RESEARCH ARTICLE

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Interleukin-23R Gene Polymophisms in Patients with Diabetic Peripheral Polyneuropathy

ABSTRACT

Objective: Proinflammatory and neurovascular changes are blamed in the pathogenesis of diabetic neuropathy. Although it is accepted that diabetes is a trigger for vascular inflammation, it has been suggested that inflammation itself may trigger diabetes. Interleukin-23 (IL-23) is a pro-inflammatory cytokine secreted by activated macrophages and dendritic cells. Interleukin-23R is known to have a critical role in chronic inflammatory diseases. The aim of this study is to determine the relationship between IL-23R polymorphism and diabetic peripheral neuropathy.

Methods: 50 diabetic peripheral neuropathy patients who applied to Neurology outpatient clinic, and 52 healthy controls compatible with the patient group in terms of age and gender were included. Electromyography was performed on all of the volunteers, who agreed to participate in the study, and 2 ml of blood samples were taken into tubes with EDTA, and the IL-23R gene polymorphism was analyzed using the pyrosequencing method.

Results: IL-23R gene variants rs2201841, rs199542433, rs201052419, rs11209026 were analyzed in diabetic peripheral neuropathy (DPN) patients and control group. While we investigate IL23R polymorphisms we didn't find any significant differences between patient and control groups. But when we use odds ratios, rs2201841 seems to have a protective role, and rs199542433 in both dominant and recessive models and rs11209026 only recessive model seem to be related 10 fold higher risks for DPN.

Conclusions: IL-23R gene polymorphism has been shown to be associated with many autoimmune and inflammatory diseases. It is known that inflammation has an important effect on diabetes. The frequency of IL-23R gene polymorphism was not significant in diabetic peripheral neuropathy. Our study is the only and first study investigating the role of IL-23R gene polymorphism in diabetic peripheral neuropathy. Ethnicity is very important in genetic studies, and it will give us more clear information for the future to carry out this study in patients with other ethnic origins and to recruit larger study groups.

Keywords: Diabetic Peripheral Neuropathy, Inflammation, Genetics, Interleukin-23R (IL-23R) Gene Polymorphism.

Diyabetik Periferik Polinöropatili Hastalarda İnterlökin-23R Gen Polimofizmleri

ÖZET

Amaç: Diyabetik nöropatinin patogenezinde proinflamatuar ve nörovasküler değişiklikler suçlanmaktadır. Diyabetin vasküler inflamasyonu tetiklediği kabul edilse de inflamasyonun da diyabeti tetikleyebileceği öne sürülmüştür. İnterlökin-23 (IL-23) aktive makrofajlar ve dendritik hücreler tarafından salgılanan proinflamatuar bir sitokindir. Interleukin-23R'nin kronik inflamatuar hastalıklarda kritik bir rolü olduğu bilinmektedir. Bu çalışmanın amacı, IL-23R polimorfizmi ile diyabetik periferik nöropati arasındaki ilişkiyi incelemektir.

Gereç ve Yöntem: Nöroloji polikliniğine başvuran 50 diyabetik periferik nöropati hastası ve hasta grubuna yaş ve cinsiyet açısından uyumlu 52 sağlıklı kontrol çalışmaya dahil edildi. Çalışmaya katılmayı kabul eden gönüllülerin tamamına elektromiyografi uygulandı ve EDTA'lı tüplere 2 ml kan örneği alındı. Pyrosequencing yöntemi ile IL-23R gen polimorfizmi analiz edildi.

Bulgular: IL-23R gen varyantları rs2201841, rs199542433, rs201052419, rs11209026 diyabetik periferik nöropati (DPN) hastalarında ve kontrol grubunda analiz edildi. IL23R polimorfizmleri sıklıkları açısından hasta ve kontrol grupları arasında anlamlı bir fark saptanmadı. Ancak, odd's oranlarına bakıldığında, rs2201841'in koruyucu rolü var gibi görünmekte, rs199542433 hem baskın hem de çekinik modellerde ve rs11209026 sadece çekinik modelde, DPN için 10 kata kadar daha yüksek risklerle ilişkili olabileceği görünmektedir.

Sonuç: IL-23R gen polimorfizminin birçok otoimmün ve inflamatuar hastalık ile ilişkili olduğu gösterilmiştir. İnflamasyonun diyabet üzerinde önemli bir etkisi olduğu bilinmektedir. Diyabetik periferik nöropatide IL-23R gen polimorfizminin sıklığı anlamlı değildi. Çalışmamız diyabetik periferik nöropatide IL-23R gen polimorfizminin rolünü araştıran tek ve ilk çalışmadır. Etnik köken, genetik çalışmalarda çok önemlidir ve bu çalışmanın başka etnik kökene sahip hastalarda yapılması ve daha geniş çalışma gruplarının alınması, bize ilerisi için daha net bilgiler verecektir.

Anahtar Kelimeler: Diyabetik Periferik Nöropati, İnflamasyon, Genetik, İnterlökin-23R (IL-23R) Gen Polimorfizmi.

INTRODUCTION

Diabetes Mellitus (DM) is an increasingly common disease. The global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 (1). Complications could begin in the first years following the diagnosis of DM, or patients can be affected by complications at the time of diagnosis. Hyperglycemia, obesity, dyslipidemia, endothelial and intima changes, hyperinsulinemia, insulin resistance and genetic factors play a role in the development of chronic complications of DM (2,3).

Nerve damage in diabetic peripheral neuropathy (DPN) occurs as a result of metabolic factors, ischemia, and neurovascular changes. Inflammation and oxidative stress are veritably important in its etiology (4).

Subunits of interleukin (IL)-23 are IL-12 p40 and IL-23 p19. The IL-23R gene located on chromosome 1p31 encodes the IL-23 receptor. IL23R does not interact with IL-12, but pairs with IL12RB1 to confer IL23 responsiveness on cells expressing both subunits. It's secreted by macrophages and dendritic cells and can affect T-cell mediated inflammation and autoimmunity by impacting the response of T helper 17 (Th17) cells (5). The capability of IL-23R knockout mice to mediate inflammation is severely reduced (6). Langrish demonstrated the role of IL-23 in Th-17 development and its importance in inflammatory diseases (7). In a mouse model, IL-23 administration has been shown to have a destructive effect on pancreatic β-cells that cause hyperglycemia, suggesting that it may be effective in the development of autoimmune DM (8). Abbasi et al. found upregulation of the IL-23 gene in unstimulated mononuclear cells in type 1 DM patients (9). However, there is also a study concluded IL-23 serum concentrations did not differ significantly between diabetic patients and controls (10). It has been shown that variants of IL-23 Alpha (IL23A), a subunit of IL-23, are protective against type 1 DM, while IL-23R variants are not associated with DM (11). In vivo administration of IL-23 triggers late-onset diabetes in mice administered multiple low-dose streptozocin below the dose that can induce DM. This effect of IL-23 was associated with the expression of IL-17 and increased expression of Tumor Necrosis Factor-alpha (TNF-α) and IL-18 immediately after DM triggering (12).

DPN is a common and important microvascular complication of DM, affects roughly one-third of patients and impairs quality of life. It can affect small and large peripheral nerve fibers particularly in the lower extremities. Cytokines play an important role in DPN. Nerve conduction study in the diagnosis of DPN is important in terms of showing whether nerve involvement is axonal damage and/or demyelination (13). Various factors such as diabetes duration, HbA1c level, smoking and gender are effective in the development and frequence of DPN. Polyneuropathies are the most common group of DPN and can cause nontraumatic limb amputations (14). Cytokines have shown to be genetic markers in the development of microvascular complications of DM, such as DPN (15).

In our study, the role of IL-23R gene polymorphism in DPN was investigated as first study on this subject.

MATERIAL AND METHODS

After obtaining the Ethics Committee approval, 54 DPN patients who applied to neurology outpatient clinic between 2020-2021 and 53 healthy controls compatible with the patient group in terms of age and gender were included.

Type 2 DM patients aged 50-90 years with electroneurophysiologically detected DPN were included in the study. Those under the age of 50 and over the age of 90, those with inflammatory diseases such as Behçet's disease, systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, ankylosing spondylitis, inflammatory bowel disease were not included in the study.

DPN was diagnosed according to Nerve conduction studies (NCS) recordings. All NCS measurements were performed with a Nihon-Kohden device (NihonKohden-Neuropack®) on three extremities of each subject including motor components of the peroneal, posterior tibial, median and ulnar nerves and sensory components of the sural, median and ulnar nerves. Nerve conduction velocities, distal latencies and amplitudes were recorded and interpreted by an experienced neurologist. Demyelinating neuropathy was diagnosed when prolonged distal motor latency, slowed conduction velocity, conduction blocks, and prolonged or absent F-wave latency while axonal neuropathy was diagnosed when low or loss of motor and sensory action potential was detected.

2 ml blood samples were taken from participants into EDTA tubes. DNA isolated with MiniBlood kit (Qiagen). After the isolation process was completed, 5 µL of DNA was distributed on the Polymerase Chain Reaction (PCR), for a total of 2 hours and 10 minutes, denaturation, bonding and elongation stages were performed as 95°C for 15 minutes, 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and 72°C for 10 minutes. Pyrosequencing was performed on the QIAGEN PyroMark Q24 instrument (QIAGEN, Inc., Valencia, CA, USA) for genotyping.

Collected data were digitalized and corrected. Among the variants studied in the IL-23R gene, rs2201841, rs199542433, rs201052419, rs11209026 were analyzed. Four from the patient group and one from the control group were excluded from the study due to the inability to obtain analysis results, and the study was evaluated on 50 patients and 52 control groups. Allele frequencies were calculated and compared between patient and control groups using

Chi Square test. When the expected value was less than five, the Fisher's exact test result was reported. The results were determined as wild, heterozygous mutant, homozygous mutant models. Odd's ratios for models for studied variants were computed and reported with 95% confidence interval (CI) limits. Test constants and absolute p values are presented for all analyses and $p<0.05$ was accepted as the general significance limit.

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RESULTS

Among the studied variants in the IL-23R gene, rs2201841, rs199542433, rs201052419, rs11209026 were analyzed in 50 DPN patients and 52 healthy controls included in the study.

In the DPN group, rs2201841 variant had 18 AA, 26 AG, 2 GT and 4 GG genotypes, rs199542433 variant had 47 CC and 3 CT genotypes, rs201052419 variant had 50 TT genotype and rs11209026 variant had 38 CG and 12 CC genotypes (Table 1).

In the control group, rs2201841 variant had 15 AA, 28 AG and 9 GG genotypes, rs199542433 variant had 49 CC and 3 CT genotypes, rs201052419 variant had 52 TT genotype and rs11209026 variant had 46 CG and 6 CC genotypes (Table 1).

Table 1. Allele frequencies

	DPN	Control	
rs2201841			
AA	18	15	X^2=4.232
AG	26	28	p=0.237
GG	4	9	
GT	2	0	
rs199542433			
CC	47	49	X^2=0.002
CT	3	3	p=0.961
rs201052419			
TT	50	52	Not calculated
rs11209026			
CC	12	6	X^2=2.724
CG	38	46	p=0.123

Odds ratio values were calculated by creating a recessive model and a dominant model for the patients and control groups (Table 2). There was no statistically significant difference between the DPN group and the control group in terms of allele frequencies ($P>0.05$). There was no significant difference between the DPN group and the control group in terms of homozygous wild and homozygous mutant genotype distribution for any variant. ($P>0.05$).

Table 2. Model results of genotype distributions

		Odd's ratio [95% CI]	Significance
rs2201841	**recessive model**	0.4155 [0.1191 - 1.4487]	Z=1.378, P=0.1681
	dominant model	0.7207 [0.3134 - 1.6573]	Z=0.771, P=0.4408
rs199542433	**recessive model**	10.396 [0.0202 - 53.4010]	Z=0.019, P=0.9846
	dominant model	10.426 [0.2003 - 53.4264]	Z=0.050, P=0.9605
rs201052419	**recessive model**	Not calculated	Not calculated
	dominant model	Not calculated	Not calculated
rs11209026	**recessive model**	10.396 [0.0202 - 53.4010]	Z=0.019, P=0.9846
	dominant model	0.4130 [0.1417 - 1.2042]	Z=1.620, P=0.1053

We found no difference between the patient and control groups in the rs2201841, rs199542433, rs201052419 variants, which may be due to the rarity of this variant in the total world population and European populations. In the rs11209026 variants our patient and control groups has higher frequency then European population, this can be because of founder affect of our small city population (Table 3).

Table 3. Frequencies of investigated IL-23R polymorphisms. (gnomAD. Genomes version 311)

	Total world population	European population
rs2201841	0.285	0.304
rs199542433 (p.Leu372Phe)	0.000215	0.0
rs201052419	0.00645	0.00494
rs11209026	0.0422	0.0586

DISCUSSION

DPN may develop in 60-70% of diabetic cases. Although the mechanisms involved in the development of neuropathy remain unclear, multifactorial risk factors included genetic predisposition and environmental factors.

Although genetic variants effective in the development of DPN have been found, it has not been clarified whether these variants are effective in the progression of the disease or they are specific for DPN (16). Although more than 60 loci that pose a risk to DM have been found, studies to determine the genetic risk factors of DPN are insufficient (17).

The IL-23 receptor complex consists of IL-23R and IL-12Rβ1. The second subunit is generally common in IL-12 receptor complexes. IL23R consists of extracellular domain (signal sequence, N-terminal immunoglobin-like domain and 2 cytokine receptor domains), a single transmembrane domain and a cytoplasmic domain. IL-23R is expressed on activated denritic cells, microglia, T cells, eosinophils, macrophages as well as on non-hematopoietic cells such as kerationocytes. Regulated expression of IL-23R plays a key role in leukocyte subset differentiation and processing. Factors that increase IL23R mRNA expression are IL-6, IL-21, T cell activation, TGFß (Transforming Growth Factor Beta) and IL-23 itself (18).

Interleukin-23R has been shown to have a critical role in a number of chronic inflammatory diseases such as chronic inflammatory bowel disease, psoriasis, Chrohn's Disease and arthritis in both mouse and human trials (19,20). IL-23 signal axis (IL-23/IL-23R) is an important inflammatory pathway (21).

Many theories have been suggested in the pathogenesis of DPN (22). One of them is oxidative stress and related inflammation. There is an increase in the levels of inflammatory cytokines such as IL-1, CRP, tumor necrosis factor (TNF)-α and IL-6 in patients with DM. Inflammatory cytokines are produced by various cell types and released into the circulation. Cytokines have local, central and peripheral effects on many different tissues. There is an increase in inflammation in type 2 DM and it is effective in the formation of many complications of DM, including DPN (23-25). On the other hand, there is also a study concluded that IL-23 serum concentrations do not differ significantly in DM patients and controls (10).

Investigated IL-23R polymorphisms didn't have any significant differences between patient and control groups. However, looking at the calculated odds ratios, rs2201841 polymorphism seems to have a protective role for DPN, rs199542433 polymorphism in both dominant and recessive model and rs11209026 polymorphism in recessive model seem to be associated with up to 10 times higher risk.

The main limitation of the study is the relatively low number of patients and controls included in the study. The representativeness of the studied sample for all diabetes and related DPN patients is uncertain. The COVID-19 pandemic has limited the number of patients and controls included in this study. New studies with larger groups will ensure that results are confirmed within the limit of significance. The study was conducted in a single center, geographical distribution differences of the investigated polymorphisms should be considered in the generalization and clinical use of the results. On the other hand, clinical and neurophysiological evaluation of patients and controls by the same neurologist is a valuable aspect of this study.

Genetic studies conducted so far have focused on the risk of developing type 2 DM. Most studies have been conducted focused on retinal and renal vascular complications diabetes. Although known effect of genetic factors and the inflammation in DPN, absolute risk factors and genetic predisposition remains unclear. Genetics studies in DPN are limited and reproducibility is lacking in most. Therefore, the investigation of new and rare variants is still important. We can reveal why many patients with the same risk factors experience different complications of DM with different severity by increasing genetic studies.

This is the first study to examine the risk of developing IL-23R gene polymorphisms in DPN patients. Our results suggest that the rs199542433 and rs11209026 polymorphisms, in particular, may be useful in identifying individuals at risk for DPN. The follow-up of patients with these polymorphisms would be the goals of future research.

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