Relationship between Parameters Linked to Surgery, Hospitalization and Single Nucleotide Polymorphism in Patients with Colorectal Cancer

A. CHIRCA* a, b, E. RADUc, D. G. MINCAD, R. COSTEAb

a*PhD student, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
b2nd Department of Surgery, University Emergency Hospital, Bucharest, Romania
cDepartment 10 – General Surgery, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
dHistology and Cell Biology Department, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
dDepartment of Public Health, “Carol Davila” University of Medicine and Pharmacy, Institute of National Health, Bucharest, Romania

ABSTRACT

Objectives: Colorectal cancer (CRC) is the third most common type of cancer, the fourth leading cause of cancer related deaths worldwide and a major public health issue. Management is difficult, especially in elderly patients, and efforts for an individualized treatment are an asset for patients’ wellbeing. Factors such as cumulated comorbidities, disease stage, and operative time may increase the length of hospitalisation (LOH) and overall costs. The aim of this paper is to assess the impact of a single nucleotide polymorphism (SNP) – rs6983267 – on CRC risk in Romanian individuals.

Materials and methods: A case-control genotyping molecular study was performed on 32 patients diagnosed with CRC (median age 67.5 years) who underwent elective surgery and 30 patients without CRC (median age 66 years). Genotyping for rs6983267 was performed on DNA extracted from peripheral venous blood.

Results: Twenty five patients were diagnosed with colonic cancer with different localizations, whereas seven had rectal cancers. Median LOH was 16.5 days (25-75 IQR 12–22 days). Genotyping for rs6983267 revealed no heterozygous (G/T) individuals within the control group, with all patients showing homozygous profiles (76.67% G/G, and 23.33% T/T), but the heterozygous (G/T) genotype was present in 59.38% of the patients in the study group (with 21.88% G/G and 18.75% T/T genotypes).

Conclusions: A higher percentage of CRC patients had at least one G allele (81.21%) when compared to controls (76.57%), although G allele frequency was higher in the control group due to an increased percentage of G/G homozygosity. When comparing clinical data between groups, we found an association...
INTRODUCTION

Due to the great improvements in basic and advanced healthcare systems, the last century has seen a rise in life expectancy in the general population. However, even with a variety of treatment possibilities, cancer mortality is continuously increasing, by 40% in the last 40 years, a percentage that is estimated to rise to 60% (13 million people) by 2030 (1). In developed countries, the rise in incidence is mainly attributed to increased life expectancy, chaotic dietary habits, low physical activity, and a gradual rise in incidence for both obesity and smoking, and not only for sporadic forms but also for hereditary types of cancer (2).

Colorectal cancer, in both sexes, is classified as the third most common type of cancer, and it accounts for almost 10% of all cancer incidence (9.2% in females and 10% in males). In 2012, in the United States, 49190 out of 134490 new cases were deaths attributed to this condition, thereby considered a major public health problem (3, 4). In 2013, approximately 771000 people died from colorectal cancer globally, ranking it as the fourth most common cause of cancer-related death, after pulmonary, hepatic and gastric cancer (5).

Carcinogenesis in this heterogenous type of neoplastic disorder is influenced by many genetic and environmental factors, with a majority of cases being sporadic – three out of four patients do not have a family history of CRC (2, 6). However, there are also hereditary colorectal cancer syndromes such as Lynch syndrome, hereditary adenomatous polyposis, Peutz-Jeghers syndrome, and juvenile polyposis (7, 8). The risk of developing colorectal cancer is mainly attributed to environmental factors such as smoking, alcohol consumption, obesity, increased intake of red meat and processed foods, and a low-fiber diet (9).

As a result of genotype-phenotype association studies, a number of genetic variants have been reported to increase the odds ratio for CRC. Biomarkers like these together with personal and family history are useful for placing the general population in a CRC risk category and the first steps in personalized medicine.

The majority of patients that have an incident form of colorectal cancer do not exhibit any symptoms, and, while increasing number of cases are diagnosed based on screening methods, as many as 70 to 90% of cases are diagnosed after the persistence of a specific symptom (10). These symptoms include altered bowel habit, abdominal pain and hematochezia, followed by iron deficiency anemia, asthenia, weight loss, and abdominal distention with nausea and/or vomiting when bowel obstruction may be present (2).

The diagnostic of colorectal cancer may be suspected after evaluation of a patient that presents specific signs and symptoms or after a positive screening test, and must be complemented with a colonoscopy exam, or other imaging examination such as CT colonography if the patient cannot tolerate a colonoscopy (1, 2). Complete surgical resection represents the only form of curative treatment for localized colorectal cancer, and depending on the type of surgical procedure chosen, a primary anastomosis should be performed, and avoided if either the patient is hemodynamically unstable or generalized peritoni-tis is present (11, 12). For patients who present locally advanced cancer, multivisceral resection was shown to increase overall survival; however, postoperative mortality was higher (13). Depending on disease stage, surgical method, operative time, and intraoperative blood loss, postoperative mortality may vary and it is higher in the cases with increased intraoperative blood loss and operation time (14). However, one of the most detrimental postoperative complications in colorectal surgery is still represented by anastomotic leakage and it requires re-intervention, furthermore increasing postoperative mortality (15).

The aim of this paper is to assess the impact of a single nucleotide polymorphism (SNP) –
rs6983267 – on CRC risk in Romanian individuals as well as the experience of our clinic in the management of this condition.

MATERIALS AND METHODS

A case-control genotyping study was undertaken in 32 patients who were diagnosed with colorectal cancer and underwent elective surgery in our department. A control group of 30 patients with no evidence of colorectal cancer was also included in this study.

Inclusion criteria for patients were histological confirmation of colorectal cancer after an elective surgery in our clinic, a proper disease staging, and then signing of an informed consent approved by the University Emergency Hospital Bucharest ethics committee. Medical charts were analyzed and the following data were collected: age, sex, symptom duration, serum CEA and CA19-9 levels, length of hospital stay, surgical procedure, and findings of the pathological exam. Operative factors such as total operative time were also recorded. The data was analyzed and descriptive statistics was performed using the NCSS data analysis package, version 10.

Peripheral venous blood was drawn from each patient in this study and an automated DNA extraction was performed using the QIAGEN QIAsymphony® DSP DNA automated extractor. Endpoint genotyping was performed using KASP genotyping assays for SNP rs6983267 and the LightCycler 480 II (Roche, SUA) real-time PCR system. Data were analyzed and descriptive statistics was performed using Light Cycler 480 Gene Scanning Software.

RESULTS

The study group included 62 patients, who were divided into two subgroups: patients recently diagnosed with CRC (n=32) and patients with no evidence of CRC (n=30). Patients with colorectal cancer had an average age of 68.03±7.10, with a median age of 67.5 years (25-75 IQR 62-72), and 17 of them were males. In the non-CRC group, the average age was 63.16±7.08, with a median age of 66 years (25-75 IQR 58-72), and 15 subjects were males.

Twenty five patients were diagnosed with colonic cancer with different localizations, whereas seven had rectal cancers. The median time between onset of symptoms and diagnosis was eight months (25-75 IQR 6-11.2). We found no significant association between time from symptom onset till diagnosis and tumor localization (eight months for colonic cancer versus seven months for rectal cancer, p=0.8).

The average length of hospital stay was 18.13±11.5 days, with a median of 16.5 days (25-75 IQR 12–22 days). Types of surgical procedures performed for the 32 patients with colorectal cancer were as follows: right hemicolectomy (five patients), left hemicolectomy (eight patients), rectosigmoid resection (nine patients), total anterior rectal excision (four patients), segmentary cholecotomy (three patients), ostomy (three patients).

When we analyzed the association between the time from symptom onset to diagnosis and serum levels of CEA and CA19-9, we found that there was a positive correlation between those variables (R=0.53, p=0.042, and R=0.58, p=0.013, respectively). The serum levels of CA19-9 were strongly associated with the number of invaded lymph nodes.

Analyzing the relationship between the type of surgical procedure and the length of hospitalization, we found a longer duration of in-hospital stay for patients who underwent ostomy (median 38 days, 25-75 IQR 7-69), segmentary cholecotomy (median 28 days, 25-75 IQR 19.7–28) and right hemicolectomy (median 16 days, 25-75 IQR 13.7–19). However, the differences did not reach statistical significance (p=0.35) due to the small number of cases.

When the operating time was analyzed comparatively to tumor stage, the longest surgery duration was shown in stage III colorectal tumors (IIIA median time of 300 minutes for one case; IIIB median time of 235 minutes, 25-75 IQR 115-365; IIIC median time of 280 minutes, 25-75 IQR 236.250-306.250; IIIB median time of 250 minutes, 25-75 IQR 236.250-306.250; IIC median time of 255 minutes for only one case). Stage IV tumors had the shortest operating time due to the fact that only ostomy was performed (210 minutes, 25-75 IQR 120-232.500). The length of surgery was also compared with the type of surgical procedure, with rectosigmoid resection being the longest procedure (293 minutes, 25-75 IQR 232.500-390.00), followed by one anterior rec-
tal excision (285 minutes, 25-75 IQR 265.00-365.00).

Genotyping for rs6983267 revealed no heterozygous (G/T) variant in the control group, with all patients showing homozygote profiles (76.67% G/G, and 23.33% T/T). The heterozygous (G/T) variant was present in the study group in a proportion of 59.38%, with 21.88% G/G, and 18.75% T/T homozygous genotypes (Figure 1). Distribution in the study population revealed that more men had G/G genotypes compared to women (71.43% vs 28.57%), more women were heterozygous G/T (52.63% vs 47.37), and distribution for the T/T genotype was equal in both sexes (Figure 2). The CRC study group had 66.67% of women and 52.94% of men with the G/T variant, 29.41% of men and 13.33% of women with the G/G genotype, and roughly 20% of both sexes were T/T (Figure 2). When the study group was divided in patients with colon tumors and patients with rectal tumors, only those with colon cancer exhibited the G/G genotype (28%), rectal cancer and colon cancer patients had 71.43% and 56% G/T genotype, while 28.57% and 16% had the low-risk T/T genotype (Figure 3). When comparing tumor sites, all patients who exhibited the G/G genotype presented with colon cancer, and also 73.68% of those with G/T, and 66.67% of those with T/T genotypes (Figure 4).

**DISCUSSION**

In a paper published in 2016, which analyzed the duration of hospitalization in 240873 patients who underwent surgery for colorectal cancer between 1998-2010, in England, the authors found that the median length of stay was 10 (7-14) days until 2006 and 8 (5-13) days between 2006-2010. As the authors stated, the length of hospitalization may be different, depending on factors like patient age, comorbidities, localization and stage of the neoplasm, and type of surgery (16). In a recent article published in 2018, Lingsma et al. showed that the mean length of stay in 26 academic hospitals from six countries, during 2007 and 2012, was 6.7 days (17). In our study, the length of hospitalization was 16.5 days (25-75 IQR 12-22 days). However, nowadays, in the European developed countries and also in the United States, the trend is to reduce the length of stay after surgery as

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**FIGURE 1.** rs6983267 distribution in patients and controls

**FIGURE 2.** rs6983267 distribution in patients with colorectal cancer according to sex

**FIGURE 3.** rs6983267 distribution in patients with colorectal cancer according to tumor localization

**FIGURE 4.** Overall rs6983267 distribution in colon and rectal cancer
much as possible. This can be achieved due to the fact that surgeons become more and more specialized and laparoscopic techniques are widely used, with the main goal to offer benefits to the their patients, including faster recovery and fewer complications. Meanwhile, the possibility of readmission or compromising patient’s safety should be evaluated when trying to reduce the period of hospitalization (18).

A correlation was found between the time from symptom onset to diagnosis and the carcinoembryonic antigen; this is in congruent to the results presented in different papers and meta-analyses according to which CEA levels are dependent of the disease stage (19), a longer duration of symptoms corresponding to a more advanced stage of the disease. In the same manner, the serum levels of CA19-9 correlated well with the time from symptom onset to diagnosis.

When the relationship between CA19-9 and the number of invaded lymph nodes was evaluated, our results were similar to other reports in the field that showed a statistically significant difference for preoperative serum CA19-9 expression in the presence or absence of lymph node metastasis (20).

The single nucleotide polymorphism rs6983267 is situated in the 128.47-128.54 MB area of chromosome 8q24.21, also known as region 3, upstream of the MYC gene, a well documented oncogene (21).

The ancestral allele is G and the variant is T, with a reported genotype distribution within Europeans of 0.2123, 0.5486 and 0.2389 for the G/G, G/T and T/T genotypes, respectively.

The presence of at least one G allele was initially associated with an increased risk of prostate cancer (22), but some studies (the largest was based on a cohort of 4000 UK patients) also suggested an association with colorectal cancer (23). Also, in a study on 2713 patients with colorectal and 2718 age and sex matched healthy subjects, Schafmayer et al. identified an association between rs6983267 and CRC, with an OR of 1.50 (24), suggesting that its presence might be a risk factor for colorectal cancer. All these studies were based on a homogenous Central and West European population. When factoring in population heterogeneity, Xiong et al showed that the presence of G/G genotype was higher in colorectal patients (OR =1.54; 95% CI 1.29-1.83) (25).

Although based on a small cohort, our results are consistent with the above mentioned information; thus, the percentage of persons carrying the G allele is higher in the CRC group than in controls (81.26% vs 76.67%). One surprising finding of our study is the clear difference between the two groups regarding the presence of heterozygous G/T genotypes: 0% in controls vs 59.38% in CRC patients. This observation also explains the apparently paradoxical higher G-allele frequency recorded within our control group when compared to CRC patients. While sampling errors cannot be excluded, our results suggest that a heterozygous G/T genotype at rs6983267 might be a risk factor for CRC.

Despite being conducted on a limited number of patients, our study showed a link between rs6983267 genotypes and colorectal cancer, with OR 88.11, 95% CI 4.9-1568.6, p=0.002. This value is attributed to the fact that no patient in the control group exhibited G/T genotype. Our findings came from a small group of patients and we presented partial results of an ongoing research on a larger population.

**CONCLUSION**

When comparing clinical data between groups, we found LOH dependent on variables such as operating time (tumor site and surgical procedure).

A higher percentage of CRC patients had at least one G allele (81.21%) when compared to controls (76.57%), although G allele frequency was higher in the control group due to the increased percentage of G/G homozygosity.

Our results suggest that the G/T genotype for the single nucleotide polymorphism rs6983267 is associated with CRC risk in a Romanian homogenous population. However, further studies on large groups are necessary in order to validate this observation.

Further research is still necessary to accurately calculate a CRC risk associated with the presence of this SNP in a Romanian population.

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REFERENCES


