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The polymorphism of catalase gene C-262T: Impact on ulcerative colitis

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Abstract

Ulcerative Colitis is a chronic inflammatory disease of the digestive tract. Reactive oxygen species (ROS) causes inflammation and are thought to play a role in the pathophysiology of Inflammatory Bowel Disease (IBD). Catalase, one of the antioxidant enzymes, decomposes hydrogen peroxide into oxygen and water and protects the cells from oxidative damage of reactive oxygen species. It has been reported that *CAT* gene promoter polymorphism has a protective effect on oxidative stress-related diseases. The aim of this study was to investigate the association of catalase *C-262T* polymorphism with ulcerative colitis and to determine whether *CATC-262T* polymorphism is a risk factor for the disease. We investigated 80 patients with ulcerative colitis and 90 controls in the Black Sea region in Turkey. After blood sampling, DNA was extracted from the peripheral blood, then we design 2 sets of primers for C and T alleles. The polymorphism was determined by using PCR technique and the statistical analysis was performed by Kolmogorov-Smirnow test. There were no significant association between frequencies of the *CATC-262T*s of the case and the control groups and genotype frequencies were very similar (P=0.996). This is the first report in regard to the association of *CATC-262T* polymorphism with ulcerative colitis in the Black Sea region in the Turkish population. According to the results of this study there were no significant differences in the genotype distribution and allelic frequency between patients and control groups.

Keywords: ulcerative colitis, catalase, ROS, allele frequency, gene polymorphism

1. Introduction

Ulcerative Colitis (UC) is a chronic inflammatory digestive system disease that occurs in the superficial part of the colon mucosa and sub mucosa, spreads from the rectum to the proximal and reaches deeper parts (1). UC can affect any age group, but the peak of the disease is between the ages of 15-25 and between the ages of 55-65, and UC is slightly more common in women than in men (2). Although its etiology still remains unclear, it is stated that environmental factors, genetic factors, immune and some infectious causes contribute to the process (3). Some clinical studies show that genetic factors increase the risk of developing (IBD) (4). The most important risk factor for IBD is positive family history. First degree relatives of 15% of IBD patients are also affected and the risk of developing IBD is 4-20 times higher in first degree relatives (5). Studies of twins have shown that the concordance rates in monozygotic twins are estimated at 16% and 4% for dizygotic twins (6).

It has been reported that the rate of compliance of this disease in monozygotic twins and dizygotic twins are 10% and 3% respectively (7). It is thought that the immune disorder and enteritis developed as a result of genetic predisposition initiates the pathogenesis of UC. Although genetic factors are primarily important for the development of IBD, genes alone are not sufficient for the progressionof the disease. In addition, complex environmental factors are important in disease formation (8). Reactive Oxygen Species (ROS) causing inflammation raise suspicion that it may play a role in the pathophysiology of IBD (9). Catalase, which enables the decomposition of hydrogen peroxide into oxygen and water in living cells, protects the cell from oxidative damages of reactive oxygen species (10). Catalase is present intensely in erythrocyte, liver, kidney and bone marrow cells, especially in peroxisome organelles of living organism . Catalase enzyme genepromoter polymorphism has a protective effect on oxidative stress related diseases (11, 12). For this purpose, the aim of this study was investigation of the role of *CATC-262T* polymorphism in the patients diagnosed with UC in the central Black Sea region of Turkiye.

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2. Materials and Methods

2.1. Study population

Blood samples were collected from 170 subjects consisting of 80 diagnosed ulcerative colitis patients (29 Females, 51 Males) and 90 healthy individuals (45 Females, 45 Males) The allele frequencies are assumed as PT = 88% and PC = 70% respectivly, therefore according to power analyse results were calculated (a power at least of 90 % and the use of 95% confidence interval) and the sample size are obtained as n1 = 80, n2 = 90, respectively.

All individuals in the patient and control groups were selected from individuals living on the Black Sea region in Turkey during 2013-2014 and gave informed consent for this study. Individuals in study groups have similar demographic characteristics. Genotype distributions of ulcerative colitis patients according to age groups were classified in three age groups as 17-29, 30-49, 50-75.

2.2. DNA Extraction

Blood samples were collected from participants into EDTA-containing (ethylenediaminetetraacetic acid) tubes and stored at -20 °C until DNA extraction. The whole genomic DNA was extracted from the blood samples by Vivantis GF-1 Kit (Vivantis, Malaysia) following the manufacturer's instructions. The quantity and quality of the extracted DNA were determined by spectrophotometer (Thermo Scientific-NanoDropOne) at a wavelength of 260/280 nm and agarose gel electrophoreses, respectively. Extracted DNA was kept at -20°C for further processing.

2.3. Polymorphism Genotyping

Determination of *CATC-262T* gene was performed according to method specified in the study of Khodayari et al. (13). The primers used in the study were shown in Table 1. Briefly, each PCR microtube was filled with the following materials and reached a volume of 50 µl: 25 µL of 2X PCR Master Mix (DNA amplification mixture containing Taq DNA Polymerase, reaction buffer, dNTPs, and MgCl2; 0.3 µM of each primer and 4 µL DNA template). The PCR program included the initial denaturation at 95 °C for 5 min, followed by 35 cycles of the denaturation step at 95 °C for 45 s, the annealing step at 56 °C for 45 s, and the extension step at 72 °C for 45 seconds, and the final extension step at 72 °C for 5 min. PCR products were separated on 2% agarose gel with an electrophoresis machine (Nanoboz-Turkey) (Vivantis, Malaysia) using a 130 V power supply for 20-30 min and then visualized under UV-transilluminator (Uvidoc HD6-England).

Table 1. Primers used to determine the genotype of catalase 262C/T polymorphism

Gene	primer	Sequence	Annealing Tm	Product size
C	F	GCCCTGGGTT CGGCTATC	56	400
	R	GGTTTGCTGTGCAGAACACT		
T	F	GCCCTGGGTTCGGCTATT	56	400
	R	GGTTTGCTGTGCAGAACACT		

2.4. Statistical Analysis

Evaluation of genotype and frequencies in controls and UC patients was done with a non-parametric Kolmogorov-Smirnow test and values of P <0.05 were considered statistically significant (14).

3. Results

The gender distribution was 51 (63.75%) males, 29 (36.25%) females in the patients, 50 (50%) males, and 50 (50%) females in the control groups. Genotype distributions of ulcerative colitis patients according to age groups were classified according to the age range of 17-29 (25%), 30-49 (33.75%), and 50-75 years (41.25%). Distribution of 17-29 and 30-49 age groups (P= 0.996ns) and distribution of 30-49 and 50-75 age groups (P= 0.518ns) is shown in Table 2 and 3. The highest and lowest frequency in patients related to age was observed in 50-75 (41.25%) and 17-29 years (25%) and for CC genotype respectively (p= 0.996). For CC genotype, the highest and the lowest frequencies in controls related to age was observed in 30-49 (38.8%) and 50-75 years (24.4%), respectively. Furthermore, the genotype and gender distribution of age for the patient and the control groups is shown in Table 4 and 5.

Table 2. Genotype age distribution of ulcerative colitis patients by groups

Age	Frequency	CAT -262C/T		
		CC	CT	TT
17-29	20 (25%)	15 (75%)	5(25%)	0
30-49	27 (33.75%)	18(66.66%)	6(22.22%)	3(11.11%)
50-75	33 (41.25%)	26 (78.78%)	5 (15.15%)	2(6.06%)

CATC-262T genotype distribution showed similarity in the patient and the control groups by gender (P= 0.996ns). CC-CT-TT genotype distribution of ulcerative colitis patients by gender was given in Table 2 and genotype distribution of the control group by gender is shown in Table 3. When *CATC-262T* polymorphism results of the ulcerative colitis and the

control groups were compared, out of 80 patients 58 (72.5%) were CC homozygous, 17 (21.25%) were CT heterozygous and 5 (6.25%) were TT homozygous. From out of 90 individuals (control group), 56 (62.22%) were CC homozygous, 30 (33.33%) were CT heterozygous and 4 (4.44%) were TT homozygous. According to the PCR results obtained, CAT gene polymorphism was detected in 60 of 78 patients. PCR products for two alleles were 340 bp in length (Fig. 1.).

Table 3. Genotype age distribution of control groups

Age	Frequency	CAT -262C/T		
		CC	CT	TT
17-29	33 (36.6%)	21 (63.63%)	10 (30.30%)	2 (6.06%)
30-49	35 (38.8%)	22 (62.85%)	13 (37.14%)	0
50-75	22 (24.4%)	8 (36.36%)	11 (50%)	3 (13.63%)

Table 4. CC-CT-TT genotype gender distribution of the ulcerative colitis patients

Gender	Frequency	CAT -262C/T		
		CC	CT	TT
Male	51	39	9	3
Female	29	19	8	2
Total	80	58 (72.5%)	17 (21.25%)	5 (6.25%)

Table 5. CC-CT-TT genotype gender distribution of the control group

Gender	Frequency	CAT -262C/T		
		CC	CT	TT
Male	45	25	18	2
Female	45	31	12	2
Total	90	56 (62.22%)	30 (33.3%)	4 (4.44%)

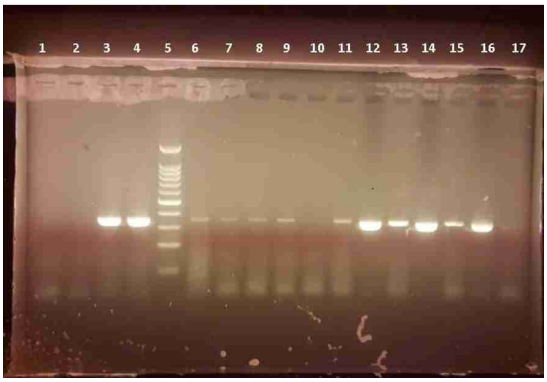

Fig. 1. Gel electrophoresis image of CAT -262C/T under UV-transilluminator 1-2: Negative control, 3: Positive control for C allele, 4: Positive control for T allele, 5: Marker (100bp) 6: DNA sample 1 amplified with the C allele primer, 7: DNA sample 1 amplified with the T allele primer, 8: DNA sample 2 amplified with the C allele primer, 9: DNA sample 2 amplified with the T allele primer, 10: DNA sample 3 amplified with the C allele primer, 11: DNA sample 3 amplified with the T allele primer, 12: DNA sample 4 amplified with the C allele primer, 13: DNA sample 4 amplified with the T allele primer, 14: DNA sample 5 amplified with the C allele primer, 15: DNA sample 5 amplified with the T allele primer, 16: DNA sample 6 amplified with the C allele primer, 17: DNA sample 6 amplified with the T allele primer, (Sample 1: CT, Sample 2: CT, Sample 3: TT, Sample 4: CT, Sample 5: CT, Sample 6: CC)

Statistical analysis showed that genotypes, ages and genders frequencies were not significantly different between the patient and the control groups (P= 0,996 ns). The SNP distribution of the control group was found compatible with Hardy-Weinberg Equation (HWE) (P= 0.007) (Table 6).

Table 6. C and T distribution in the ulcerative colitis and the control groups

	Ulcerative colitis (n=80)	Control (n= 90)	T test
C	133 (83.12%)	142 (78.88%)	$\chi^2 = 7.23$ p= 0.007
T	27 (16.87%)	38 (21.11%)	

4. Discussion

The results of our study indicated that the C allele frequency was found as 83% and 78% in the patient and the control groups, while the T allele frequency was 16% and 21%in the patient and the control groups. When the control and the patient groups were compared, the difference between TT and CC genotype distribution and T and C frequencies was found insignificant. These results reveal that there is no causal relationship between *CATC-262T* polymorphism and UC. The results obtained from this study are compatible with the results of other studies conducted in Turkey (15).

Many research studies show, it is informed that T is less common in the Asian population than C (15,17). On the other hand, "Khodayari et al. (13)" reported that the CC genotype had a protective role. Relation between catalase gene polymorphism and the risk of UC formation is not fully

understood (13). However, catalase activity seems to decrease in many diseases. This situation is associated with increased reactive oxygen species that inactivate the enzyme and cause DNA damage (18,19). In additional to many factors, oxidative stress is thought to be important in the development of ulcerative colitis. The catalase enzyme, which is among the antioxidant defense systems, shares this task with glutathione peroxidase enzyme (GPx) While GPx is effective at low H_2O_2 concentrations, catalase becomes more effective at higher concentrations. However, when the oxygen radicals increase excessively and the proxy/antioxidant balance shifts in favor of the proxydanes, oxidative stress damages the organism by various mechanisms (20, 21). Although the mechanism underlying the change in promoter activity under the influence of the catalase gene *CATC-262T* polymorphism is not fully understood, database searches are not shown that various hypothetical binding sites exist near the polymorphic site for transcription factors such as AP-2 and Sp-1 (20).

There are also conflicting reports in several studies about the role of the *CATC-262T* polymorphism C/C genotype on disease progression. "Ahn et al. (11)" reported that the C/C genotype reduced the risk of breast cancer by 17% compared to the C/T and T/T genotypes (22). Also, the role of the catalase *CATC-262T* gene polymorphism on the risk of prostate cancer (PCa) was investigated in some studies with contradictious results.

In a meta-analysis conducted by "Hu et al. (26)", researchers examined five studies with a total of 3865 cases and 28224 controls (12, 23, 24, 25) and stated that there is a positive correlation between *CATC-262T* polymorphism and the development of prostate cancer (26). In other study, "Jamhiri et al. (27)", investigated CAT mRNA levels in the blood with mutations in the CAT promoter region and reported that CAT mRNA levels in the TC/TC and TT/TT diploids were 2 to 4 times higher than AC/AC diploids. While "Forsberg et al. (20)" reported that T transcription was higher in their study (20), "Ahn et al. (11)" reported that the C was associated with higher enzyme activity in red blood cells (22). Also, "Chistiakov et al. (28)" and "Zotova et al. (29)" reported that T reduced the risk of diabetic nephropathy compared to the C (28, 29). "Mak et al. (17)" reported that the T reduced the risk of asthma in non-smokers. Contradictory results were obtained in the studies in which gene expression was followed by transferring cloned genes into cells (17). It is thought that these contradictory results between studies may be due to usingdifferent techniques to track enzyme activity or mRNA levels, genetics and living conditions between patient and control populations (nutritional, contact with infectious agents, vital stress, etc.). For example, in a study conducted in France, it is reported that thecatalase enzyme activity decreased with age, while an increase by aging to lesser extent was observed in Turkey (15). Another reason for the difference in results is that the relationship between *CATC-262T* polymorphism and UC stages is not evaluated. Considering oxidative stress and oxidative damage caused by free radicals lead to the development of disease in some people, while in others, not causing diseases on other people highlights the genetic difference between individuals (15, 30).

In this study, we investigated the relationship between *CATC-262T* polymorphism and UC. Statistically no significant relationship was observed. However, it is recommended to conduct more comprehensive studies in wider experimental groups including factors such as expression of the catalase enzyme gene and different UC stages.

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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None to declare.

Authors' contributions

Concept: N.N.M., H.B., Design: N.N.M., H.B., Data Collection or Processing: B.Y., İ.B., T.A., Analysis or Interpretation: N.N.M., H.B., Literature Search: N.N.M., H.B., Writing: N.N.M., H.B.

Ethical statement

Ethical committee permission was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee (2013/311).

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