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Evaluation of TNF alpha G308A promoter gene polymorphism and serum TNF alpha levels in patients with inflammatory bowel disease in Turkish population

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Abstract

Tumor necrosis factor alpha (TNF-α) plays an important role in the pathophysiology of inflammatory bowel diseases (IBD). In this study, we aimed to investigate the role of TNF-α G308A promoter gene polymorphisms and TNF-α level in patients with IBD and their associations with clinical features of the patients. This study included 91 patients with inflammatory bowel disease (46 patients with ulcerative colitis (UC) and 45 patients with Crohn's disease (CD)) and 129 healthy controls (HC). Polymerase chain reaction and restriction fragment length polymorphism techniques were both used to explain the frequency of TNF-α polymorphisms and the relationship about clinical outcomes of patients with IBD. There was no statistically significant difference about genotype frequencies and serum TNF-α level between the three groups (UC, CD, HC) (all p>0.05). TNF-α G308A genes polymorphisms were compared in terms of disease localization, behaviour, activity, the use of anti TNF-α treatment and operative status in patients with CD and UC. Similarly, there was no statistically significant difference about genotype frequencies in terms of all these parameters in patients with CD (p> 0.05). However, In patient with UC, the frequency of A allele and AG genotype was significantly increased in moderate and severe UC cases (p= 0.004). These findings suggest that TNF-α G308A gene polymorphism may be helpful in predicting the behavior of the disease in patients with ulcerative colitis but further studies are needed

Keywords: TNF-α G308A, polymorphism, serum levels, inflammatory bowel diseases

Introduction

Today, inflammatory bowel diseases getting an important global health problem in the world and the rate of incidence is increasing day by day mostly in industrialized and westernized countries. Inflammatory bowel diseases (IBD) are chronic, recurrent inflammatory diseases of the gastrointestinal tract. IBD has two main phenotypes that characterized by clinical and histopathological features: Crohn's disease (CD) and Ulcerative Colitis (UC). Environmental, immunological and genetic factors play a role in the etiopathogenesis of IBD [1]. The increase in IBD prevalence in family members of IBD patients (15% of patients with CD have an affected family member) and the increase in IBD prevalence in monocytic twins with IBD disease (twin studies for CD have shown 50% concordance in monozygotic twins) support the role of genetic factors in the pathogenesis of IBD [2].

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In recent years, several gene regions have been shown to be associated with IBD in the data that obtained from molecular genomic studies in families with a large number of IBD patients [3]. Some of these are; 16q12 (IBD1), 12q13 (IBD2), 6P13 (IBD3), 14q11 (IBD4), 5q31-33 (IBD5), 19P13 (IBD6), 1P36 (IBD7), 16P (IBD8), 3P (IBD9) chromosomal loci [4]. Most of the genes in these regions are considered to be candidate genes that involved in the pathogenesis of the disease. The IBD region includes the tumor necrosis factor-α (TNF-α) gene [5]. The TNF-α gene region is a potential candidate gene region for IBD genetic predisposition due to its localization and functionality. It is also known that TNF-α, which is an important pro-inflammatory cytokine, has also an important function in initiating and regulating the cytokine cascade. In addition to this; it plays an important role in the pathophysiology of IBD [6]. Else, it has been shown that tumor necrosis factor alpha levels increase significantly in serum and intestinal mucosa in inflammatory bowel disease patients [7,8].

Anti-TNF-α monoclonal antibody treatments are an important treatment option in both CD and severe UC treatment [9]. In a

study in mice, genetic mutant mice causing increased transcription of TNF-α have been shown to have histopathological findings similar to Crohn's disease associated with increased TNF-α biosynthesis [10]. In addition, in a study performed on TNF-α gene knockout mice, it was also shown that there were significant reductions in chemically induced chronic intestinal inflammation [11]. It has been determined that the TNF-α G308A promoter gene region affects TNF-α expression [12]. The replacement of the A allele in place of the promoter gene G allele in the TNF-α 308 region has been shown of increasing TNF-α transcription 6-7 fold [13]. The disease was found to be more severe in IBD patients with TNF-α 308A allele [14].

In this study, we aimed to investigate the presence of serum TNF-α level and TNF-α G308A polymorphisms on the patients from Turkish population with IBD and the association of these polymorphisms with the clinical features of the patients with CD and UC that compared with healthy subjects.

Materials and Methods

Subjects

In total, 220 Turkish subjects, 91 patients with IBD (46 UC and 45 CD) and 129 healthy control (HC) subjects were enrolled consecutively in this study. The characteristics and demographic data of the HC group and patients with CD, UC are shown in Table 1. The patient group was selected among patients who were followed-up at the Gastroenterology IBD outpatient clinic between January 2012 and January 2014 with the diagnosis of IBD (UC and CD) according to the criteria of the European Crohn's and Colitis Organization [15]. The control group included volunteers from hospital staff and volunteers among individuals who were admitted to the Gastroenterology Clinic for dyspeptic complaints. Subjects in the control group were individuals that dont have an inflammatory disease or systemic disorder. Informed consent was obtained from all the participants following an approval of the Umraniye Education and Resarch Hospital ethics committee (Number: 141). Blood specimens were collected in tubes containing EDTA. DNA was extracted from peripheral blood lymphocytes using the salting-out procedure. The disease activity and severity were assessed by the Crohn's Disease Activity Index (CDAI) in patients with CD and the Truelove-Witts index (TW) in patients with UC [16]. Patients with CD were divided into three groups according to the CDAI; mild disease (CDAI = 150-220), moderate disease (CDAI = 220-450) and severe disease (CDAI> 450). The location and the behavior of the disease are classified according to the Montreal classification. [17]. The CD location is classified as L1 terminal ileum with or without cecum involvement (L1), colon (L2), ileocolon (L3). According to the CD behavior, three groups were separated as nonstricturing, nonpenetrating (B1), stricturing (B2) and penetrating (B3). Patients with UC were divided into three groups according to the extent of the disease: distal (proctosigmoiditis) colitis was determined as inflammation limited to the rectum and sigmoid colon; left-sided colitis, determined as inflammation limited to distal of the splenic flexure; and pancolitis, involvement exceeding the splenic flexure. Patients were also divided into two groups according to whether they had a surgery or not before. According to their treatment, the patients were also divided into mesalazine, azathioprine and anti-tumor necrosis factor alpha drugs (infliximab and adalimumab).

Laboratory tests

Blood samples were collected after 8-12 hours of fasting. Complete blood count, sedimentation and C-reactive protein (CRP) levels were measured on the same day. All blood samples collected for TNF-α measurement were centrifuged at 5000 rpm for 6 minutes and 1-2 ml serum samples were stored at -80^{0}C. All the samples that were used during the study were in accordance with the specifications of the kits as described in the instruction manual, and the application steps in the user manual were followed during each test. TNF-α (AssayMax Human ELISA, USA) levels were examined.

Polymorphism analysis

Genomic DNA was isolated from lymphocytes by standard procedure. The isolated DNA was used to detect the following polymorphic genes. Polymerase chain reaction (PCR) and then restriction fragment length polymorphism (RFLP) were performed for genotyping. First, PCR was applied to identify polymorphic regions using appropriate primers. PCR products of TNF-α G308A were also cut with restriction enzyme NcoI (Table 1). The PCR products were visualized by agarose gel electrophoresis. All genotype results were readed by two researchers. In addition, in case of any confliction, the genotypes were repeated again.

Statistical analysis

SPSS 11.0 software was used for statistical analysis. The Chi-square test and Fisher's test were used to assess the differences of genotype and allele frequency between the two groups. The comparison of intergroup demographic data was determined using Student's t-test. ANOVA and t tests were used to compare the average of two or more variables. Chi-square test was used to calculate the differences between the genders. Fisher Freeman Halton test and Continuity (Yates) correction were also used. Pearson and Spearman correlation analysis were performed to compare the relationship between parametric and non-parametric variables. Quantitative variables are shown as mean ± SD (standard deviation) and median (Min / max). Categorical variables are shown as n (%). Variables were determined in the 95% confidence interval. A p value of <0.05 was accepted as the statistically significant.

Results

The demographic and laboratory data of patients with CD, UC and the control group are presented on Table 1. There were no significant differences between the three groups in terms of age and sex. Table 3 summarizes the distributions of genotypes TNF-α G308A genes in patients with IBD including CD, UC and healthy controls.

There was no statistically significant difference in genotype frequencies between all three groups (all $p>0.05$). TNF-α G308A polymorphisms were compared in terms of disease location, disease behavior, activity index (CDAI), use of anti-TNF-α treatment, and operative status in patients with CD (Table 4). There was no significant difference in terms of all these parameters.

Table 1. PCR and RFLP method

	TNF-α G308A
Primers	5'AGGCAATAGGTTTTGAGGGCC-3' 5' TCCTCCCTGCTCCGATTCCG-3'
Restriction Enzyme	NcoI

TNF-α; tumor necrosis factor alpha

Table 2. Characteristics of patients with CD and UC patients and control group

	CD	UC	HC	P1	P2	P3
Number (n)	45	46	129			
Age	40.42±11	41.96±11	42.18±12	0.09	0.49	0.31
Sex Female / Male	24/21	22/24	79/50	0.52	0.18	0.19
BMI	24.48±4.3	25.28 ±4.6	26.26±3.7	0.08	0.48	0.26
CRP	1.58±3.4	0.74±0.9	0.47±0.3	0.04	0.14	0.08
TNF-α	0.238±0.5	0.242±0.67	0.233±0.62	0.67	0.49	0.12
CD Behavior, n(%)						
Non-stricturing, non-penetrating	22(49)					
Stricturing	11(24)					
Penetrating	12(27)					
CD Location, n(%)						
Ileal	20(44)					
Ileocolonic	18(40)					
Colonic	7(16)					
Disease Activity Index*, n(%)						
Mild	23(51)	22(48)				
Moderate	14(31)	21(46)				
Severe	8(18)	3				
UC Location, n(%)						
Proctitis		14				
Left colon		11				
Pancolitis		21				
Treatment, n (%)						
Mesalazine	37 (77)	46 (100)				
Azathioprine	39 (80)	18 (32)				
Anti-TNF-α treatment	13 (20)	2(7)				

Mean± standard deviation, CD; Crohn's disease, UC; ulcerative colitis, HC; healthy control, CRP; C-reactive protein, BMI; body mass index, TNF-α; tumor necrosis factor alpha, p1; p-value between CD and HC, P2; p-value between UC and HC, p3; p-value between CD and UC, *;Truelove-Witts index (TW) in patients with UC, Crohn's Disease Activity Index (CDAI) in patient with CD,

TNF-α G308A polymorphisms in patients with UC were evaluated according to disease type, localization, activity index (TW), treatment and operative status. There was a significant relationship between disease activity and genotype in patients with UC disease (p=0.004), but there was no significant difference in other parameters (localization, anti-TNF-α treatment, or operative status) (Table 5).

Table 3. Distributions of genotypes of TNF-α G308A polymorphism in patients with inflammatory bowel disease including UC and CD and control groups

TNF-α 308 A/G polymorphism	AA	AG	GG
Healthy Control	1 (0.8%)	16 (12.4%)	112 (86.8%)
Ulcerative Colitis	1 (2.2%)	5 (10,9%)	40 (87%)
Crohn's Disease	3 (6.7%)	3 (6.7%)	39 (86.7%)

Chi-square test (all p>0.05)
TNF-α; tumor necrosis factor alpha

Table 4. Evaluation of TNF-α G308A polymorphisms in patients with CD in relation to disease type, localization, activity, treatment and operative status

Crohn's Disease		TNF-α G308A polymorphism			
		AA n(%)	AG n(%)	GG n(%)	P
Localization	Ileal	1(5)	1(5)	18(90)	
	Ileocolonic	0	2(11)	16(89)	0.106
	Colonic	2 (29)	0	5(71)	
Disease Behaviour	Non-stricturing, non-penetrating	2(9)	1(5)	19(86)	
	Stricturing	1(9)	1(9)	9(82)	0.835
	Penetrating	0	1(8)	11(92)	
Disease Activity (CDAI)	Mild	2(5)	1(5)	21(90)	
	Moderate	1(8)	1(8)		0.91
	Severe	0	1(12)		
Surgery	No	3(10)	1(3)	27(87)	0.283
	Yes	0	2 (14)	12(86)	
Anti-TNF-α treatment	No	1(8)	1(8)	12(84)	1.000
	Yes	2(6)	2(6)	28(88)	

CDAI; Crohn's Disease Activity Index, TNF-α; tumor necrosis factor alpha, Anti-TNF-α treatment; tumor necrosis factor alpha inhibitor treatment

Table 5. Evaluation of TNF-α G308A polymorphisms in patients with UC in relation to disease type, localization, activity, treatment and operative status

Ulcerative Colitis		TNF-α G308A polmorphism			
		AA n(%)	AG n(%)	GG n(%)	P
Localization	Proctitis	0	1(6)	13(94)	
	Left Colon	0	2 (25)	9 (75)	0.106
	Pancolonic	1 (4)	2 (9)	18(87)	
Disease Activity (TW)	Mild	0	0	22(100)	
	Moderate	0	5(24)	16 76)	0.004
	Severe	1(12)	0	2(88)	
Surgery	No	1(2)	5(11)	38(86)	1.000
	Yes	0	0 (14)	1 (86)	
Anti-TNF-α treatment	Yes	1(2)	1(5)	38(86)	1.000
	Yes	0	0	2(100)	

TW; Truelove-witts index, Anti TNF-α treatment; tumor necrosis factor alpha inhibitor treatment

Discussion

In this study, we investigated the association of TNF-α G308A polymorphism with disease characteristics and TNF-α levels in patients with IBD. In our study, the frequency of TNF-α G308A gene polymorphism was similar in IBD patients and healthy controls. However, there were a relationship between genotype and disease activity in patients with UC.

TNF-α is a major proinflammatory cytokine that has some local roles about inflammation and tissue damage on IBD pathogenesis [18]. Due to its important role on the pathogenesis of IBD, anti-TNF-α therapy gains an important role on the treatment of moderate and severe UC patients and Crohn's disease. Else, the role of TNF-α G308A gene polymorphism in IBD pathogenesis has been investigated in various populations. In the studies that conducted in Australia, France, Spain, Czech Republic, Italy and Belgium, there were no relationship between TNF-α polymorphism and IBD susceptibility [19-24]. In addition, there were no significant difference in the frequency of TNF-α G308A polymorphisms in the Iranian Azeri IBD population study [25] and another study that conducted in our country [26]. In our study, similar to these studies, no significant difference was found between TN group and UC and CD disease in terms of the frequency of TNF-α G308A gene polymorphism. In contrast, TNF-α 308A polymorphism has been reported to be associated with UC in two studies conducted in Chinese populations and in a study conducted in Japan [27-29]. Else, in another study conducted in the Saudi IBD population, TNF-α G308A polymorphism was found to be significantly higher when comparing with the control group [30]. In a systematic review and meta-analysis of IBD genetics, genetic mutations have been reported to differ from Asians to Caucasians. The difference between all these results may be related to the genetic differences of these populations.

On the other hand, several studies had reported that TNF-α G308A polymorphism is associated with IBD severity [19,22, 23]. It had been shown that individuals with TNF-α G308A polymorphism A allele were associated with an increased risk of pancolitis and a higher risk of surgical intervention [22]. In studies with CD patients, the TNF-α 308A allele and the AG genotype were significantly increased in those who did not respond to TNF-α inhibitors and steroid therapy [23]. The authors attributed these findings to the fact that this genotype causes more intense TNF-α release in the mucosa [22, 23]. Else, Individuals with TNF-α 308A allele showed greater CRP value and disease activity in the UC and CD groups [23]. In the study conducted by Wilson et al., Serum TNF-α levels were found about 6 to 7 times higher in individuals with TNF-α 308A allele [13]. Similarly, in our study, TNF-α levels were higher in individuals with AA genotypes. In our study, the frequency of A allele and AG genotype was found to be significantly increased in moderate and severe UC patients. These results are similar to the above mentioned studies [19,22,23] and may be associated with high levels of TNF-α in patients with TNF-α 308A alleles [13]. Similar to the above studies, GG genotype was significantly higher in mild UC patients. Some studies have reported that the A allele is associated with more severe diseases in patients with CD (22,23). However, as in our study, there is no relationship between disease severity and A allele and TNF alpha G308A genotypes in patients with CD. This may be related to sample size, ethnicity and ethnic genotypic differences. The limitation of the our study is the small sample size.

Conclusions

In conclusion, this study showed that there were no significant difference between the prevalence of TNF-α G308A polymorphism in Turkish IBD patients and healthy controls. However, TNF 308 A allele and AG polymorphism were associated with the severity of the patients that have ulcerative colitis. This gene polymorphism may be helpful for predicting the behavior of the disease in patients with ulcerative colitis but of course further study is needed in this regard.

Competing interests
The authors declare that they have no competing interests.

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Ethical approval
Written informed consent from the participants and approval from Umraniye Education and Resarch Hospital ethics committee (Number: 141). Committee based on World Medical Association Declaration of Helsinki.

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