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RESEARCH ARTICLE/KLİNİK ÇALIŞMA

FLORA 2024;29(1):105-115 • doi: 10.5578/flora.202401777

An Investigation on the Correlation Between Diabetic Foot Infection, Osteomyelitis, Venous Insufficiency, and IL-6 (-174 G>C) and TNF-α (-238 G>A) Gene Polymorphisms

Diyabetik Ayak İnfeksiyonu, Osteomiyelit, Venöz Yetmezlik ile IL-6 (-174 G>C) ve TNF-α (-238 G>A) Gen Polimorfizmleri Arasındaki İlişkinin İncelenmesi

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Cite this article as: *İnan Sarıkaya R, Parlak E, Yüce Kahraman Ç, Yılmaz S. An investigation on the correlation between diabetic foot infection, osteomyelitis, venous insufficiency, and IL-6 (-174 G>C) and TNF-α (-238 G>A) gene polymorphisms. FLORA 2024;29(1):105-115.*

ABSTRACT

Introduction: *This study aims to investigate the correlation between interleukin-6 (IL-6) (-174 G>C) and tumor necrosis factor-alpha (TNF-α) (-238 G>A) cytokine gene polymorphisms and diabetic foot infection (DFI).*

Materials and Methods: *This study involved the collection of blood samples from a total of 207 participants who were grouped as follows: a control group of 60 healthy individuals, a group of 60 patients with type 2 diabetes mellitus (T2DM) but without diabetic foot ulcer (DFU), and a group of 87 patients with DFI. The genotypes of IL-6 (-174 G>C) and TNF-α (-238 G>A) polymorphisms were determined using real-time PCR and the TaqMan method.*

Results: *The findings indicate that there were no statistically significant variations in the distribution of IL-6 (-174 G>C) and TNF-α (-238 G>A) polymorphisms genotypes among individuals with T2DM who had DFI, individuals with T2DM without DFU, and the control group (p> 0.05). A higher prevalence of osteomyelitis was detected in patients with DFI possessing the GG genotype (53.1%) of the IL-6 (-174 G>C) polymorphism, compared to those with the mutant genotypes (GC + CC) (28.9%) (p= 0.024). The study revealed a higher prevalence of venous insufficiency (VI) in individuals with DFI possessing TNF-α (-238 G>A) GA genotype compared to those with the GG genotype (80.0%, 24.4%, respectively) (p= 0.007). The study indicated that patients with DFI possessing the TNF-α (-238 G>A) GA genotype had a higher mean body mass index (BMI) compared to those with the GG genotype (p= 0.012).*

Conclusion: *The study has demonstrated that the IL-6 (-174 G>C) and TNF-α (-238 G>A) polymorphisms cannot serve as reliable indicators for determining an individual's vulnerability to DFI and T2DM. The IL-6 (-174 G>C) polymorphism's GG genotype exhibited a significant association with osteomyelitis in patients with DFI. Findings suggest that individuals with the TNF-α (-238 G>A) GA genotype in DFI patients may experience VI and higher average BMI than patients with the GG genotype.*

Key Words: *Diabetic foot infection; Interleukin-6; Osteomyelitis; Polymorphism; Tumor necrosis factor-alpha*

Received/Geliş Tarihi: 20/01/2023 - Accepted/Kabul Ediliş Tarihi: 12/10/2023

Available Online Date: 22.03.2024

ÖZ

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Giriş: *Bu çalışmada interlökin-6 (IL-6) (-174 G>C) ve tümör nekroz faktörü-alfa (TNF-α) (-238 G>A) sitokin gen polimorfizmlerinin diyabetik ayak infeksiyonu (DAİ) ile arasındaki ilişkiyi araştırmayı amaçladık.*

Materyal ve Metod: *Bu çalışmada 60 sağlıklı birey kontrol grubu, 60 diyabetik ayak ülseri (DAÜ) olmayan tip 2 diyabetes mellitus (T2DM)'lu hasta ve 87 DAİ ile izlenen T2DM'li hasta olmak üzere toplam 207 katılımcıdan alınan kanlardan real-time PZR ve TaqMan yöntemi ile IL-6 (-174 G>C) ve TNF-α (-238 G>A) polimorfizmlerinin genotipleri belirlendi.*

Bulgular: *IL-6 (-174 G>C) ve TNF-α (-238 G>A) polimorfizmleri genotip dağılımı DAİ tanısı olan T2DM'li hastalar, DAÜ tanısı olmayan T2DM'li hastalar ve kontrol grubu arasında farklılık göstermedi (p> 0.05). Diyabetik ayak infeksiyonu tanılı hastalarda IL-6 (-174 G>C) polimorfizmin GG genotipinde (%53.1), mutant genotiplere (GC + CC) kıyasla (%28.9) daha yüksek osteomiyelit sıklığı gözlendi (p= 0.024). TNF-α (-238 G>A) GA genotipi olan DAİ tanılı hastalarda venöz yetmezlik (VY) sıklığı GG genotipi olanlara göre (sırasıyla %80.0, %24.4) daha yüksek bulundu (p= 0.007). TNF-α (-238 G>A) GA genotipi olan DAİ tanılı hastalarda vücut kitle indeksi (VKİ) ortalaması GG genotipi olanlara göre daha yüksek bulundu (p= 0.012).*

Sonuç: *IL-6 (-174 G>C) ve TNF-α (-238 G>A) polimorfizmlerinin bir bireyin DAİ ve T2DM'ye yatkınlığını tahmin edemeyeceği gösterilmiştir. Diyabetik ayak infeksiyonu hastalarında IL-6 (-174 G>C) polimorfizminin GG genotipi osteomiyelit ile ilişkilendirildi. Diyabetik ayak infeksiyonu hastalarında TNF-α (-238 G>A) GA genotipinin GG genotipi olanlara göre VY sıklığı ve VKİ ortalaması yüksekliği için bir risk faktörü olabileceği ortaya çıkmıştır.*

Anahtar Kelimeler: *Diyabetik ayak infeksiyonu; İnterlökin-6; Osteomiyelit; Polimorfizm; Tümör nekroz faktör-alfa*

INTRODUCTION

Diabetic foot infection (DFI) often arises from the proliferation and infiltration of microorganisms into the soft tissue or bone. This process induces an inflammatory response in the host, leading to tissue damage in patients with diabetes mellitus[1]. Diabetic foot infection is usually the result of a pre-existing diabetic foot ulcer (DFU) or foot ulcer[2]. The etiology of DFI and DFU is complicated, including a combination of genetic and environmental factors. The wound healing process typically follows a sequential progression, beginning with a short inflammatory phase, followed by the proliferative phase, and ending with the remodeling phase, which is crucial to ensuring timely wound closure[3]. Chronic low-level inflammation and extended inflammatory response in the diabetic foot result in impairments in the process of wound healing. During the extended inflammatory phase, there is an apparent elevation in the levels of proinflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) which contribute to the process of wound healing[4]. The presence of compromised immune function in patients with type 2 diabetes mellitus (T2DM) results in reduced cell proliferation and poor wound-healing processes. Persistent inflammation results in prolonged infection[3]. Therefore, genetic, and microbiological factors and local cytokine response determine the severity and treatment outcome of DFI[5].

The involvement of single nucleotide polymorphisms (SNPs) and epigenetic processes in the etiopathogenesis of DFU is significant for growth factors and cytokines[6]. During the wound healing process of the

foot, immune cells exhibit overexpression of proinflammatory cytokines, including IL-1, IL-6, and TNF-α, as well as chemokines such as stromal cell-delivered factor-1 (SDF-1). Stromal cell-delivered factor-1 is a cytokine that is classified under the chemokine family. Chemokines are known to stimulate leukocytes and are frequently produced in response to proinflammatory triggers, such as lipopolysaccharide, TNF, or IL-1. These cytokines and chemokines regulate wound repair, which consists of inflammation, collagen synthesis, extracellular matrix formation, angiogenesis, and epithelialization[5,7]. Single nucleotide polymorphisms in cytokine and chemokine genes have a crucial role in the pathogenesis of DFUs by coordinating the three distinct stages of wound healing[7]. IL-6 (-174 G>C/rs1800795), TNF-α (-238 G>A/rs361525), and TNF-α (-308 G>A/rs1800629) are SNPs that are well characterized, previously shown to be genetically related to other diabetic complications[8-10]. In a study, an important correlation was observed between TNF-α SNP and a heightened susceptibility to high-grade ulcers. In the aforementioned study, there was no observed correlation between SNPs of IL-6 and TNF-α and DFU patients who suffered major amputation. However, it was revealed that a specific SNP of SDF-1 was linked to a heightened risk for amputation[5]. Nevertheless, there has been a lack of adequate research on the genetic aspects of DFU and DFI in comparison to retinopathy and nephropathy[11].

The most common factors contributing to delayed healing of DFU include neuropathy associated with diabetes, inadequate arterial perfusion, and venous insufficiency (VI). The relationship between diabetes and chronic venous insufficiency (CVI) is often observed, while it remains unclear if DFU is the causative factor for CVI[12]. The etiopathogenesis of CVI is influenced by several variables, including as age, gender, genetics, and a sedentary lifestyle. Recent studies have investigated the genetic predisposition to CVI by examining particular variations in DNA sequences, known as polymorphisms. Although these studies provide evidence of a genetic element in CVI, the precise genes accountable for this condition remain unknown[13,14]. Another objective of our study is to examine the correlation between VI, which is prevalent in diabetic foot, and IL-6 and TNF-α SNPs.

In genetic polymorphism studies, genotype prevalence differs by race and ethnicity. Genetic factors involved in the pathogenesis of DFI may contribute to population-specific differences in outcome. Our review of the literature revealed that the majority of studies focused on DFU and identified infection in a minority of patients[15]. Our study is distinct from others since all of the patients in it were diagnosed with DFI. In this study, the aim was to investigate the correlation between two SNPs, IL-6 (-174 G>C/rs1800795) and TNF-α (-238 G>A/rs361525), which have been implicated in the pathogenesis of diabetes and DFU, and the development of DFI in the Turkish population. Additionally, the study aimed to explore the classification of DFI and its impact on treatment results.

MATERIALS and METHODS

Study Population

This study includes a group of 207 patients who submitted applications to the Faculty of Medicine at Atatürk University throughout the year 2019-2020. The participants were categorized into three distinct groups for the study. The first group, referred to as group 1, consisted of 60 individuals who were in good health and had normal glucose tolerance (NGT), serving as the control group. Group 2 comprised 60 patients diagnosed with T2DM but without DFU. Lastly, group 3 consisted of 87 T2DM patients who were being monitored for DFI. All participants were Turkish citizens. The study received approval from the Ethics Committee (Approval date: 30/05/2019, decision number: 02). All participants provided written informed consent. The study was carried out in compliance with the Declaration of Helsinki.

Sample Size Calculation and Influence of the Study

In our study, the required sample size was calculated to be 157 for a confidence level of 95%, a power of 90%, and $\alpha = 0.05$.

Inclusion Criteria

The study comprised exclusively adult participants who had DFI, T2DM, and a regular blood count while excluding those with any chronic diseases.

Exclusion Criteria

Patients younger than 18 years of age, type 1 diabetes, patients in pregnancy and postpartum period, patients with chronic liver disease, cancer patients under treatment, patients with active infectious diseases other than DFI, and patients with acute or chronic inflammatory diseases were excluded.

Anthropometric Measurements, Clinical and Biochemical Parameters

A comprehensive medical history was collected from each participant. Data on age, gender, demographic parameters, duration of diabetes, presence of additional comorbidities, blood pressure levels, smoking status, usage of antidiabetic medications, and relevant clinical information were recorded. Standard procedures were employed to collect anthropometric data, including measurements of height, body weight, and body mass index (BMI). Elaborate descriptions of DFI were provided. The term "infection" was operationally defined as the presence of a minimum of two clinical symptoms, including local swelling, erythema, pain, local temperature rise, and purulent discharge[16]. The wounds of patients with DFI have been recorded using the PEDIS classification. The diagnosis of peripheral sensory neuropathy was established by the utilization of electroneuromyography, while the diagnoses of peripheral artery disease and VI were confirmed with Doppler ultrasonography. The diagnosis of osteomyelitis was made clinically or by imaging methods such as direct X-ray, magnetic resonance imaging, scintigraphy, or histopathology. Various biochemical tests were conducted to assess the complete blood count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), hemoglobin A1c (HbA1c), fasting blood sugar, total serum cholesterol, LDL cholesterol, blood urea nitrogen (BUN), and creatinine levels. The biochemical parameters were analyzed using the Roche Cobas 8000 autoanalyzer, while the total blood count was assessed using the Roche Sysmex result Xn 9000 autoanalyzer.

Genetic Analysis

Genomic DNA was extracted from blood samples by the Gene JET DNA Blood Mini Kit (ThermoFisher) protocols; the extracted DNA was quantified by a spectrophotometer (Maestro-Nano; Maestrogen) and stored at approximately -20°C until analysis. Genotypes were determined by real-time PCR (Rotor-Gene Q) with TaqMan™ Genotyping Master Mix and TaqMan SNP Genotyping Assay designed by ThermoFisher for rs1800795 (IL-6 -174 G>C) and rs361525 (TNF-α -238 G>A) SNPs. Amplification conditions were the same for both SNPs; denaturation at 95°C for 10 min, followed by 40 amplification cycles of 15 s at 95°C, and 60 s at 60°C. The allelic determination of the patients was determined by Rotor-Gene Q software.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS v20) software was used to analyze the research data. Categorical variables were presented as numbers and percentages, and numerical variables were presented as the mean and standard deviation. The Kolmogorov-Smirnov test, z values for skewness and kurtosis, and graphing methods were used to find out if the numerical variables fit the normal distribution. The T-test and Mann-Whitney U test were used when comparing numerical variables between two independent groups. ANOVA was used to analyze variance, and the Kruskal-Wallis test was used when necessary. Bonferroni corrected Mann-Whitney U tests were used in post hoc analyses, and χ^2 tests were used to investigate the relationships between categorical variables. The level of statistical significance was set at $p< 0.05$ in all analyses.

RESULTS

Clinical and Biochemical Characteristics of the Study Groups

Table 1 displays the demographic, clinical characteristics, and serum biomarkers of both the patient and control groups. There were notable disparities in gender representation, level of

Table 1. Demographic, anthropometric data, clinical characteristics and laboratory findings at the time of admission of the groups participating in the study

Clinical parameters	Healthy controls n (%)/mean ± SD	Diabetic patients without diabetic foot ulcer n (%)/mean ± SD/median (Q1-Q3)	Diabetic patients with diabetic foot infection n (%)/mean ± SD/median (Q1-Q3)	p
Gender (female/male) (number)	28/32	39/21	21/66	**<0.001**
Age (years)	42.3 ± 14.5	56.3 ± 14.0	60.0 ± 10.1	**<0.001**
Systolic blood pressure (mmHg)	106.5 ± 9.9	116 ± 10.7	119.7 ± 14.0	**<0.001**
Diastolic blood pressure (mmHg)	61. ± 6.7	70.3 ± 7.8	72.4 ± 9.9	**<0.001**
Diabetes duration (years)	-	10.3 ± 7.8	13.7 ± 6.5	**0.001**
Antidiabetic drugs				
OAD	-	16 (26.7)	14 (16.1)	
Insulin	-	18 (30.0)	52 (59 .8)	**0.002**
OAD + Insulin	-	26 (43.3)	21 (24.1)	
CRF	-	1 (1.7)	15 (17.2)	**0.003**
WBC (/mm^3)	-	7924.5 ± 1816.9	10876.3 ± 4851.5	**<0.001**
Hemoglobin (g/dL)	-	14.0 ± 2.0	12.1 ± 2.2	**<0.001**
Platelet (/mm^3)	-	267483.3 ± 80741.2	321126.4 ± 94144.6	**<0.001**
ESH (mm/h) (<15)	-	14.1 ± 15.6	61.0 ± 31.3	**<0.001**
Albumin (g/dL)	-	4.0 ± 0.4	3.0 ± 0.5	**<0.001**
Total-cholesterol (mg/dL) (<200)	-	201.9 ± 54.1	165.8 ± 46.4	**<0.001**
LDL-cholesterol (mg/dL) (<130)	-	131.5 ± 39.3	107.3 ± 31.4	**<0.001**
Urea (mg/dL)	-	15.4 (11.7-21.0)	26.0 (17.0-40.0)	**<0.001**
Creatinine (mg/dL) (0.5-0.9)	-	0.8 (0.7-0.9)	1.1 (0.8-1.5)	**<0.001**
CRP (mg/L) (0-5)	-	4.0 (3.0-9.0)	50.0 (14.0-129.0)	**<0.001**

SD: Standard deviation, n: Number of induviduals, BMI: Body mass index, OAD: Oral antidiabetic drug, CRF: Chronic renal failure, WBC: White blood cell, ESH: Eritrocyte sedimentation rate, HbA1c: Hemoglobin A1c, LDL: Low density lipoprotein, CRP: C-reactive protein.

education, and employment status among all three groups. Type 2 diabetes mellitus patients were substantially older and had higher systolic and diastolic blood pressures than NGT individuals. There was a statistically significant difference in duration of diabetes, white blood cell (WBC), hemoglobin, thrombocyte, total cholesterol, LDL-cholesterol, albumin, ESR averages, CRP, BUN, and creatinine between the patients with DFI and the group with only T2DM. 24 (27.6%) DFI patients had VI, and 44 (50.6%) patients had peripheral sensory neuropathy. The proportion of patients with PEDIS classification grades 2, 3, and 4 was as follows: 28 (32.2%), 54 (62.1%), and five (5.7%). Since our investigation included patients with infection, there were no grade 1 patients. Thirty-seven (42.5%) patients exhibited active osteomyelitis.

Interleukin-6 -174 G>C Gene Polymorphism

The study has revealed that the genotype and allele distributions of the IL-6 (-174 G>C) rs1800795 and TNF-α (-238 G>A) rs361525 SNPs were consistent with the expectations of the Hardy-Weinberg equation. Table 2 presents a comparison of the genotype and allele frequencies of IL-6 (-174 G>C) and TNF-α (-238 G>A) SNPs across the different study groups. IL-6 (-174 G>C) genotype distribution in diabetic patients with DFI (GG 56.3%, GC 32.2%, CC 11.5%), T2DM patients without DFU (GG 61.7%, GC 30.0%, CC 8.3%), and the control group (GG 56.7%,

Table 2. Genotype and allele distribution of IL-6 (-174 G>C) and TNF-α (-238 G>A) SNPs

Cytokine gene polymorphisms		Healthy controls (n= 60)	Diabetic patients (n= 147)		p
			Diabetic patients without diabetic foot ulcer (n= 60)	Diabetic patients with diabetic foot infection (n= 87)	
IL-6 (-174 G>C)	Genotype n (%)				
	GG	34 (56.7)	37 (61.7)	49 (56.3)	>0.05*
	GC	19 (31.7)	18 (30.0)	28 (32.2)	
	CC	7 (11.7)	5 (8.3)	10 (11.5)	
	Alleles n (%)				
	G	87 (72.5)	92 (76.6)	126 (72.4)	>0.05*
	C	33 (27.5)	28 (23.4)	48 (27.6)	
TNF-α (-238 G>A)	Genotype n (%)				
	GG	57 (95.0)	54 (90.0)	82 (94.3)	>0.05*
	GA	3 (5.0)	6 (10.0)	5 (5.7)	
	AA	-	-	-	
	Alleles n (%)				
	G	117 (97.5)	114 (95.0)	169 (97.1)	>0.05*
	A	3 (2.5)	6 (5.0)	5 (2.9)	

* Statistically significant difference wasn't determined between three groups (p> 0.05).

GC 31.7%, CC 11.5%) did not significantly differ (p> 0.05) (Table 2). There was no significant difference in the frequency of G alleles among diabetic patients with DFI, T2DM patients without DFU, and the control group. The frequencies of G alleles were 72.4%, 76.6%, and 72.5%, respectively (p> 0.05) (Table 2). IL-6 genotype distribution did not differ significantly between T2DM patients (GG 58.5%, GC 31.3%, CC 10.2%) and the control group (GG 56.7%, GC 31.7%, CC 11.7%) (p> 0.05). There was no significant difference in the frequency of G alleles between patients with T2DM and the control group (74.1% and 72.5%, respectively) (p> 0.05).

Tumor Necrosis Factor-alpha -238 G>A Gene Polymorphism

Genotype distribution of TNF-α (-238 G>A) diabetic patients with DFI (GG 94.3%, GA 5.7%), patients with T2DM without DFU (GG 90.0%, GA 10.0%), and the control group (GG 95.0%, GA 5.0%) did not differ significantly (p> 0.05) (Table 2). G allele frequency was similar between diabetic patients with DFI, T2DM patients without DFU, and the control group (97.1%, 95.0%, 97.5%, respectively) (p> 0.05) (Table 2). AA genotype was not detected in all three groups. TNF-α genotype distribution did not differ significantly between T2DM patients (GG 92.5%, GA 7.5%) and the control group (GG 95.0, GA 5.0%) (p> 0.05). The frequency of G alleles was similar between T2DM patients and the control group (96.2%, and 95.5%, respectively) (p> 0.05).

Genetic Association of the Immunogenotypes with Ulcer Grade in DFU

The findings have indicated that grade 3 had the highest level of dominance within the PEDIS classification for patients with DFI. In the IL-6 (-174 G>C) gene polymorphism, when the wild-type genotype (GG) and mutant genotypes (GC + CC) were compared using PEDIS classification grades, grade 2 revealed 53.6% GG and 46.4% GC + CC, while grade 3 revealed 53.7% GG and 46.3% GC + CC. The GG genotype was present in all grade 4 patients. In TNF-α (-238 G>A)

Table 3. The relationship of IL-6 (-174 G>C) and TNF-α (-238 G>A) genotype distributions with clinical findings in patients diagnosed with DFI

IL-6 (-174 G>C)			
	GG (mean ± SD)/ median (Q1-Q3)	**GC + CC (mean ± SD)/ median (Q1-Q3)**	**p**
Osteomyelitis (n)(%)	26 (53.1)	11 (28.9)	**0.024**
TNF-α (-238 G>A)			
	GG (mean ± SD)/ median (Q1-Q3)	**GA (mean ± SD)/ median (Q1-Q3)**	**p**
BMI (kg/m^2)	27.3 ± 3.4	31.7 ± 4.3	**0.012**
VI (n)(%)	20 (24.4)	4 (80.0)	**0.007**

SNP, grade 2 showed 89.3% GG, and 10.7% GA + AA genotypes, while grade 3 showed 96.3% GG and 3.7% GA + AA. The GG genotype was present in all grade 4 patients. In grade 4, neither SNP exhibited a mutant genotype.

Genetic Association of the Immunogenotypes with Surgical Treatment Methods in DFU

Debridement was conducted on 66 patients with DFI (75.9%), whereas amputation was carried out on 24 patients (27.6%). The wild-type genotype (GG) of IL-6 (-174 G>C) was observed in 59.1% of the debrided patients, whereas the mutant genotypes (GC + CC) were observed in 40.9% of the patients. Among the patients who underwent amputation, 66.7% exhibited the GG genotype, whereas 33.3% had the GC + CC genotype. There were no statistically significant differences seen in the surgical treatment methods between the distributions of IL-6 (-174 G>C) in the wild-type and mutant genotypes (p> 0.05). The wild-type genotype (GG) of TNF-α (-238 G>A) was observed in 97.0% of the debrided patients, whereas the mutant genotypes (GA + AA) were present in 3.0% of the patients. All amputated patients had the GG genotype, while no patients with a mutant genotype were identified.

Correlation Between IL-6 (-174 G>C) and TNF-α (-238 G>A) Genotypes and the Clinical Characteristics of Subjects

Subsequently, an investigation was conducted to analyze the correlation between demographic characteristics, anthropometric measurements, clinical observations, and laboratory results based on the distribution of the wild-type and mutant genotypes of IL-6 and TNF-α in diabetic patients with DFI. In patients with DFI, the IL-6 (-174 G>C) GG genotype was associated with a substantially higher incidence of osteomyelitis than the mutant genotypes (GC + CC) (p= 0.024). The incidence of VI was notably greater in patients with DFI who possessed the TNF-α (-238 G>A) GA genotype compared to those with the GG genotype (80.0% vs. 24.4%, respectively) (p= 0.007). In patients with DFI, the mean BMI was significantly higher in the GA genotype (31.7 ± 4.3 kg/m^2) than in the GG genotype (27.3 ± 3.4 kg/m^2) (p= 0.012; Table 3). Patients with DFI exhibited no correlation with other demographic, anthropometric, clinical, and laboratory findings.

DISCUSSION

Although comparable studies on DFU have been identified in other ethnic communities, no study on the Turkish population has been found analyzing the association between DFI and polymorphisms in the gene locus of IL-6 (-174 G>C) and TNF-α (-238 G>A). The significance of our findings is further heightened due to the potential variation of polymorphisms across people based on ethnic factors. The objective of this study was to investigate the impact of IL-6 (-174 G>C/rs1800795) and TNF-α (-238 G>A/ rs361525) SNPs on the susceptibility to DFI in the Turkish population.

IL-6 is a significant proinflammatory cytokine that has possible pathological implications in the progression of diabetic foot. Studies examining the association between the -174G/C SNP in the IL-6 gene and metabolic syndrome, obesity, insulin resistance, T2DM, and DFU have shown

inconsistent findings[17-21]. In this study, there was no correlation between the IL-6 (-174 G>C) SNP and DFI or T2DM in the Turkish population. Erdoğan et al. reported that IL-6 (-174 G>C) polymorphism is an independent risk factor for diabetes but not for DFU[15]. A similar result was observed by Dhamodharan et al[17]. On the other hand, the study by Viswanathan et al. showed that IL-6, TNF-α, and SDF-1 SNPs were essential risk factors for severe infection in DFU[5]. It has been shown that IL-6 (-174 G>C) polymorphism is associated with T2DM in Indians, Native Americans, and Caucasians, while it does not play a role in Taiwanese[22-24]. In their study, none of the participants carried the "C" allele[24]. The "C" allele is a minor allele. It may not be found in some ethnic populations.

Although SNP has been detected in many parts of the TNF-α gene, the most investigated ones are those in the -308 (G/A) and -238 (G/A) positions. The -308A and -238A alleles are known to play a role in releasing high amounts of TNF-α. Therefore, it is emphasized that polymorphisms in these two positions may be genetic markers related to the high amount of TNF-α production[25]. Different results were obtained in studies on the relationship between TNF-α (-238 G>A) SNP and insulin resistance, metabolic syndrome, T2DM, and DFU[26-30]. This study found no significant association between the TNF-α (-238 G>A) SNP and DFI or T2DM in the Turkish population. Our findings are consistent with the study conducted by Stappers et al., which did not demonstrate a genetic association between TNF-α (-238 G>A) and complex skin infections in Caucasians. The study also revealed a significant genetic association between TNF-α (-308 G>A) and IL-6 (-174 G>C) SNPs and skin infections[31]. The study findings of Dhamodharan et al. showed that the TNF-α (-308 G>A) "A" allele confers genetic susceptibility to both T2DM and DFU, but not TNF-α (-238 G>A)[17]. The study conducted by Viswanathan et al. revealed that SNPs in IL-6, TNF-α, and SDF-1 were associated with an elevated susceptibility to microbial infection in patients with DFU. Nevertheless, the SNP that was shown to be linked to severe microbial infections was TNF-α (-308 G>A), while it was observed that TNF-α (-238 G>A) did not exhibit such an association[5]. These results may be attributable to the well-documented racial disparities among population-based genetic studies. The findings of our study align with previous publications that have demonstrated a lack of correlation between the TNF-α (-238 G>A) polymorphism with the development of T2DM in many populations, including Arab, Chinese, Taiwanese, European, and Asian populations[26-28,30].

In the Indian population, IL-6 (-174 G>C), TNF-α (-308 G>A), TNF-α (-238 G>A), and SDF-1 (+801 G>A) SNPs showed some association with Wagner grade 1, 2, 3 but none of the SNPs showed association with grade 4. The presence of TNF-α (-308 G>A) and TNF-α (-238 G>A) variants has been associated with a heightened vulnerability to high-grade ulcers, indicating a potential genetic predisposition[5]. While the sample size of 207 participants in our study was deemed enough for investigating gene polymorphism, statistical analysis was not able to be performed due to the limited number of participants when they were further separated into subgroups. The lack of a grade 4 mutant genotype in both SNPs shown in our results indicates a potential protective effect of mutant genotypes against high-grade DFI. However, further studies are required to validate this hypothesis.

Amputation is the undesirable consequence of impaired wound healing in diabetic patients. There are relatively few studies in the literature investigating the relationship between surgical treatment results and gene polymorphisms in patients with diabetic foot. There was no difference between debridement and amputation and IL-6 (-174 G>C) SNP of DFI patients in our study. Our results are in line with the report of Viswanathan et al., which showed no association between IL-6 (–174 G>C), TNF-α (-238 G>A) SNPs, and DFU patients undergoing major amputation in the Indian population[5].

Cytokines, including IL-1β, IL-6, TNF-α, and interleukin-4, are actively implicated in the control of bone resorption and osteoclast activity in osteomyelitis, therefore contributing to the pathophysiology of this condition. Tsezou et al.

reported that individuals with the CC genotype at IL-6 -174 G/C in the Greek population had a seven-fold increased risk for osteomyelitis and that IL-6 was involved in the pathogenesis of osteomyelitis[32]. In our research, we observed a discrepancy with the findings of Tsezou et al., since our study revealed a heightened susceptibility to osteomyelitis in individuals with the GG genotype of IL-6 -174 G/C. Upon evaluating the findings of our investigation, it is reasonable to propose that the IL-6 (-174 G>C) SNP might serve as a potential biomarker for the identification of osteomyelitis. Limited research has been conducted to investigate the association between osteomyelitis and cytokine gene polymorphisms. This study is the initial documentation of a significant association between the IL-6 (-174 G>C) SNP and the occurrence of osteomyelitis in individuals with diabetic foot. Further studies are required to elucidate its function in the pathophysiology of osteomyelitis. TNF-α produced by adipocytes is a proinflammatory cytokine that plays a role in developing obesity, insulin resistance, and metabolic syndrome. The presence of obesity poses a substantial risk for the onset of T2DM. Previous studies have demonstrated that there is no significant association between TNF-α (-238 G>A) SNP and the incidence of obesity in both Spanish and Iranian populations[33,34]. A correlation was observed in the Chinese population between the presence of the TNF-α (-238 G>A) gene polymorphism and elevated insulin resistance, as well as BMI[35]. In our study, we observed that Turkish patients with DFI exhibited a statistically significant increase in mean BMI among those with the GA genotype of the TNF-α (-238 G>A) polymorphism, as compared to those with the GG genotype. Further study is needed to explore the association between polymorphisms and obesity, particularly in regard to the variations observed across different ethnic groups and the potential involvement of inflammation in the development of obesity.

An increasing body of research substantiates the assumption that VI is a condition characterized by inflammation and is intricately associated with blood pressure. There exists a potential for diverse genetic variants to serve as risk factors for CVI[14]. The available literature does not provide any evidence about the impact of TNF-α and IL-6 cytokine gene polymorphisms on the development of VI in individuals with diabetic foot conditions. Our study possesses unique value in this regard. In this study, we observed a notable disparity in the prevalence of VI among patients with DFI based on their TNF-α (-238 G>A) genotype. Specifically, those with the GA genotype exhibited a considerably greater frequency of VI compared to those with the GG genotype (80.0%, and 24.4%, respectively). This finding represents a novel contribution to the existing literature on this subject matter. This study posits the involvement of cytokine gene polymorphisms in the etiology of VI.

In our study, notable differences were seen in many clinical parameters, including age, systolic blood pressure, diastolic blood pressure, total cholesterol, LDL-cholesterol, ESH, CRP, BUN, and creatinine levels, between patients DFI and other patient groups. These findings indicate substantial variations in these factors across the different groups. This finding is similar to the polymorphism study findings in which DFU patients were evaluated in the literature[5,15,17]. The presence of systematic variances among different groups may potentially influence the outcomes of our research. Hence, to reduce systematic disparities among demographic groups, it is imperative to do more research that employs age and race-matched samples while controlling for confounding factors.

A noteworthy feature of this study is its contribution to the limited body of research investigating the association between DFI and the genetic factors of IL-6 and TNF-α. One of the primary constraints of this study is the limited sample size seen within the subgroups of patients. We believe that our study can be extended by including more comprehensive sample groups.

Consequently, the findings of this study indicate that the IL-6 (-174 G>C) and TNF-α (-238 G>A) polymorphisms do not possess predictive value in determining an individual's vulnerability to DFI and susceptibility to T2DM. The presence of the IL-6 (-174 G>C) GG genotype seems to be associated with a higher susceptibility to osteomyelitis development in individuals with DFI. The presence

of the TNF-α (-238 G>A) polymorphism in patients with DFI suggests that this genetic variant may serve as a promising genetic marker for predicting the occurrence of VI and the average increase in BMI in individuals with the GA genotype, as compared to those with the GG genotype. Further study including a larger sample size is necessary to have a better understanding of the genetic influence of proinflammatory cytokines in the development of DFI.

ETHICS COMMITTEE APPROVAL

This study was approved by the Atatürk University Faculty of Medicine Clinical Research Ethics Committee (Decision no: 02, Date: 30.05.2019).

CONFLICT of INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

AUTHORSHIP CONTRIBUTIONS

Concept and Design: EP, RİS

Analysis/Interpretation: RİS, SY, ÇYK

Data Collection or Processing: RİS, SY, ÇYK

Writing: RİS

Review and Correction: EP

Final Approval: All of authors

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