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ORIGINAL ARTICLE

Evaluation of the Relationship between TLR1(RS4833095) Gene Polymorphism and Disease in Patients with Ulcerative Colitis in the Turkish Population

Türk Popülasyonundaki Ülseratif Kolitli Hastalarda TLR1(RS4833095) Gen Polimorfizminin Hastalıkla İlişkisinin Değerlendirilmesi

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ABSTRACT

Background: Ulcerative colitis is a multifactorial disease which is characterized by recurrent episodes of inflammation in the mucosal layer of the colon. Toll-like receptors (TLRs) are a class of transmembrane pattern recognition receptors that play a key role in the induction of pro/anti-inflammatory genes and in the control of adaptive immune responses. In this study, we aimed to evaluate the relation between TLR1(rs4833095) single nucleotide polymorphism and ulcerative colitis.
Methods: The study included 90 patients with ulcerative colitis and a healthy control group consisting of 90 people. Of the patients included in the study; medical treatments received, laboratory data, colonoscopy findings, and extraintestinal manifestations were recorded. TLR1(rs4833095) single nucleotide polymorphism was studied using RT-PCR methods.
Results: There was no increased risk for ulcerative colitis in patients with TLR1(rs4833095) single nucleotide polymorphism in Turkish population (p>0.05). There was no association between TLR 1(rs4833095) single nucleotide polymorphism and the spread of the disease to the colon, severity of disease and treatment required for remission in our study (p>0.05).
Conclusion: In the Turkish population, TLR1 (rs4833095) single nucleotide polymorphism was evaluated and no significant difference was found between the patients with ulcerative colitis and the control group.

Keywords: Ulcerative colitis, Toll-like receptors, Inflammatory bowel disease, Single nucleotide polymorphism

ÖZ

Amaç: Ülseratif Kolit, kolonun mukozal tabakasında tekrarlayan inflamasyon dönemleri ile karakterize olan çok faktörlü bir hastalıktır. Toll benzeri reseptörler (TLR'ler), pro/anti-inflamatuar genlerin uyarılmasında ve adaptif immün yanıtların kontrolünde anahtar rol oynayan transmembran, patern tanıma reseptörleridir. Bu çalışmada TLR1(rs4833095) tek nükleotid polimorfizmi ile ülseratif kolit arasındaki ilişkiyi değerlendirmeyi amaçladık.
Yöntem: Çalışmaya 90 ülseratif kolit hastası ve 90 kişiden oluşan sağlıklı kontrol grubu dahil edildi. Çalışmaya dahil edilen hastaların aldıkları tıbbi tedaviler, laboratuvar verileri, kolonoskopi bulguları, bağırsak dışı bulguları kaydedildi. TLR1(rs4833095) tek nükleotid polimorfizmi RT-PCR yöntemiyle çalışıldı.
Bulgular: Türk toplumunda TLR1(rs4833095) tek nükleotid polimorfizmi olan ülseratif kolit hastalarında ülseratif kolit riskinde artış saptanmadı (p>0,05). Çalışmamızda TLR 1(rs4833095) tek nükleotid polimorfizmi ile hastalığın kolondaki yayılımı, hastalığın şiddeti ve remisyon için gerekli tedavi arasında ilişki saptanmadı(p>0,05).
Sonuç: Türk toplumunda TLR1 (rs4833095) tek nükleotid polimorfizmi değerlendirilmiş ve ülseratif kolitli hastalar ile kontrol grubu arasında anlamlı fark saptanmamıştır.

Anahtar Kelimeler: Ülseratif kolit, Toll benzeri reseptörler, İnflamatuar barsak hastalığı, Tek nükleotid polimorfizmi

Introduction

Ulcerative Colitis is a multifactorial disease which is characterized by recurrent episodes of inflammation in the mucosal layer of the colon, resulting from the combined effect of genetic and environmental factors (1). There is a common consensus that chronic mucosal immune dysregulation plays a role in the etiopathogenesis of ulcerative colitis (1).

Normally, the mucosal immune system is unresponsive to mucosal content due to oral tolerance. When soluble antigens are taken orally instead of subcutaneously or intramuscularly, antigen-specific non-responsiveness is induced (1). Several processes including the deletion and anergy of antigen-reactive T cells, and secretion of inhibitory cytokines such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) cause the development of oral tolerance (1).

When infections evolve in the normal host, the intestinal-associated lymphoid tissue is fully activated, but

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the inflammation is regulated and inactivated in a controlled manner by controlling, suppressing, and repairing the immune response. This immune regulation is impaired in inflammatory bowel diseases (1).

Genetic susceptibility to inflammatory bowel diseases

Chronic inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are complex diseases. Many genetic and environmental factors play a role in their development (2). Toll-like receptors (TLRs) are a class of transmembrane pattern recognition receptors that act integral role in the induction of pro/anti-inflammatory genes and in the control of adaptive immune responses (3,4).

Genetic association studies have demonstrated polymorphism of Toll-Like receptors (5,6), apoptosis (7,8), IL-23/IL-17 (7,8) and interferon-gamma (IFN-γ) (7,8) pathways related to susceptibility to IBD.

Pathogen-associated molecular patterns such as bacterial or viral DNA, flagellin, lipopolysaccharide (LPS) includes all of these pathways and may cause autoimmune response (9).

Pathogen-associated molecular patterns can be bound by membrane-bound toll-like receptors (TLR), the connection between them provoke inflammation through activating many pro- and anti-inflammatory cytokines (9).

Genetic differences in TLRs can alter host-commensal interactions. Even if small changes in TLRs can affect ligand recognition, mucosal tolerance and commensal composition, resulting in natural/adaptive immune hypo- and hyperactivity (5).

In this trial, our intention is to evaluate the relation between TLR1(rs4833095) single nucleotide polymorphism and ulcerative colitis.

Materials and Methods

Patient population

This study was planned as a prospective study. Ninety ulcerative colitis patients and 90 healthy controls were assigned into this study. All participants had to give informed consent before participating in our study. The necessary approval for the study was obtained from the local ethics committee of our university. Diagnoses of patients were confirmed by examining clinical features, as well as colonoscopy findings and histological findings. Medications received, laboratory and colonoscopy data, and extraintestinal manifestations of the disease included in this study were recorded via examination of patient files.

DNA isolation and genotyping

Three to four cc blood taken from the patients and control group were transferred into EDTA (Ethylenediaminetetraacetic acid)-containing tubes. Samples were stored at -80°C in the freezer (Haier ULT Freezer) until DNA isolation. DNAs of the patient and control group were isolated by standard procedures and using a commercial kit (Roche-Highpure PCR template). The quality of the DNAs was controlled using nano spectrophotometry. High-quality DNAs were kept at -20°C until examination.

TLR1 (rs4833095) single nucleotide polymorphism was determined by real-time polymerase chain reaction (RT-PCR) (Lightcycler 96) using Tag Man Genotyping Assay.

RT-PCR was conducted in a total volume of 50µl, using the following reagents: 25 µl TaqMan assay master mix, 2.5µlTaqMan assay primer probe, 22.5µl genomic DNA.

PCR conditions included denaturation at 95°C for 10 min. Amplification included denaturation at 95°C for 15 second, elongation at 60°C for 60 second, and cooling process at 37°C for 60 second. This was repeated in 40 cycles.

Genotype determination (Endpoint analysis)

All VIC/FAM marked probes were synthesized by thermo fischer. VIC and FAM hydrolysis probes were used for genotype determination. C nucleotide was detected with VIC probe, whereas T nucleotide was detected with FAM probe. Natural and mutant types separated from each other with endpoint analysis

Statistical Analysis

SPSS for Windows software package version 15.0 was used for data entry and analysis. The compliance of the data to normal distribution was examined using visual and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). In summarizing numerical data; arithmetic mean, standard deviation values were used. Numbers and percentages were used in summarizing categorical data. Associations between categorical data were evaluated using chi-squared test. Results with a p less than 0.05 were accepted to indicate statistical significance.

Results

Patients' demographics and clinical data

The mean age of the 90 patients was 39.7±12.9 years.

While 52.2% patients were male, 47.8% were female. The mean age of the 90 healthy controls was 36.9±10.6 years. Fifty percent of control group were male and 50% were female. According to the colonoscopy findings, the disease spread was as follows; 61.8% had distal colitis and 38.2% pancolitis. Of the patients, 5.5% had extraintestinal manifestations. Two patients had sclerosing cholangitis, one ankylosing spondylitis, one arthritis and one sacroiliitis. While 70.8% patients were taking one of the 5-ASA preparations alone or in combination with azathioprine 29.2% patients were receiving anti-TNF treatment alone or in combination with other treatments for remission of the disease.

The association between TLR 1(rs4833095) single nucleotide polymorphism and patient and control groups

Genotype was found to be C/C (normal) in 12 (13.3%) T/T (homozygous variant) in 33 (36.7%) and C/T (heterozygous) in 44 (48.9%) patients. No results were obtained by PCR in 1 (1.1%) patient. Genotype was found to be C/C (normal) 15 (16.7%) controls, T/T (homozygous variant) in 30 (33.3%) controls and C/T (heterozygous) in 44 (48.9%) controls. No results were obtained by PCR in 1 (1.1%) control subject.

There was no significant difference in TLR1 (rs4833095) single nucleotide polymorphism distribution between patient and control groups according to Pearson's Chi-squared test (Table 1). TLR1 (rs4833095) single nucleotide polymorphism was not associated with increased risk for ulcerative colitis in the Turkish population (p>0.05).

Table 1. TLR1(rs4833095) snp distribution between patient and control groups

	TLR1(RS4833095) snp				
	C/T n (%)	T n (%)	C n (%)	Total n (%)	
Control	44(50.0)	33(52.4)	12(44.4)	89(50.0)	
Patient	44(50.0)	30(47.6)	15(55.6)	89(50.0)	p>0.05
Total	88(100.0)	63(100.0)	27(100.0)	198(100.0)	

TLR: Toll like receptor, snp: single nucleotide polymorphism

There was no significant correlation between TLR1 (rs4833095) single nucleotide polymorphism and colon spread in the Turkish population (p>0.05) (Table 2).

There was no significant correlation between TLR1 (rs4833095) single nucleotide polymorphism and the required treatment for remission of the disease in the Turkish population (p>0.05) (Table 3).

Table 2. Association between TLR1(rs4833095) snp and the spread of disease

	TLR1(RS 4833095) snp				
	C/T n (%)xxx	T n (%)	C n (%)	Total n (%)	
Distal colitis	28(63.6)	18(60.0)	9(60.0)	55(61.8)	
Pancolitis	16(36.4)	12(40.0)	6(40.0)	34(38.2)	p>0.05
Total	44(100.0)	30(100.0)	15(100.0)	89(100.0)	

TLR: Toll like receptor, snp: single nucleotide polymorphism

Table 3. Association between the TLR 1(rs4833095) snp and the treatment for remission of the disease

	TLR1(RS4833095) snp				
	C/T n(%)	T n(%)	C n(%)	Total n(%)	
5-ASA and the other treatments	31(70.5)	23(76.7)	9(60)	63(70.8)	
Anti-TNF	13(29.5)	7(23.3)	6(40)	26(29.2)	p>0.05
Total	44(100)	30(100)	17(100)	89(100)	

TLR: Toll like receptor, snp: single nucleotide polymorphism

Discussion

Recent trials have shown that TLR signaling act a significant role in the pathogenesis of IBD (5). There is a significant difference in TLR function and regulation between normal intestinal mucosa and damaged intestinal mucosa due to inflammation. (5). This reflects the fine line between the protection and damage of the intestinal mucosa of the host (5).

TLRs can be induced or structurally expressed in different combinations by various cell types throughout the gastrointestinal tract. These types of cells in the gastrointestinal tract include intestinal epithelial cell family (absorptive enterocytes) (10-15), paneth cells (16,17), goblet cells (18), enteroendocrine cells (19,20), subepithelial myofibroblasts (21,22), monocyte/ macrophage (23,24), dendritic cells (25,26), and

CD4+T cells (27).

TLRs are localized on the cell surface (TLR 1/2/4/5/6) or in intracellular compartments (TLR 3/7/8/9) (28). In normal gastrointestinal tract, TLR 2 and TLR 4 are found in small amounts in intestinal epithelial cells and mononuclear cells in lamina propria. Therefore, the recognition of environmental factors in these cells is minimized and the basal state of activation is maintained (10-15, 24).

In healthy hosts, TLR signaling maintains required basal immune mechanisms to maintain host barrier integrity and maintain tolerance, as well as commensal composition (5) However, in a sensitive host, dysfunctional or abnormal TLR signaling might cause deterioration of commensal-mucosal homeostasis, thereby leading to persistance of damage on tissue, resulting with chronic inflammation of IBD on the ongoing process (5).

TLR inhibition is involved in avoiding inappropriate activation against intestinal microbiota. Cellular mechanisms and several inhibitory regulators can reduce or eliminate TLR activation in the intestinal mucosa (5). When the host encounters threats, these inhibitory mechanisms may be suppressed and stimulant regulators can thus induce significant immune response by allowing TLR signaling to eliminate threats (5). However, continuous TLR hyperactivation may cause damage and chronic inflammation in mucosa (5).

Genetic differences in TLRs may alter host-commensal interactions. Even if small changes in TLRs can affect ligand recognition, mucosal tolerance and commensal composition, resulting in natural/adaptive immune hypo- and hyperactivity (5).

The multidimensional and highly interactive regulatory triad which consists of environmental factors, genetic factors and host immunity controls TLR functions in the intestinal mucosa (5). Unbalanced associations in this triad may induce abnormal TLR signaling that contributes acute and chronic inflammatory process in the IBD colitis and IBD-associated cancer (5). Impaired balance between mucosal immunity and commensal bacteria may result in bowel inflammation in genetically susceptible individuals (28, 29).

Multiple experiments have shown that inhibiting the TLR4 signaling pathway can prevent Dextran sodium sulfate(DSS)-induced colitis (30,31).

A recent trial shown that the expression of TLR4 is up-regulated by DSS, and down-regulated after fecal microbiata transplantation (FMT). It therefore stands to reason that, by inhibiting TLR4, a protective effect from intestinal inflammation will be induced (32).

In a Danish polymorphism study by Bank et al. (2015), TLR1 743 T>C (rs4833095) (OR:2.92, 95% CI:1.42–6.00, p=0.004) homozygous variant genotype seemed to be associated with increased risk for ulcerative colitis (30). The association between single nucleotide polymorphism in TLR1 743 T>C (rs4833095) and ulcerative colitis was demonstrated for the first time in this study (33).

In a Danish cohort including 411 patients with ulcerative colitis, all patients had severe disease. Patients were either receiving tumor necrosis alpha inhibitor therapy (anti-TNF) for the remission of the disease or anti-TNF treatment was planned for the remission of the disease (33).

In our trial, no significant difference was demonstrated in genotype distribution of TLR1(rs4833095) between ulcerative colitis patients and healthy control group. The polymorphism described above had no significant effect on the phenotypic spread of the disease. There was no significant association between TLR1 (rs4833095) single nucleotide polymorphism and disease severity.

Ninety patients and 90 healthy individuals were included in our study. The characteristics of the patient arm and control arm were similar. The limitations of our study included lower number of patients than that in the Danish cohort and that the disease was controlled with 5-aminosalicylic acid (5-ASA) derivatives and azotiopurine. In the Danish cohort, patients were receiving anti-TNF therapy for disease remission or anti-TNF therapy was planned for disease remission (33).

Conclusion

Toll-like receptors (TLRs) are a class of transmembrane pattern recognition receptors that act an integral role in the induction of pro/anti-inflammatory genes and in the control of adaptive immune responses. TLR mutations and dysregulation have been proposed to be important factors contributing to predisposition and susceptibility to IBD. Therefore, modulating TLRs represents an innovative immunotherapeutic approach in the treatment of IBD.

Although genetic features are one of the important causes for the development of inflammatory bowel disease, it remains unclear which steps are responsible

of disease development. Disruption of any step in TLR signaling may cause hyperactivation of TLRs and increase the inflammatory response. Various genetic polymorphisms that may occur in any of the 9 types of TLRs may lead to disease development and the development of different phenotypic features of the disease.

In this study, we investigated single nucleotide polymorphism in TLR1 (rs4833095), possibly one of these steps, and we did not observe any difference between patient and control groups in Turkish population.

Genetic polymorphism studies may be affected by sociodemographic characteristics such as geographical distribution and race. Furthermore, the reflection of the genotype to the phenotype may not be the same in all individuals or all races.

We may have more information about the disease by looking at larger and various inflammasome pathways.

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