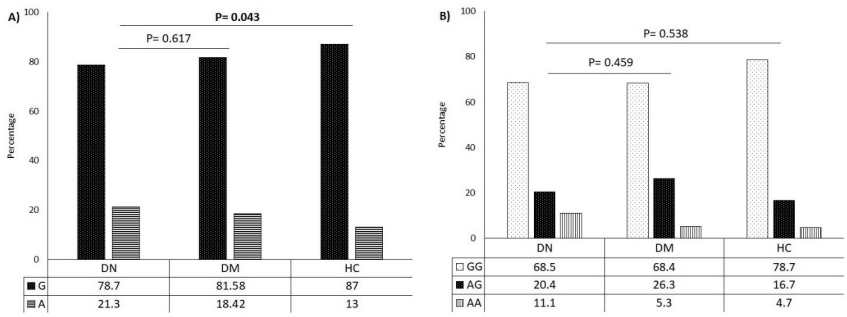

Figure 1. Frequencies of IL-17A (rs2275913) genotypes and alleles in the assessed groups. Variables were assessed via independent t-test and $P< 0.05$ was statistically considered significant. Allele frequency was calculated by https://wpcalc.com/en/equilibrium-hardy-weinberg. DN: diabetic nephropathy, DM: diabetes mellitus, HC: healthy controls.

Table 2. Association between IL-17A (rs2275913) genotypes and serum creatinine among diabetic patients

Groups	Serum creatinine level (mean± standard deviation)				*P* value
	GG	AG	AA	Total	
DN patients	1±0.28	1.12±0.52	0.95±0.22	1.0±0.43	0.535[a]
DM patients	0.92±0.14	0.99±0.15	0.88±0.02	0.94±0.14	**<0.001**[b]
P value[c]	<0.001	<0.001	<0.001	<0.001	

* Variables were assessed via independent t-test and P<0.05 was statistically considered significant. DN: diabetic nephropathy, DM: diabetes mellitus, HC: healthy control.

[a] Comparison of serum Cr levels in DN patients with different IL-17R (rs2275913) genotypes.

[b] Comparison of serum Cr levels with different IL-17R (rs2275913) genotypes of DN versus DM patients.

[c] Comparison serum Cr levels between DN and DM groups with different IL-17R (rs2275913) genotypes.

study investigating the frequency of IL-17A rs2275913 polymorphism among 70 patients with renal insufficiency, no statistically significant difference between the frequency of AG genotype of these patients and 30 healthy individuals as a control group was found. In other words, the presence of rs2275913 polymorphism is significantly associated with renal failure (23). In addition, no significant difference in IL-17A genotype and allele frequencies have been observed between healthy individuals and patients with renal diseases (24).

Conclusion

The nephropathic complications of T2D are very complex and are related to several genetic and environmental factors. It can be concluded that the diabetic patients who carry A allele of rs2275913 gene polymorphism (homozygous AA genotype) had kidney complication.

Limitations of the study

This study had some limitations. In fact, the low sample size could affect the obtained results and it is suggested to investigate IL-17A polymorphisms along with other related polymorphisms in future studies. In addition, it is better to evaluate serum levels of inflammatory factors to determine if the presence of these polymorphism could effect on nephropathy occurrence in diabetic patients or not.

Authors' contribution

MM, MA and SZV designed the study and prepared the final version of the manuscript. EA and JS prepared the draft manuscript. SMH analyzed the data. AN collected samples. MH performed experimental sections. All authors read and signed the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

Informed consent was obtained from all included individuals. All obtained information remained completely confidential. The study was approved by ethics committee of Tabriz university of medical sciences (Ethical code#IR.TBZMED.REC.1397.331). This study was extracted from residency thesis of Akbar Nazarian at the deparment of internal medicine at this university (Thesis#60737). Accordingly, the authors completely have observed the ethical issues including data fabrication, falsification, plagiarism, double publication misconduct, or submission and redundancy.

Funding/Support

This study financially was supported by the Kidney Research Center at Tabriz University of Medical Sciences, Tabriz, Iran (Grant #60737).

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