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The Role of Mannose-Binding Lectin-2 Gene Polymorphisms in Patients with Colorectal Cancer

[Kolorektal kanserli hastalarda mannoz bağlayıcı lektin-2 gen polimorfizmin rolü]

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ABSTRACT

Purpose: The mannose-binding lectin pathway of innate immunity is part of the first line of defense against microorganisms. Variations in mannose-binding lectin levels or activity are caused by single-nucleotide polymorphisms in the promoter region. Mannose-binding lectin may play a role as a phase reactant due to inflammatory processes related to cancer disease. Such inflammatory processes are well known in colorectal cancer and have been shown to be independent of stage of disease. We aimed to investigate whether profile of mannose-binding lectin-2 -221G>C genotyping may be associated with the risk of colorectal cancer.

Materials and Methods: The study group consisted of 107 patients were diagnosed with histologically confirmed cancer of the colon or rectum. The control group consisted of 99 were selected among healthy people. Genomic DNA of control and patients was extracted from whole blood using High Pure PCR template preparation kit. Genotyping of mannose-binding lectin-2 polymorphisms were detected by using a mannose-binding lectin-2 mutation detection kit in real-time PCR. Chi-square or Fisher's Exact Tests were used to evaluate the distribution of the -221G>C genotypes among the patients and control subjects. Associations between -221G>C genotype and colorectal cancer risk were analyzed by binary logistic regression**.**

Results: Logistic regression analyses showed that the mannose-binding lectin-2 GC genotype was associated with a significantly increased risk of colorectal cancer. The odds ratio of colorectal cancer for the mannose-binding lectin-2 GC genotype was 2.095 (95% CI=1.76-3.731, *P*=0.012) compared with the control group.

Conclusion: These results suggest that mannose-binding lectin-2 G/C allele gene polymorphisms may be associated with genetic susceptibility of colorectal cancer.

Keywords: Colorectal cancer, mannose-binding lectin, polymorphism

ÖZET

Amaç: Doğal bağışıklığın mannoz-bağlayıcı lektin yolağı mikroorganizmalara karşı ilk savunma hattıdır. Promotör bölgedeki tek nükleotid polimorfizmlerin mannoz-bağlayıcı lektin düzeyinde ya da aktivitesinde değişime neden olmaktadır. Mannoz-bağlayıcı lektin kanser ilişkili enflamatuvar süreçten dolayı faz reaktantı olarak önemli rol oynamaktadır. Bunun gibi enflamatuvar süreçler kolorektal kanserde bilinir ve hastalığın evrelerinden bağımsız olduğu gözlenmiştir. Bu çalışmamızda, mannoz-bağlayıcı lektin-2 -221G>C genotip dağılımın kolorektal kansere ilişkin risk faktörü olup olmadığını araştırmayı amaçladık.

Gereç ve Yöntemler: Çalışma grubu cerrahi ve histopatolojik olarak kolon veya rektal kanseri tanısı konulan 107 hastadan oluşmaktadır. Kontrol grubu ise 99 sağlıklı birey arasından seçilmiştir. Kontrol ve hastaların genomik DNA'ları High Pure PCR template preparation kiti kullanılarak tam kandan izole edildi. Mannoz-bağlayıcı lektin-2 polimorfizmi mannoz-bağlayıcı lektin-2 mutasyon belirleme kiti kullanılarak Real-time PCR ile saptandı. Hasta ve kontrol grubu arasındaki -221G>C genotip ve allel dağılımlarını değerlendirmede Ki kare ya da Fisher's Exact testi kullanıldı. -221G>C ve kolorektal kanser arasındaki risk ilişkisi binary logistik regresyon ile analiz edildi.

Bulgular: Mannoz-bağlayıcı lektin-2 GC heterozigot genotipin kolorektal kanserdeki artmış riskle ilişkili olduğu logistik regresyon analizi ile saptandı. Mannoz-bağlayıcı lektin-2 GC genotipi kontrol grubuna göre kolorektal kanser için risk oranı 2.095 (95% Güven Aralığı=1.76-3.731, *P*=0.012) olarak belirlendi.

Sonuç: Bu araştırma sonuçları; mannoz-bağlayıcı lektin-2 G/C allel gen polimorfizmlerinin kolorektal kansere genetik yatkınlık ile ilişkili olabileceğini düşündürmektedir.

Anahtar Kelimeler: Kolorektal kanser, mannoz-bağlayıcı lektin, polimorfizm

Introduction

In developed countries colorectal cancer is the second most common cancer. Epidemiologic evidence suggests that many of the geographical variations reflect variations in environmental or lifestyle exposures, perhaps acting with variations in genetic factors [1]. Infections and inflammation promote the development of human cancer [2,3]. Both the adaptive and innate immunity regulate survival and migration of tumor cells, and provide important protection against malignancies [2,4,5]. Complement activation is a central mechanism in innate immune response and leads to the formation and liberation of pro-inflammatory factors as well as activation of inflammatory cells, which in turn, contribute to tumorigenesis [6-8]. Mannose-binding lectin (MBL) may play a role as a phase reactant due to inflammatory processes related to cancer disease. Such inflammatory processes are well known in colorectal cancer tissue and have been shown to be independent of stage of disease [9]. MBL-2 is a key regulator of the innate immune response and a prime candidate for genetic association studies in cancer because of its genomic heterogeneity that causes low and high expression phenotypes [10]. Functional relationships between MBL-2 secretor haplotypes and alterations in complement activation have been demonstrated [11-15]. MBL is encoded by the MBL-2 gene and is a central component of the innate immune system. MBL binds to specific carbohydrate structures on the surface of microorganisms and activates both the lectin complement system and complement-independent opsonization by monocytes [16]. Variations in MBL levels or activity are caused, in part, by two single-nucleotide polymorphisms (SNPs) in the 5' regulatory region at positions C-550G (H/L) and G-221C (Y/X) and in the 5' untranslated sequence at position C+4G (P/Q), and three SNPs in the first exon, known as the D (codon 52 CGT to TGT), B (codon 54,GGC to GAC),, and C (codon 57,GGA to GAA) variant alleles; the A allele for the SNPs in the first exon is the wild-type allele [17]. Pine and *et al*. found that GC (XY) or CC (X/Y) genotype, corresponding to lower levels of serum MBL [17]. Previous studies reported that MBL2 -221G>C genotypes frequency were found 78.9% for GG, GC 19.3% and CC 1.8% in Japanese population, 51%, 40.8% and 8.1% in Caucasian, 75%, 22.9% and %2.1 in African-American, 81.1%, 18.9% and in Hispanic, respectively [18].

In numerous studies, associations between variant MBL-2 alleles and susceptibility to infectious and autoimmune diseases have been reported [19-22]. There is also emerging evidence that MBL-2 variants are risk factors in human cancer [23].

Although much work has been conducted on the relationship between MBL-2 genetic polymorphisms and some cancers such as gastric [24], breast [25], ovarian [26], lung [17] and stomach cancer [23]. Our knowledge, this is the first report on the association between MBL-2 genetic polymorphisms and colorectal cancer published in the English. The purpose of this study was to examine the association between profile of MBL-2 -221 G>C genotyping and the risk of colorectal cancer.

Material and Methods

Patients

This case-control study was conducted between 2008 September to 2009 July. This study includes all cases of colorectal cancer consecutively hospitalized in the surgery departments of Mersin University Hospital. The 107 newly diagnosed colorectal cancer patients (48 female, 59 male) and 99 unrelated controls (46 female, 53 male) were recruited from hospital. Cases of consist of patients with positive colonoscopic results for malignancy and histologically confirmed as the colon or rectum carcinomas. Cases with a prior history of partial or total removal of the colorectum and inflammatory bowel disease were excluded from study. Controls were sampled at the same time as cases and chosen among subjects undergoing colonoscopy due to macroscopic bleeding, positive fecal occult blood test, or abdominal pain of unknown origin. Only subjects whose colonoscopic results were negative for malignancy, colorectal adenomas or inflammatory bowel diseases were chosen as controls. Control subjects with history of cardiovascular disease, cancer, chronic degenerative neurological disease, chronic obstructive pulmonary disease, hepatitis, diabetes, hypertension, atopy, autoimmune diseases and history of familial colorectal cancer or allergies in general were excluded from study. Data were collected through a medical interview, based on a standardized questionnaire. Information on tumor site was abstracted from the medical records of the patients. All subjects were systematically interviewed about their smoking status. The investigations were approved by the Medical Ethical Review Committee of the Academic Hospital of the University of Mersin, and informed consent was obtained from all patients and controls according to the Helsinki Declaration II (1975, revised 1983).

DNA extraction and genotyping of MBL-2 -221G>C

Heparinized venous blood samples were collected from women in the study and control groups. Immediately after collection, whole samples were stored at +4 ^{0}C until use. Genomic DNA was extracted from circulating leukocytes by high pure real-time polymerase chain reaction (PCR) template preparation kit (Roche diagnostics, GmbH, Mannheim, Germany).

The genotyping of polymorphisms of MBL-2 -221G>C was performed using a Real-time PCR instrument (Light Cycler 2.0, Roche Diagnostics, Germany) using hybridization probes in combination with a Light Cycler DNA Master Hybridization Probes Kit (Roche Diagnostics). Both the PCR primers and hybridization probes were

synthesized by Tib Molbiol (Berlin, Germany). For MBL-2 -221G>C genotyping the following primer and probe sequences were used. For genotyping of the variants located upstream of the coding sequence, the forward primer 5'-CCT GCCAGAAAGTAGAGAGG-3' and the reverse primer 5'-CCTCACCTTGGTGTGAGAAA-3' were used to generate PCR fragments of 706 bp spanning the 5' regulatory region at position -221G>C (X/Y). For GC the PCR included two hybridization sets, one for each locus since multiplexing by colour and Tm allows simultaneous genotyping of each mutation. The GC probe set included a detection probe 5'-TCTCACTG-CCACGGAAGCAT-3' FLU, which was complementary to the C allele and an anchor probe 5'LC Red 640-TTTA-TAGTCTTCCA GCAGCAACGCCA3'-P. The anchor probes were able to bind within a distance of one and two bases from the detection probe for GC. The PCR conditions used were essentially those described by Steffensena et al [27].

Statistical analysis

Chi-square (χ^2) or Fisher's Exact Tests were used to evaluate the distribution of the MBL-2 -221G>C genotypes among the patients and control subjects. Associations between MBL-2 -221G>C genotypes and colorectal cancer risk were analyzed by binary logistic regression. The age of both groups were compared with Independent Sample t-test. χ^2 test were used to evaluate the distribution of the sex and smoking among the patients and control groups. Test for Hardy–Weinberg equilibrium was conducted by comparing observed versus expected genotype frequencies using a χ^2-test. Age values are represented as mean and standard deviation (SD). Power calculations were performed using PASS software package program (Utah, USA), version 11.0 for Windows. To test for this difference (desired study power, 80%; α-error = 0.05, two-tailed) the number of subject for each group enrolled in the study would be 90. Therefore, the study included 107 patients and 99 controls. All statistical calculations were performed using the SPSS software package version 11.5 for Windows (SPSS Inc., Chicago, IL). All tests were conducted at the $P < 0.05$ level of significance.

Results

Table 1 summarizes the distribution of ages, sex and smoking between the groups. The mean age of the colorectal cancer group was 59.1±12.8 years (range, 56 to 61 years) and 50.1±12.5 years (range, 47 to 52 years) in the control subjects. The mean age was significantly different between the groups significantly ($P<0.05$). The sex did not differ between the groups significantly ($P>0.05$). In this study, smoking status did not differ between the colorectal cancer and control groups.

The frequency distribution of MBL-2 -221G>C genotypes in 99 healthy subjects and 107 patients was determined by using real-time PCR. Regarding both groups (controls and patients with colorectal cancer); the distribution of MBL-2 -221G>C genotypes was in Hardy–Weinberg equilibrium (P=0.125, P=0.956 respectively). Statistically significant differences were found between the patient and control groups in the frequency of gene polymorphism at MBL-2 -221G>C As shown in Table 2, the frequencies of GG, GC and CC were 36.4, 55.2 and 8.4%, respectively in the colorectal cancer group and 54.5, 39.4 and 6.1%, respectively in the control group. The relative risk for the GC genotype was 2.095 (95% CI: 1.176-3.731 P=0.012) for the colorectal cancer cases compared with the controls. The relative risk for the CC genotype was 2.077 (95% CI: 0.683-6.315) for the colorectal cancer cases compared with the controls. But there was no significant association with the CC and developing colorectal cancer. Additionally, the frequency of MBL-2 C allele was found to be 25.8% in colorectal cancer and 36% in the controls, indicating that the individuals carrying this C allele had an increased risk of colorectal cancer (OR: 1.62, CI: 1.061-2.474, p=0.026).

We determined the distribution of colorectal cancer sites in the population studied (left, right and rectal; Table 3). The majority of the tumors were rectal tumors (66.4%). Left-sided tumors constituted 21.4% and right-sided tumors constituted 12.2% of the total number. We then determined the distribution of MBL-2 GC genotypes in relation to tumor site. Our data revealed an over-representation of the GC genotype in rectal, left and right-side tumors, where 57.7, 47.8, 53.8% respectively.

All the 107 cases were adenocarcinoma among these, 79.4% (56 moderately differentiated, 29 well differentiated) were reported as non-mucinous adenocarcinoma, 15.9% (7 moderately differentiated, 10 well differentiated) as mucinous adenocarcinoma and 4.7% (n=5) as signet-ring cell type carcinoma. In this study, the association between MBL2 -221G>C genotypes and various histological types of colorectal carcinomas showed that the over-representation of the GC genotype in adenocarcinomas, mucinous adenocarcinoma and as signet-ring cell type carcinoma, where 54.1, 58.8, 60% respectively. But there was no significant association with the MBL-2 -221G>C genotypes and histological types of colorectal carcinomas ($P>0.05$).

Discussion

In the present study, we analyzed the relationship between colorectal cancer and MBL-2 221G>C genotype. In the our study, we reveal that GC genotype is more frequent in rectal, left and right-side tumors than in CC and GG genotypes.

In the present study, we found that patients carrying GC genotype have a 2.095 fold colorectal cancer risk. CC genotype is more frequent in patients with colorectal cancer than in controls, but statistical significance is lacking. The absence of statistical significance may be a result of the insufficient number of patients and controls.

Table 1. Demographic characteristics of the patients and controls.

Variable	Cases (n=107)	Controls (n=99)	OR (95% CI)	*P* value
Age (years)	59.07±12.89	50.12±12.55		0.001
Sex				
Female	48 (55.1)	46 (44.9)		0.654
Male	59 (53.5)	53 (46.5)		
Smoking habit (n %)				
Non-smoking	47 (50)	47 (50)	(Reference)	
Smoking	60 (53.6)	52 (46.4)	1.154 (0.666-1.998	0.609

n: number of sample, p: values of significance with difference of each group. Note that values are given as the mean ± SD or n (%). OR (odds ratio); CI (confidence interval)

Table 2: MBL2 -221G>C genotypes and the risk of developing colorectal cancer.

Position of SNP on MBL gene	Colorectal cancer group N (%)	Control group N (%)	OR (95% CI)	P value
-221G>C				
GG*	39 (36.4)	54 (54.5)	(Reference)	
GC	59 (55.2)	39 (39.4)	2.095 (1.176-3.731)[a]	0,012
CC	9 (8.4)	6 (6.1)	2.077 (0.683-6.315)[a]	0.198
Allel				
G	137 (74.2)	147 (64)	(Reference)	
C	51 (25.8)	77 (36)	1.62 (1.061-2.474)	0.026

n: number of sample**,** Ors; odds ratio, CI; confidence interval, *GG genotypes are used as reference.

Table 3: MBL2 -221G>C genotypes frequencies related to site of colorectal cancer

	MBL-2		
	GG	***GC***	***CC***
Rectal-sited tumor	27 (38)	41 (57.7)	3 (4.3)
Right-sited tumor	7 (30.5)	11 (47.8)	5 (21.7)
Left-sited tumor	5 (38.5)	7 (53.8)	1 (7.7)

Our results suggest that the GC genotype indicating an association between this genotype and rectal, left, right-sided tumors.

Wang *et al*. [28] investigated the associations between polymorphism at codon 54 of exon 1 of the MBL gene and ulcerative colitis. They found the frequency of polymorphism at codon 54 of exon 1 of the MBL gene was significantly higher in the relapsing cases than that in controls. It indicates that polymorphism of the MBL gene may be associated with an increased risk for the flare-ups of ulcerative colitis. Recent studies indicate that enteric pathogens could cause initial onset of ulcerative colitis and are associated with reactivation of quiescent disease. Despite their self-limited character, these infections initiate a cascade of inflammatory events leading to chronic, relapsing disease in a genetically susceptible host ("hit-and-run" hypothesis) [29,30]. Epidemiological and microbiologic studies also suggest that enteropathogenic microorganisms play a substantial role in the clinical initiation and relapses of inflammatory bowel disease [31]. Based on these results, MBL gene polymorphism may increase susceptibility of an individual to enteric infection, which induces the relapse of inflammation in patients with chronic ulcerative colitis. Also, these results indicated that MBL-2 gene polymorphism may be an important role in colon inflammatory pathway. In present study, patients carrying GC genotype may leads to the lower levels of plasma MBL, this results in lower levels of circulating MBL in colorectal cancer. Thus, the presence of high frequencies of variant MBL alleles that are responsible for low MBL levels may confer relative protection against some infections or, alternatively, may result in a reduction of the deleterious effects of complement-mediated inflammation.

Infections and inflammation promote the development of human cancer [2,3]. MBL has also been suggested to

have a cytotoxic effect on colon adenocarcinoma cells in vitro [32,33] and in vivo [34]. The MBL concentration in serum and the activity of the MBL pathway is generally increased in patients with colorectal cancer [35]. Insufficient levels of MBL and low activity of the MBL/MASP complexes, are associated with an increased risk of postoperative pneumonia and possibly also with other postoperative infectious complications in patients with colorectal cancer [36,37]. In a previous study, low MBL levels could not be associated with recurrent cancer disease or survival [36].

In conclusion, our study suggests that patients carrying MBL-2 GC genotype may be a determinant in susceptibility to colorectal cancer. Large-scale molecular epidemiological studies that investigate the role of MBL-2 GC genotype together with other susceptibility gene polymorphism and biomarkers of carcinogen exposure are necessary to expand our current understanding of the MBL-2 polymorphism in colorectal cancer.

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