



Investigation of Interleukin 1 Alpha Gene Promoter Polymorphism in hemodialysis patients with arteriovenous fistula thrombosis

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ORIGINAL INVESTIGATION

ABSTRACT

Objective: Hemodialysis (HD) requires a well-functioning temporary or permanent vascular access. Arteriovenous fistula (AVF) is frequently used in chronic kidney disease (CKD) patients for vascular access. Cytokines direct inflammation and immune response. Inflammation is one of the prevalent risk factors in renal patients. Inflammatory cytokine gene polymorphisms have an effect on vascular access. Therefore, we aimed to investigate a cytokine gene, IL-1 α -889 C>T, promoter polymorphism in HD patients with and without AVF thrombosis.

Materials and Methods: Eighty-one HD patients and 62 healthy controls were enrolled in the study. Polymorphism was studied using a polymerase chain reaction and restriction fragment length polymorphism. The Mann-Whitney U test and Pearson chi-square analysis were used for statistical analysis.

Results: There was no significant difference between the groups. However, the wild genotype was found to be much higher in the group without thrombosis compared to the thrombosis group (59.1% versus 48.6%). This difference was not statistically significant. In the patients' group, the heterozygous genotype was found to be more prevalent (37.8% versus 27.3%).

Conclusions: Polymorphisms in the promoter region of the genes affect the transcriptional activity and also the gene products. According to our results, the IL-1 α -889 C>T gene promoter polymorphism is not associated with a risk of thrombosis in HD patients.

Keywords: Chronic kidney disease, cytokine, interleukin 1 alpha, arteriovenous fistula, thrombosis

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INTRODUCTION

The prevalence of end stage renal disease (ESRD) continues to increase worldwide (1, 2). Cardiovascular disease (CVD) is highly prevalent in dialysis patients and is major factor for mortality and morbidity (3, 4). Hemodialysis (HD) requires a well-functioning temporary or permanent vascular access (5). In ESRD, arteriovenous fistula (AVF) is preferred for creating vascular access over other types with respect to patency, morbidity, and mortality rates (6-8). Vascular access failure mostly depends on thrombotic factors leading to mortality and morbidity in HD patients (9-11). AVF and grafts are also at risk of infectious complications (5).

Cytokines mediate and regulate immunity, inflammation, and hematopoiesis (12). Therefore, it is not surprising that these molecules have been investigated in genetic association studies. Genetic changes associated with the construction and connections of these molecules are important changes affecting the immune response. Genetic polymorphism in cytokine genes can alter cytokine production, and some of these polymorphisms are single base changes. Others have been classified as dinucleotide repeats and microsatellites (13, 14). Mutant genotypes for high producer alleles are generally associated with high cytokine production, heterozygotes with intermediate production, and wild type for the low producer alleles with low cytokine production (14). Polymorphisms in the promoter region of the genes affect the level of transcription and translation. A number of methodologies, such as those based on polymerase chain reaction (PCR), are used for the detection of genetic variations (13). Inflammation is very common and a risk factor in atherosclerosis and CVDs and specifically in HD patients (15). Interleukin-1 (IL-1) is a potent proinflammatory cytokine associated with several chronic kidney diseases (CKDs) (16, 17).

The *IL-1* gene family is located in the 2q13 region, is flanked with 415 base pairs, and codes the *IL-1 α* , *IL-1 β* , and *IL-1RA* genes. IL-1 is the primary important mediator in the pathogenesis of many inflammatory and immunologically mediated diseases. A polymorphism (a nucleotide change from C to T) at position -889 of the promoter

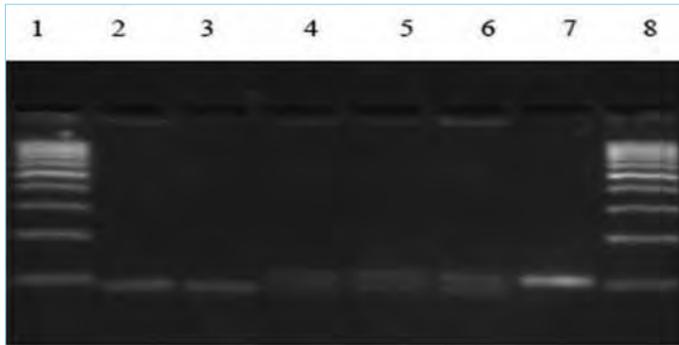


Figure 1. Image of agarose gel electrophoresis after *Nco* I enzyme digestion.

region of the *IL-1 α* gene has been reported, and this change suppresses the transcription of the *IL-1 α* gene (18, 19).

Genetic polymorphisms can contribute to vascular access failure due to thrombotic factors and endothelial dysfunction. However, the roles of inflammatory factors or immune system gene polymorphisms are not well understood in this condition. The role of pro- and anti-inflammatory cytokine gene polymorphisms as prognostic factors for vascular access failure in HD patients has not been clearly defined (5). In addition, *IL-1 α* gene polymorphisms (including promoter polymorphisms) have not been investigated as a risk factor for thrombosis. Therefore, with this study, we aimed to reveal the relationship of promoter polymorphism in the *IL-1 α* gene and AVF thrombosis in HD patients.

MATERIAL AND METHODS

Patient Selection

The *IL-1 α* gene -889 C>T promoter polymorphism was investigated in 81 HD patients and 62 healthy controls of similar ethnicity. The study was performed in accordance with the Declaration of Helsinki. The study protocol was approved by the local ethics committee in the Erciyes University Medical Faculty, and informed consent was obtained from all of the participants. All patients were chosen from the department of Nephrology and AVF was implemented in the department of Cardiovascular Surgery, Erciyes University. The fistula from the radial artery to the cephalic vein was created at the wrist. The anastomosis was constructed in a side-to-side fashion, by using a 6-0 or 7-0 monofilament suture. After a flow is established and a thrill is palpated in the proximal vein, the distal vein was ligated to pathway for venous hypertension. The patients were followed up for 8 weeks after the first puncture. The patients with more than one AVF creation were excluded from this study. In all patients, blood samples were obtained after the first puncture of AVF. After an 8-weeks observation period, 37 (14 females and 23 males) HD patients developed AVF thrombosis, which did not result from known factors such as hypotension. Forty-four (26 females and 18 males) patients did not develop AVF thrombosis, and 62 healthy controls (34 females and 28 males) were enrolled as a control group. Demographic, clinical, and biochemical features of the groups (age, gender, white blood cell (WBC) count, total cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides, blood urea nitrogen [BUN], and creatinine) were evaluated.

Laboratory Studies and Genotyping

Genomic DNA was extracted from 2 mL peripheral blood leukocytes using Magna Pure LC according to the manufacturers' instructions (Roche, Germany). All genotypes were identified using PCR-restriction fragment length polymorphism (RFLP) methods. A forward primer 5'-TTACATATGAGCCTTC-CATG-3' and a reverse primer 5'-AAGCTTGTCTACACCT-GAAGTAGGC-3' were used for *IL-1 α* -889 C>T promoter polymorphism. DNA samples from each patient and control were amplified in a 50 μ L reaction mixture volume, containing 200 ng of DNA, 2.5 mmol/L magnesium chloride ($MgCl_2$), deoxyribonucleotide triphosphates (dNTP) mix (2.5 mmol/L each), oligonucleotide primers (10 pmol each), and *Taq* DNA polymerase (2.5 U/ μ L). PCR was performed using the following profile: initial denaturation at 94°C for 5 min followed by denaturation at 94°C for 1 min, annealing at 62°C for 1 min, and extension at 72°C for 2 min for 35 cycles. The final extension was carried out at 72°C for 5 min. The PCR product of 108-bp was mixed with *Nco* I restriction enzyme and the reaction mixture was incubated overnight at 37°C. To detect different genotypes, 25 μ L of the digestion product was loaded into 3% agarose gel electrophoresis stained with ethidium bromide (Figure 1).

Statistical analysis

The Shapiro–Wilk's test was used to assess the data normality. To compare the differences between groups, one-way analysis of variance and Pearson chi-square analysis were used for continuous and categorical variables, respectively. The Mann–Whitney U test was used to compare nonparametric variables between the two groups. Values are expressed as frequencies and percentages and mean and standard deviation. A p value of <0.05 was considered statistically significant. The R 3.0.2 program (www.r-project.org) was used for statistical analysis.

RESULTS

Forty-four patients without AVF thrombosis (26 females and 18 males) and 37 patients with AVF thrombosis (14 females and 23 males) were enrolled in this study. Demographic and biochemical parameters of patients with AVF thrombosis were compared with those without AVF thrombosis, shown in Table 1. There were no significant differences between the two groups in terms of age, all lipid parameters, WBC count, and BUN and serum creatinine levels ($p>0.05$). Although the heterozygous genotype was higher in the patients with AVF thrombosis than in those without AVF thrombosis (37.8% versus 27.3%) and normal genotype was higher in the patients without AVF thrombosis than in those with AVF thrombosis (59.1% versus 48.6%), there was no statistically significant difference between the two groups in terms of *IL-1 α* -889 C>T ($p=0.576$). When we compared the groups with healthy control subjects, no significant difference was detected ($p=0.781$). Comparison of the genotypes among the three groups was summarized in Table 2. There was no significant difference between the three groups for the distribution of alleles according to gender ($p>0.05$), and these findings were summarized in Table 3.

Table 1. Comparison of clinical parameters of patients with and without thrombosis

	Patients with thrombosis (n=37)	Patients without thrombosis (n=44)	p
Age (years)	58±16	53±15	0.137
Gender			0.046
Female (%)	14 (37.8)	26 (59.1)	
Male (%)	23 (62.2)	18 (40.9)	
Total cholesterol (mg/dL)	181±46	198±40	0.093
Low-density lipoprotein (mg/dL)	98±30	101±38	0.670
High-density lipoprotein (mg/dL)	34±12	38±12	0.090
Triglyceride (mg/dL)	180 (46–1260)	190 (56–550)	0.253
White blood cell count (mm ³)	6450 (3350–19380)	6000 (1800–21920)	0.129
Blood urea nitrogen (mg/dL)	44±25	39±21	0.334
Serum creatinine (mg/dL)	5.2±2.7	4.4±3.6	0.266

Table 2. Comparisons of the genotypes among the groups

Polymorphism IL-1 α	Patients with thrombosis (n=37)	Patients without thrombosis (n=44)	Control (n=62)	p
CC	18 (48.6%)	26 (59.1%)	30 (48.4%)	0.781
CT	14 (37.8%)	12 (27.3%)	24 (38.7%)	
TT	5 (13.5%)	6 (13.6%)	8 (12.9%)	
C allele	0.68	0.73	0.68	
T allele	0.32	0.27	0.32	

Table 3. Distribution of alleles according to gender in patients with and without thrombosis and the control group

Gender/polymorphism IL-1 α -889 C>T	Patients with thrombosis (n=37)	Patients without thrombosis (n=44)	Control (n=62)	p
Male				
CC	13 (56.5%)	13 (72.2%)	16 (57.1%)	0.742
CT	8 (34.8%)	4 (22.2%)	8 (28.6%)	
TT	2 (8.7%)	1 (5.6%)	4 (14.3%)	
C allele	0.46	0.34	0.68	
T allele	0.16	0.07	0.32	
Female				
CC	5 (35.7%)	13 (50%)	14 (41.2%)	0.685
CT	6 (42.9%)	8 (30.8%)	16 (47.1%)	
TT	3 (21.4%)	5 (19.2%)	4 (11.8%)	
C allele	0.22	0.39	0.68	
T allele	0.16	0.20	0.32	

DISCUSSION

Cytokine production is controlled by several genes and is altered by polymorphisms in the regulatory regions of the genes encoding cytokines. An impaired immune response against infectious

agents in HD patients may be related to the overproduction of proinflammatory cytokines, such as IL-1 β , IL-6 and tumor necrosis factor (TNF)- α (14, 20). Inflammation is regulated by the genes of the *IL-1* cluster (21). In recent years, some studies have been

conducted in this field (22). In dialysis patients, plasma IL-6 level is a better inflammatory marker than other cytokine markers. However, the impact of the alteration of inflammatory markers on CV events in ESRD patients is not known (21). Therefore; it is of great importance to investigate different markers to overcome the lack of studies in this area. In a study with HD patients, it has been revealed that the levels of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, were significantly higher in HD patients than in healthy subjects and uremic patients who had not yet started on dialysis (20). Polymorphisms of -511C/T, -31C/T, and +3954C/T in the *IL-1β* gene were associated with inflammation in ESRD patients between the genders. Therefore, the authors suggested that these polymorphisms may have a prognostic utility for predicting inflammation in ESRD patients (23). In a study, *IL-1β* (promoter -511 and exon-5 +3953) genes and a variable number of tandem repeats (VNTR) in the *IL-1* receptor antagonist gene (*IL-1RA*) were investigated in ESRD patients. According to their results, these three polymorphisms within the IL-1 cluster are strongly associated with ESRD (23). Bensen et al. (24) evaluated polymorphisms in the *IL-1α* 3' untranslated region (UTR) for association with type 2 diabetes mellitus-associated (DM) and non-DM-associated ESRD in two ethnic groups. They found that a polymorphism in the 3'UTR of human *IL-1α* gene was associated with ESRD and *IL-1α* protein expression and concluded that the association of the TN7 (del-TTCA) A haplotype with higher levels of IL-1α expression and reduced risk for ESRD is consistent with involvement of cytokines in the risk for developing nephropathy. Goicoechea et al. (22) investigated the relationship between CV outcomes and variations of inflammatory markers, such as C-reactive protein (CRP), IL-6, IL-1β, and TNF-α in 90 ESRD patients who had not yet started dialysis and observed that IL-6 is a better inflammatory marker than CRP, TNF-α, and IL-1β in predicting CV events in such patients. In our previous study, *ACE* and *TNF-α* genes were investigated in vascular access failure. A G>A genotype in the *TNF-α* gene was statistically significant, and at least a four-fold high inflammation risk was determined between the groups of AVF thrombosis (25).

Controversial results were obtained in studies performed on interleukins. The impact of gene polymorphisms encoding donor or recipient *IL-1α*, *TNF-α*, or *IL-4* on acute rejection after renal transplantation has been investigated, and it was observed that there is no association between cytokine gene polymorphisms and the incidence of post-transplant acute rejection (19). Hahn et al. (26) investigated the association between single nucleotide polymorphisms (SNPs) of the *IL-1* gene cluster and childhood immunoglobulin A nephropathy (IgAN). SNPs in *IL-1α*, *IL-1β*, and *IL-1RA* genes were analyzed in 182 patients with IgAN and in 500 healthy controls. They found that *IL-1β* and *IL-1RN* genes were associated with increased susceptibility to IgAN. In a study performed by Buckham et al. (27), 664 kidney transplant recipients and 577 kidney donors were genotyped to determine whether common variants in the interleukin genes, including *IL-1α*, *IL-1β*, *IL-1RN*, *IL-6*, and *IL-10*, are associated with ESRD. SNP genotype data for each gene were downloaded for the European population. They found that SNPs in these five interleukin genes are not major risk factors for ESRD in white Europeans. In a recent study, polymorphisms of *IL-1α* C-889T, *IL-1β* C-511T, *IL-1β* C+3954T, and *IL-1RA* (intron 2) and transcript levels were investigated in a total of 246 individuals with CKD and periodontitis. They observed a possible

evidence for the association of *IL-1B* and *IL-1RA* alleles with susceptibility to ESRD and PD. But there were no significant differences according to *IL-1α* C-889T polymorphism (p=0.588) (28).

The present study investigated whether there is a relationship between *IL-1α* gene promoter polymorphism and AVF thrombosis in HD patients. Our results showed that there is no significant difference between patients with AVF thrombosis and those without AVF thrombosis in terms of *IL-1α* gene promoter polymorphism. According to our results, we suggest to investigate the other genes responsible for the production of cytokines and their polymorphisms in a larger sample size for evaluating whether there is an effect of the cytokine on the development of AVF thrombosis in HD patients.

CONCLUSION

Polymorphisms in the promoter region of the genes affect the transcriptional activity and also the gene products. According to our results, the *IL-1α* -889 C>T gene promoter polymorphism is not associated with a risk of thrombosis in HD patients.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Erciyes University Medical Faculty.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Conceived and designed the experiments or case: EFŞ., ST. Performed the experiments or case: EFŞ., ST. Analyzed the data: EFŞ., ST. Wrote the paper: ÖNE., AT., EFŞ., ST., AÜ., YÖ. All authors have read and approved the final manuscript.

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