

Interleukin-4 Gene Polymorphisms in Type 2 Diabetic Patients With Nephropathy

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Introduction. The impact of several environmental and genetic factors on diabetes mellitus and its complications is well documented. It has also been established that cytokines play key roles in the pathogenesis of nephropathy. Polymorphisms of the -590 region of interleukin (IL)-4 are associated with the regulation of expression of this gene. In this study, we aimed to find polymorphisms of this region in nephropathic type 2 diabetic patients.

Materials and Methods. Peripheral blood samples were collected from 100 type 2 diabetic patients with nephropathy and 150 healthy controls. DNA was extracted and a polymerase chain reaction-restricted fragment length polymorphism technique was performed to examine polymorphisms in the -590 region of the IL-4 gene.

Results. Our results showed a significant difference between the C/C, T/C, and T/T genotypes and the C and T alleles of the -590 region of IL-4 in nephropathic patients in comparison with the healthy controls.

Conclusions. Results of this study suggest that the functional gene polymorphisms of IL-4 play an important role in the pathogenesis of diabetic nephropathy in patients with type 2 diabetes mellitus.

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INTRODUCTION

The prevalence of diabetes mellitus (DM) is increasing globally and it is expected that this latent disorder will affect 200 000 000 people in 2010 and 300 000 000 in 2025.¹ Type 2 DM is the most prevalent type.² Recent studies have shown that several genetic and environmental parameters are associated with type 2 DM.³ It has been suggested that DM is an immune-dependent disease in which the pattern of cytokine expression are changed.⁴ As an example, in type 2 DM, peripheral blood monocytes produce inflammatory cytokines.⁵ Cytokines and the cytokine-receptor axis are the subject of several recent studies for their crucial roles in DM and its complications.³ The important role of cytokine imbalance in type 2 DM with and without nephropathy has been reported.^{6,7} Increased serum levels of inflammatory cytokines,

including interleukin (IL)-18, IL-6, interferon- γ , IL-17, IL-12, and tumor necrosis factor- α , are documented in type 2 DM and its nephropathic complications.⁷⁻⁹

The association of IL-4 in immunological disorders such as multiple sclerosis, systemic lupus erythematosus (SLE), nephrotic syndrome, graft rejection, asthma, and type 1 and 2 DM is well established.¹⁰⁻¹⁷ The key roles of IL-4 as an inhibitory cytokine of autoimmunity and inflammations raise questions concerning the impacts of this cytokine on the pathogenesis of some diseases including nephropathic type 2 DM.¹⁸ Previous studies showed that secretion of IL-4 can be affected by its polymorphisms in -590 region.^{17,19} Our previous study showed that the mentioned polymorphisms are not associated with type 2 DM without nephropathy,¹⁷ but their

relation with nephropathic type 2 DM was not studied. Therefore, this study was designed to investigate the relation between the polymorphisms in the -590 region of IL-4 in type 2 diabetic patients with nephropathy.

MATERIALS AND METHODS

Subjects

Peripheral blood samples were collected from 100 patients with type 2 DM showing nephropathic complications and 150 healthy controls. The diabetic patients were selected based on peripheral blood glucose levels higher than 130 mg/dL, proteinuria of at least 500 mg/24 h, and a glomerular filtration rate (GFR) less than 25 mL/min/73 m² calculated based on creatinine-based equations.^{20,21} Information about serum lipid and glucose levels, proteinuria, estimated GFR, and drug therapy of the patients are listed in Table 1. The patients and the control group were selected within Rafsanjan population matched for demographic characteristics including sex and age (Table 1). The ethical approval of this study was granted by the Ethical Committee of Rafsanjan University of Medical Sciences. A written consent form was filled out by both patients and controls, and their

Table 1. Characteristics of Type 2 Diabetic Patient With Nephropathy and Healthy Controls*

Variable	Diabetic Patients	Healthy control
Age, y	40 ± 6	40 ± 7
Sex		
Female	62 (62.0)	90 (60.0)
Male	38 (38.0)	60 (40.0)
Weight, kg	50 ± 9	60 ± 7
Duration of diabetes	10 ± 4	...
Economic status		
Low	24 (24.0)	31 (20.7)
Medium	46 (46.0)	72 (48.0)
High	30 (30.0)	47 (31.0)
Drug therapy	Insulin	...
Serum triglyceride, mg/dL	350 ± 12	100 ± 4
Serum cholesterol, mg/dL	290 ± 10	150 ± 6
Serum HDL, mg/dL	24 ± 2	40 ± 3
Serum LDL, mg/dL	180 ± 11	100 ± 9
Blood glucose, mg/dL	230 ± 40	90 ± 15
Proteinuria, mg/dL	899 ± 50†	25 ± 1†
Estimated GFR, mL/min	72 ± 3‡	120 ± 5‡

*Ellipses indicate not applicable. Values in parentheses are percents. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and GFR, glomerular filtration rate.

†P = .002

‡P < .001

blood collection was performed. Previous studies have shown that some factors such as infections, allergic conditions, and smoking are considered as confounders in DM and nephropathy²²⁻²⁷; hence, patients with these factors were excluded from the study.

Assays

Fasting blood glucose, urine albumin level, blood pressure, and clinical presentations were assessed for each participant.

DNA Extraction

Peripheral blood was collected on ethylenediamine tetra-acetic acid-precoated tubes, and then, genomic DNA was extracted by a commercial kit (Bioneer, Seoul, South Korea). The extracted DNA samples were stored at -20°C for further use.

Detection of Polymorphisms

Interleukin-4 gene polymorphism (-590 C/T) was analyzed by polymerase chain reaction (PCR)-restricted fragment length polymorphism method. The primers sequences were as following sense:

5'-TAAACTTGGGAGAACATGGT-3'

The antisense was as follows:

5'-TGGGGAAAGATAGAGTAATA-3'

The PCR reaction mixture was made up of addition of the following reagents to a 0.2-mL microcentrifuge tube on ice: 2.5-μL of Taq DNA polymerase buffer (10x), 0.5-μL of magnesium chloride (stock concentration, 1.5 mM), 0.5-μL of each dNTP (dATP, dCTP, dGTP, dTTP; stock concentration of 10 mM), 1-μL of each primer (forward and reverse, stock concentration of 25 ng/μL), and 1-μL of prepared DNA and sterile double-distilled water to a final volume of 25 μL. The PCR condition was an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of melting at 95°C for 50 seconds, annealing at 53°C for 50 seconds, and extension at 72°C for 45 seconds, with a final extension step of 5 minutes at 72°C, using thermal cycler (C1000, Bio-Rad, Hercules, California, USA). The PCR product of IL-4 (-590 C/T) was a 195-bp fragment and was digested with *Ava*II into 175-bp and 20-bp fragments. The digested products were run on a 2.5% agarose gel (Cinnagen, Tehran, Iran) and studied on a Chemi-Doc model XRS (Bio-Rad, Hercules, California, USA) after staining with ethidium bromide.

Statistical Analyses

Hardy-Weinberg equilibrium was assessed using genotype data. Allele and genotype frequencies were calculated in patients and healthy controls by direct gene counting. Statistical analyses of the differences between groups were determined by the chi-square test using the SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, Ill, USA). *P* values less than .05 were considered significant. The study power was also calculated for each allele and genotype.

RESULTS

Evaluation of the polymorphisms at -590 of IL-4 by *Ava*II restriction enzyme showed that the frequency of the C/C genotype was 69 (69.0%) in the patients and 108 (72.0%) in the controls. Our results also revealed that the frequency of the T/C genotype was 29 (29.0%) and 38 (25.3%) in the patients and controls, respectively. The frequency of the T/T genotype was 2 (2.0%) in the patients and 4 (2.7%) in the controls (Table 2). A significant difference between the two groups regarding genotypes was confirmed ($P < .001$).

The frequency of the C allele was 167 (83.5%) and 254 (84.6%) in the patients and controls, respectively. Thirty-three cases of the C allele (16.5%) were observed in the patients, while the frequency of this allele was 46 (15.4%) in the controls. Statistical analysis of alleles also displayed a significant difference between the patients and the controls ($P < .001$; Table 2).

DISCUSSION

The main etiological cause of type 2 DM and its inflammatory complications such as nephropathy has yet to be clarified. It seems that immune-related factors play important roles in the etiology and pathogenesis of type 2 DM and its associated renal

complications.^{7,17} The crucial role of the cytokines network in orientation of immune responses is documented.^{2,7} Several factors such as infectious agents, hormonal conditions, and cytokine gene polymorphisms regulate expression and secretion of cytokines.²⁸ Our findings indicated a significant difference between type 2 diabetic patients with nephropathy and healthy controls regarding genotypes and alleles of the -590 region of IL-4 gene. Our previous data revealed that there was no relation between these polymorphisms and type 2 diabetic patients without nephropathy¹⁷; therefore, based on the current and previous studies¹⁷ it can be concluded that the polymorphisms are associated with nephropathic complications rather than type 2 DM in our studied population (south-east Iranian patients).

To our knowledge this is the first study performed to evaluate the polymorphisms in the -590 region of IL-4 gene in nephropathic patients with type 2 DM; however, some studies have investigated these polymorphisms in type 1 and 2 DM without nephropathy and in nondiabetic nephropathies.^{17,29-31} For example, Ikeuchi and colleagues²⁹ and Parry and coworkers³² showed that the polymorphisms in the IL-4 gene are not associated with minimal change nephrotic syndrome. Mittal and Manchanda reported that these polymorphisms are related with susceptibility to end-stage renal disease.³³ Another study in a Japanese population showed that IL-4 polymorphisms could influence disease susceptibility and progression in immunoglobulin A nephropathy.³⁴

A significant relation between IL-4 polymorphisms and type 2 DM was reported by Bid and colleagues, in the north Indian population.³¹ A probable reason for the discrepancy between our previous results and those of Bid and colleagues could be that Indian populations are different in race and genetics from our studied population. Another study demonstrated that there were no significant differences in the IL-4 polymorphisms between patients with type 1 DM and healthy controls.³⁵

CONCLUSIONS

Overall, based on the results of these and the present study, it may be concluded that the polymorphisms in IL-4 can affect nephropathy in type 2 diabetic and nondiabetic^{33,34} patients. Nephropathic complications of type 2 DM are

Table 2. Polymorphisms of Interleukin-4 Gene in Nephropathic Type 2 Diabetic Patients and Controls*

Genetic Parameter	Diabetic Patients	Controls	<i>P</i>
Genotype			
C/C	69 (69.0)	108 (72.0)	
T/C	29 (29.0)	38 (25.3)	
T/T	2 (2.0)	4 (2.7)	< .001
Allele			
C	167 (83.5)	254 (84.6)	
T	33 (16.5)	46 (15.4)	< .001

*Values in parentheses are percents.

very complex that are associated with several environmental and genetic factors, and these aspects of the disease should be examined by further studies.

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CONFLICT OF INTEREST

None declared.

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