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Possible relationship between the resistin gene C-420G polymorphism and colorectal cancer in a Turkish population

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Original Article

ABSTRACT

Background/Aims: In recent years, with improvements in genotyping, a possible relationship between obesity-related gene polymorphisms and colorectal cancer (CRC) has been studied. The promoter region C-420G of the resistin gene is believed to have an important role in the development of malignancy. We prospectively evaluated the possible effect of the resistin C-420G polymorphism on the risk and prognosis of CRC.

Materials and Methods: One hundred twenty-three patients with CRC and 79 healthy individuals were included in the study. Blood samples were genotyped, and the relationship between the resistin C-420G polymorphism and demographic characteristics and tumor features was evaluated.

Results: No statistically significant difference was found in genotype distribution between the patient and control groups and among patients in the means of gender, biochemical findings, and tumor characteristics ($p>0.05$).

Conclusion: The relationship between the C-420G polymorphism and various diseases has been evaluated in many studies to date. With the increased importance of obesity in etiopathogenesis, studies have focused on CRC. According to our results, the GG genotype may be associated with a decreased CRC risk. Our study is important because to our knowledge, it is the first one to be conducted in a Turkish population to date, but we believe that more patients and controls are needed to obtain statistically significant results.

Keywords: Colorectal cancer, gene polymorphism, resistin

INTRODUCTION

Colon and rectum cancers are together termed as colorectal cancer (CRC), which is one of the most common malignancies with high mortality and morbidity rates worldwide. Nearly 1 million newly diagnosed cases are observed every year (1). As in many cancer types, the patient's characteristics and familial predisposition as well as additional factors such as nutritional factors, alcohol, smoking, and obesity play a role in the development of CRC (2). In recent years, increasing information about the molecular and biological characteristics of CRC provides details on the relationship between genetic predisposition and environmental factors and the pathogenesis of CRC. Numerous genetic association studies have revealed possible interactions between certain genetic polymorphisms and the development of cancer. Various epidemiological studies have shown a relationship between CRC and adiposity (3,4).

Today, obesity is thought to be an important risk factor in cancer development and is also thought to be independent of gender and geographical characteristics (5). Adipocytes in adipose tissue include all enzymes necessary for lipogenesis and lipolysis. Adipose tissue is metabolically active in visceral fat. Adipocytokine differentiation is controlled by nuclear transcription factor and peroxisome proliferator-activated receptor. In vitro studies have shown that adipocytokines play a role in carcinogenesis (6).

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Resistin is a peptide having a molecular weight of 12.5 kDa and is rich in cysteine. It is released from the bone marrow, peripheral mononuclear cells, lungs (7), placental tissues (8), and pancreatic beta cells (9). It has been shown that resistin has strong proinflammatory effects on tissues by activating proinflammatory cytokines (10). In previous studies, nine different single-nucleotide polymorphisms were found on the human resistin locus. The resistin gene (RETN) C-420G polymorphism (r1862513) is one of the most common polymorphisms among them. It is a single nucleotide transition on the first exon of the gene (11), and it is found to be associated with a high body mass index in males and GG genotype with insulin resistance (12). Chung et al. (13) found that the C-420G allele stimulates the promoter activity of the gene, and Cho et al. (14) reported that this allele increases serum resistin levels. The G allele in the C-420G polymorphism has been found to stimulate promoter activity and is associated with high resistin mRNA levels in abdominal fat (15). Wagsater et al. (16) found higher levels of resistin protein in the tumor tissues of CRC patients than in those of healthy people. The proinflammatory effect has been suggested to be an alternative mechanism in the relationship between CRC development and resistin (17).

In our study, we aimed to evaluate the effect of resistin gene polymorphism on CRC development and prognosis. The polymorphism of the RETN C-420G promoter region of the gene has been studied in different populations to date to determine its relationship with CRC. Although the serum levels of the resistin gene have been previously studied, to our knowledge, our study is the first one to date that investigates the RETN C-420G promoter region polymorphism in CRC patients in a Turkish population and may contribute to understanding the clinical course of the disease.

MATERIALS AND METHODS

Study participants and clinical investigation

The study group comprised 123 colorectal cancer (CRC) patients among patients of general surgery and internal medicine clinics. The control group comprised 79 healthy volunteers, and they were selected from among patients in the general surgery clinic of the same hospital and who were treated because of non-neoplastic diseases, such as inguinal hernia and pilonidal sinus. The patients were all histopathologically confirmed to have CRC and were surgically treated before any radiotherapy or chemotherapy. Blood specimens were analyzed for the RETN C-420G polymorphism for both groups, and the results were compared in the means of patient demographic characteristics, biochemical results, and tumor characteristics.

The study protocol was approved by the Ethics Committee of İstanbul Education and Research Hospital, and all participants signed an informed consent form in accordance with the ethics guidelines regarding the study (240/2013).

Polymerase chain reaction-based detection of the RETN C-420G polymorphism/genotyping

Blood specimens were collected in tubes containing EDTA, and DNA samples were extracted from whole blood with a salting out procedure (18). The DNA samples were analyzed for the RETN C-420G polymorphism by polymerase chain reaction (PCR) with locus-specific primers and subsequent analysis of a restriction fragment length polymorphism (RFLP). The primers for the PCR amplification of the 420 locus were 420F (5′- TGT CAT TCT CAC CCA GAG ACA -3′) and 420R (5′- TGG GCT CAG CTA ACC AAA TC -3′). The PCR products were excised by BpiI restriction enzyme, and the DNAs were then separated on a 2% agarose gel; genotyping was conducted by visualization under ultraviolet light.

Statistical analysis

Statistical analysis was performed using SPSS software package (version 11; SPSS, Inc., Chicago, IL, USA). Differences in the distribution of genotypes and alleles between the two groups were tested using the chi-square test and were associated with patient and tumor characteristics. A p value of less than 0.05 was considered to be statistically significant. The associations in numerical values between the groups were analyzed by Student's t-test or ANOVA and Mann–Whitney U or Kruskal–Wallis tests depending on data distribution. The chi-square and/or Fischer's test were also performed to compare the results. Spearman's coefficient was applied to test for bivariate correlations. The values of negative results are not mentioned in the text and are shown in the tables.

RESULTS

The characteristics of the patients with CRC and the controls are shown in Table 1. We did not observe any associations in age or sex in any of the participants ($p>0.05$). In the blood specimens of the participants in both groups, various biochemical parameters that may show significance for CRC were studied as well as DNA isolation. These results are shown in Table 2 along with the physical characteristics of the participants. We did not observe any significant differences between these results and genotype frequencies with either the chi-square and/or Fischer's tests ($p>0.05$). However, there were significant differences in the numerical values of albumin, cholesterol, LDL, and HDL ($p<0.05$).

In the CRC group, tumor localization and stage are shown in Table 1. When the specimens were histopathologically studied, it was observed that 22.2% of the tumors were well differentiated, 65.3% were moderately differentiated, and 12.5% were poorly differentiated. The most common site for tumor localization was the sigmoid colon, and most common tumor stage was 2A. The respective frequencies of the CC, CG and GG genotypes were 43.1%, 49.6%, and 45.6% in the CRC patients and 39.2%, 45.6%, and 15.2% in the controls. There was no significant association between the RETN C-420G genotypes and alleles in the CRC patients and controls ($p>0.05$ each) (Table 3).

Table 1. Characteristics of patients with colorectal cancer and controls

	Patients n (%)	Controls n (%)
No. of patients	123	79
Age (years) (mean±standard deviation)	60.56±13.67	60.92±11.40
Male	75 (61.0)	42 (53.2)
Female	48 (39.0)	37 (48.8)
Tumor localization		
Left colon	20 (16.2)	-
Right colon	17 (13.8)	-
Transverse colon	6 (4.8)	-
Sigmoid	44 (35.7)	-
Caecum	7 (5.6)	-
Rectum	29 (23.5)	-
Stage		
1	17 (13.8)	-
2	42 (34.15)	-
3	37 (30.09)	-
4	27 (21.95)	-

n: number of individuals; CRC: colorectal

Table 2. Physical characteristics and biochemical findings

	Group	Average	p value
Age	CRC	60.56±13.67	>0.05
	Control	60.92±11.40	
Weight (kg)	CRC	71.47±10.91	>0.05
	Control	73.0±7.26	
Height (cm)	CRC	167.98±8.31	>0.05
	Control	168.73±8.99	
Albumin (g/dL)	CRC	3.62±0.71	<0.05
	Control	3.95±0.60	
Cholesterol(mg/dL)	CRC	164.36±33.78	<0.001
	Control	196,72±40,20	
Triglyceride (mg/dL)	CRC	120.46±45.67	>0.05
	Control	148.24±80.80	
HDL (mg/dL)	CRC	41.10±12.54	<0.05
	Control	49.70±12.78	
LDL (mg/dL)	CRC	99.08±25.26	<0.01
	Control	121.27±27.88	
LDH (Unit/L)	CRC	242.39±93.74	>0.05
	Control	244.70±99.74	

n: number of individuals; CRC: colorectal

The distribution of the RETN C-420G genotype was shown in the CRC patients in the means of tumor characteristics. There were no statistically significant results between the RETN C-420G genotypes and tumor grade, lymph node positivity, distant metastasis presence, angiolymphatic invasion, perineural invasion, and mucinous component (p>0.05 for each parameter) (Table 4).

Table 3. Genotype and allele distribution of the RETN C-420G polymorphism

Genotype and allele	CRC n (%)	Control n (%)	p value
Resistin			
CC	53 (43.1)	31 (39.2)	
GG	9 (7.3)	12 (15.2)	>0.05
CG	61 (49.6)	36 (45.6)	
C allele	167 (67.9)	98 (62.0)	>0.05
G allele	79 (32.1)	60 (38.0)	

n: number of participants; chi-square (χ^2) test was used between the groups.
RETN: resistin; CRC: colorectal

DISCUSSION

Like all other cancer types, CRC is caused by defects in genetic and epigenetic mechanisms, which consequently effect angiogenetic factors, cell cycle regulators, growth factors, or DNA repair genes. In recent years, because of developments in molecular polymorphism studies, individual characteristics of patients have gained importance in cancer development.

Resistin is a peptide hormone that is released from not only adipose tissue but also monocyte/macrophages and is related to proinflammatory cytokines such as C-reactive protein, TNF-α, and IL-6 (10), which affect the NF-κB signaling pathway. Till today, conflicting results were reported about the relationship between resistin and metabolic syndrome, obesity, various chronic diseases, and CRC.

Danese et al. (19) suggested in their study that the serum level of resistin is an independent risk factor for CRC. Nakajima et al. (20) reported that serum resistin levels are significantly higher in CRC patients than controls and that these levels also correlate with tumor stage. Salageanu et al. (21) found increased levels of resistin in both pre- and postoperative periods when compared to controls.

Nine nucleotide polymorphisms have been declared in RETN to date, and the C-420G polymorphism, which is a single nucleotide transition on the first exon of the gene, is the most important among them (11). The polymorphism on this promoter region has been reported to increase the serum levels of resistin by inducing mRNA synthesis (22). Wagsater et al. (16) found high levels of resistin in the tumor tissue of CRC patients in a Swedish population but no significant relationship between the C-420G polymorphism and CRC development.

In our study that was conducted in a Turkish population, we evaluated the same promoter region and its relationship with clinical and histopathological findings in CRC patients and compared it with a healthy control group. We did not find any statisti-

Table 4. Distribution of the RETN C-420G genotypes with different histopathological parameters

	Resistin C-420G		
	CC n (%)	**CG n (%)**	**GG n (%)**
Gender			
Female	18 (37.5)	29 (60.4)	1 (2.1)
Male	35 (46.7)	32 (42.7)	8 (10.7)
T stage			
T3+T4	43 (44.3)	47 (48.5)	7 (7.2)
T1+T2	10 (40.0)	13 (52.0)	2 (8.0)
Lymph node status			
N1+N2	24 (43.6)	25 (45.5)	6 (10.9)
N0	27 (45.0)	30 (50.0)	3 (5.0)
Metastasis			
Positive	14 (48.3)	13 (44.8)	2 (6.9)
Negative	32 (42.7)	36 (48.0)	7 (9.3)
Angiolymphatic inv.			
Positive	13 (48.1)	13 (48.1)	1 (3.7)
Negative	45 (49.5)	38 (41.8)	8 (8.8)
Perineural inv.			
Positive	13 (36.1)	19 (52.8)	4 (11.1)
Negative	38 (47.5)	37 (46.2)	5 (6.2)
Differentiation			
Well	6 (33.3)	11 (35.0)	1 (5.0)
Moderate-poor	26 (48.1)	24 (44.4)	4 (7.4)
Mucinous component			
Positive	8 (38.1)	12 (57.1)	1 (4.8)
Negative	20 (33.3)	34 (61.1)	3 (5.6)

n: number of patients; chi-square (χ^2) test was used between the groups.
inv: invasion

cally significant result in the means of genotype distribution and allele frequency (p>0.05). In the CRC patients, the frequencies of the CC, CG, and GG genotypes were 43.1%, 49.6%, and 45.6%, respectively. Although the results were not statistically significant (p>0.05), the GG genotype may be related to a decreased CRC risk, which differs from the study by Mahmoudi et al. (23) in an Iranian population, where they found that the CC genotype was related with a decreased CRC development in the population.

In the control group, the frequencies of the CC, CG, and GG genotypes were found to be 39.2%, 45.6%, and 15.2%, respectively. The values were similar to the frequencies in the healthy control group in a study by Suriyaprom et al. (24) in a Thailand population, in which the frequencies were 37.0%, 39.4%, and 13.6%, respectively. Wagsater et al. (16) reported frequencies of 53.5%, 40.2%, and 6.3%, respectively, in their control group in a Swedish population, similar to the results of Pechlivanis et al. (25) in a Czech population. These results were different from those of the Turkish and Thailand population. Without any statistical significance (p>0.05), the GG genotype had a lower frequency in females and in patients with metastases and angiolymphatic invasion.

Our study is a preliminary study evaluating the RETN C-420G promoter region polymorphism and its relationship with CRC in a Turkish population. Although we did not establish statistically significant results, our findings show that various allele polymorphisms of this promoter region may be related to CRC development. One of the limitations of our study was the number of CRC patients. We believe that further studies with larger study groups including the serum levels of resistin are needed to define an exact interaction between CRC and the resistin gene, which will show ethnic differences between different populations in the world.

As authors of this manuscript, we declare that we do not have any conflict of interests.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul Education and Research Hospital 240/2013.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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