A case-control study of *CYP2E1* (*Pst*I) and *CYP1A1* (*Msp*I) polymorphisms in colorectal cancer

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ABSTRACT. Polymorphisms in genes encoding P450 cytochrome enzymes may increase the risk of sporadic colorectal cancer (SCRC). Here we investigated the association between SCRC and *CYP2E1* (*Pst*I) and *CYP1A1* (*Msp*I) polymorphisms in a case-control study. Moreover, we sought to determine any possible associations between this disease and the sociodemographic factors. We included 273 individuals (74 patients and 199 controls); the gender, age, tobacco usage, and alcohol consumption of the included subjects, and the clinico-histopathological parameters of the tumors, were analyzed. Molecular analyses were performed using PCR-RFLP. The effect of polymorphisms on SCRC development, and the association between this disease and sociodemographic factors

were determined by multiple-logistic regression analyses. The combined genotype was also evaluated. Statistically significant differences between the patients and controls regarding the male gender (odds ratio, OR = 0.19, 95% confidence interval, CI = 0.08-0.46; P ≤ 0.05) and age ≥44 years (median = 44; OR = 96.84, 95%CI = 21.78-430.49; P ≤ 0.05) were observed. The evaluated polymorphisms were not associated with SCRC (*Pst*I-CYP2E1: OR = 0.93, 95%CI = 0.30-2.85; P = 0.897; *Msp*I-CYP1A1: OR = 0.75, 95%CI = 0.35-1.61; P = 0.463); the combined genotypes were not associated with the risk of disease. Thus, individuals aged ≥44 years are more sensitive to SCRC, while men are less susceptible. Additionally, polymorphisms in *CYP2E1* (*Pst*I) and *CYP1A1* (*Msp*I) were not associated with SCRC in the evaluated Brazilian population.

Key words: Colorectal neoplasms; Cytochrome P-450 CYP2E1; Alcohol; Cytochrome P-450 *CYP1A1*; Genetic polymorphisms; Smoking

INTRODUCTION

Sporadic colorectal cancer (SCRC) is a term used to designate malignancies that occur in the large intestine (colon) and rectum (INCA, 2015) in individuals with no family history of cancer. It occurs mostly in the sigmoid colon and rectum (Santos, 2007). The most frequent histological type of SCRC is adenocarcinoma, which accounts for more than 90% of the cases (Zampino et al., 2009). Studies have predicted the emergence of approximately 32,600 new cases of SCRC in Brazil in 2014 (INCA, 2015).

The most common etiological factors for SCRC include age over 60 years (INCA, 2015), alcohol consumption, tobacco habits, diet poor in calcium and folate, and rich in saturated fat, red meat, bread, pasta, and refined sugar. On the other hand, the increased intake of polyunsaturated fatty acids (chiefly derived from olive oil and seed oils), and the consumption of fruits and vegetables may provide protection against this type of cancer. Other factors that may influence the development of SCRC are obesity and a sedentary lifestyle (Giacosa et al., 2002; Zampino et al., 2009; Dolatkthah et al., 2015).

The carcinogenesis of SCRC involves damage to the DNA of somatic cells caused by environmental factors, forming DNA adducts. Some genetic variants codify highly polymorphic enzymes that may accelerate this process. Therefore, the development of SCRC is a consequence of both environmental factors and genetic mutations (Gertig et al., 1998).

The balance between the rate of absorption and elimination of xenobiotics has an important role in preventing DNA damage caused by chemical carcinogens (Hatagima, 2002). Cigarettes and alcohol cause a deleterious effect on the body because of the many carcinogens produced as a result of their degradation and metabolization. The oxidative reactions caused by these substances to the tissues result in the production of free radicals during different cellular events. Reactive oxygen can cause mutagenesis and alterations in the cellular cycle by damaging the proteins, carbohydrates, lipids, and DNA within the body (Zain et al., 2001).

Two types of enzymes participate in xenobiotic metabolism: the phase I and phase II enzymes. The major phase I enzymes belong to the cytochrome P450 (CYPs) superfamily, and are responsible for the conversion of many compounds in highly reactive metabolites. In this context,

the *CYP2E1* and *CYP1A1* genes are responsible for the metabolic activation of xenobiotics in the human carcinogenesis process, thereby contributing to the development of diseases such as cancer (Zhang et al., 2006).

Polymorphisms in the *CYP2E1* and *CYP1A1* genes can modify the expression or function of enzymes, resulting in the activation of pro-carcinogen compounds (Zhang et al., 2006). These polymorphisms can be potential candidates for predictive biomarkers that may help in the identification of the subset of patients with an increased risk of development of SCRC (Zhou et al., 2010). The polymorphism *CYP1A1**2A (for the restriction enzyme *Msp*I) in the *CYP1A1* gene results from a single base substitution (T→C) at position 3801 of the poly-(A) tail in the 3'-untranslated region of the gene, and is responsible for higher enzyme stability and/or activity (Shah et al., 2009). The *Pst*I polymorphism (-1293 G→C) in the *CYP2E1* gene is another potential indicator of susceptibility to cancer. This polymorphism, located within the promoter region in the gene, is associated with an increased enzyme activity (Hayashi et al., 1991). The *CYP2E1* polymorphic allele (c2) has been associated with increased risk SCRC and combined with alcohol consumption, smoking, and red meat consumption may significantly increase the susceptibility to colon cancer (Zhou et al., 2010).

Based on the above evidence, the aim of this study was to investigate the association between SCRC and *CYP2E1* (*Pst*I) and *CYP1A1* (*Msp*I) polymorphisms in a case-control study. Moreover, we sought to identify any possible associations between this disease and sociodemographic factors.

MATERIAL AND METHODS

Ethics statement

This study was approved by the Research and Ethics Committee of the Faculdade de Medicina de São José do Rio Preto - FAMERP (No. 216/2009).

Patients

This study was conducted on 273 individuals (74 patients and 199 controls) with a mean age of 47.2 ± 13.4 years. All adult individuals agreed to participate in the study, and written informed consent was obtained from all individuals. The patients and controls resided in the city of São José do Rio Preto or one of the many surrounding towns in this region.

The case group consisted of 74 patients who were diagnosed with SCRC based on their clinical histopathological parameters at the Hospital de Base, São José do Rio Preto, SP, Brazil. Patients with hereditary cancer and those who had been previously treated for cancer were excluded from this study. The control group included 199 Brazilian healthy blood donors with aged 40 years and over and no previous history of cancer diagnosis. The subjects with a family history of cancer were excluded from this study.

Methods

The variables analyzed were gender, age, and tobacco and alcohol consumption, extension of the tumor (T), lymph node involvement (N), and the polymorphisms. Individuals who had smoked

more than 100 cigarettes over their lifetime, and those who continued to smoke on some or all days of the week around the time of the interview, were classified as smokers. Individuals who drank four doses of alcohol per week were considered alcohol consumers (Ahrendt et al., 2000).

Each eligible subject was interviewed in order to determine their age, gender, smoking habit, and alcohol consumption rate. The clinical histopathological parameters were gathered from the medical records of the patients. The tumors were classified according to the parameters of the International Union of Cancer Control (UICC), 2004, based on three criteria (UICC, 2012): extension of the tumor (T), presence of regional lymph node involvement (N), and presence of metastasis at distance (M).

Genomic DNA was obtained from peripheral blood leukocytes according to the modified salting out technique described by Miller et al. (1998). The genotypes of the *Pst*I-*CYP2E1* and *Msp*I-*CYP1A1* polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to Cury et al., 2012. The following primers were used for these reactions: sense, 5'-CCA GTC GAC TCT ACA TTG TCA-3' and anti-sense, 5'-TTC ATT CTG TCT TCT AAC TGG-3' to detect the *Pst*I-*CYP2E1* polymorphism; and sense, 5'-TAG GAG TCT TGT CTC ATG CCT-3' and anti-sense 5'-CAG TGA AGA GGT GTA GCC GCT-3' for the *Msp*I-*CYP1A1* polymorphism. The products of the *CYP2E1* and *CYP1A1* polymorphisms were digested with the *Pst*I and *Msp*I restriction enzymes, respectively. The *Pst*I-*CYP2E1* polymorphism produced a 410-bp fragment in the presence of the *c1* allele, and 290- and 120-bp fragments in the presence of the *c2* polymorphic allele. The polymorphic *Msp*I-*CYP1A1* gene produced a 340-bp fragment in the presence of the *wt* allele and 206- and 134-bp fragments in the presence of the *m1* polymorphic allele.

The data were statistically analyzed using the Minitab computer program for Windows (Version 14). The allele and genotype frequencies were calculated, and the chi-square test was used to analyze the Hardy-Weinberg equilibrium. The groups were compared by gender, age, smoking habit, drinking habit, and the presence of polymorphisms. Multiple-logistic regression models were used to determine the association between the genetic polymorphisms and SCRC. These models included factors such as the gender (reference: female), age (reference: <44 years; median) smoking habit (reference: non-smokers), drinking habit (reference: non-drinkers), and polymorphisms (reference: wild-type homozygote genotypes).

The clinical and histopathological parameters were also analyzed by multiple-logistic regression. Extensions of the tumor were classified as small (T1, T2) and large (T3, T4). The N classification was dichotomized into no lymph node involvement (N0) and lymph node involvement (N1, N2, and N3).

The *CYP2E1 c1c1* and *CYP1A1 wt/wt* genotypes were used as a reference (Risk 0) for the analysis of combined genotypes. The *CYP2E1 c1c1* and *CYP1A1 wt/m1* or *CYP2E1 c1c2* and *CYP1A1 wt/wt* genotypes (wild-type homozygote and heterozygote) were classified as risk 1; the *CYP2E1 c1c1* and *CYP1A1 m1/m1* genotype (wild-type homozygote and polymorphic homozygote) was defined as risk 2 (in this case, the *CYP2E1 c2c2* and *CYP1A1 wt/wt* combination was not possible because of the absence of the *CYP2E1 c2c2* genotype in the sample); *CYP2E1 c1c2* and *CYP1A1 wt/m1* (both heterozygote genotypes) was classified as risk 3; and the *CYP2E1 c1c2* and *CYP1A1 m1/m1* genotype (heterozygote and polymorphic homozygote); or two polymorphic genotypes) was classified as risk 4 (in this case, the *CYP2E1 c2c2* and *CYP1A1 m1/m1* or *CYP2E1 c2c2* and *CYP1A1 wt/m1* combinations did not occur because of the absence of the *CYP2E1 c2c2* genotype in the sample).

P values <0.05 were considered to be statistically significant. The results are reported as odds ratio (OR) and 95% confidence intervals (95%CI).

RESULTS

The analysis showed statistically significant differences between patients and controls with respect to the male gender (OR = 0.19; 95%CI = 0.08-0.46; P < 0.001) and age ≥44 years (OR = 96.84, 95%CI = 21.78-430.49; P < 0.001). The patients and controls did not differ significantly with respect to the smoking habit (OR = 1.36, 95%CI = 0.63-2.93; P = 0.436) and alcohol consumption (OR = 0.89, 95%CI = 0.40-1.97; P = 0.771). The evaluated polymorphisms were not associated with SCRC (*Pst*I-*CYP2E1*: OR = 0.93, 95%CI = 0.30-2.85; P = 0.897; *Msp*I-*CYP1A1*: OR = 0.75, 95%CI = 0.35-1.61; P = 0.463) (Table 1).

Table 1. Distribution of gender, age, risk factors, *Pst*I-*CYP2E1*, and *CYP1A1-Msp*I genotypes between patients and controls in CRC odds ratio (OR).

Variables	Patients [N (%)]	Controls [N (%)]	Multiple-logistic regression	
			OR (95%CI)	P
No. of individuals	74	199		
Age (median)				
<44 years	2 (2.7)	133 (66.8)	Reference	
≥44 years	72 (97.3)	66 (33.2)	96.84 (21.78-430.49)	**<0.001[a]**
Gender				
Female	34 (45.9)	45 (22.6)	Reference	
Male	40 (54.1)	154 (77.4)	0.19 (0.08-0.46)	**<0.001[a]**
Smoking habit				
Non-smokers	41 (44.6)	116 (58.3)	Reference	
Smokers	33 (55.4)	83 (41.7)	1.36 (0.63-2.93)	0.436
Drinking habit				
Non-drinkers	47 (63.5)	108 (54.3)	Reference	
Drinkers	27 (36.5)	91 (45.7)	0.89 (0.40-1.97)	0.771
Genotypes				
*Pst*I-*CYP2E1*				
*c1c1**	66 (89.2)	171 (85.9)	Reference	
c1c2 and *c2c2***	8 (10.8)	28 (14.1)	0.93 (0.30-2.85)	0.897
*Msp*I-*CYP1A1*				
*wt/wt**	54 (73.0)	129 (64.8)	Reference	
wt/m1 and *m1/m1***	20 (27.0)	70 (35.2)	0.75 (0.35-1.61)	0.463

Adjusted for age, gender, tobacco usage, and alcohol consumption. P ≤ 0.05 was considered to be significant in a multiple-logistic regression model. [a]Bold values indicate P ≤ 0.05. *Wild type. **Mutant.

The genotypic distributions (Table 2) of *Pst*I-*CYP2E1* were observed to be in Hardy-Weinberg equilibrium (HWE) in both groups (case: χ^2 = 0.2416; P = 0.6230, and control: χ^2 = 1.1396; P = 0.2857); the genotypic distributions of *Msp*I-*CYP1A1* were in equilibrium in the case group, and disequilibrium in the control group (case: χ^2 = 1.1538; P = 0.2827 and control: χ^2 = 6.2501; P = 0.0124).

Multiple-logistic regression analyses of the polymorphisms with clinical histopathological parameters did not detect any association between the tumor extension and lymph node involvement (Table 3).

The genotypes were combined and the group with zero risk alleles was used as the reference. We did not observe a statistically significant increase in SCRC risk with the number of risk alleles (risk 1 - OR = 0.91; 95%CI = 0.42-1.99; P = 0.817; risk 2 - OR = 0.57; 95%CI = 0.10-3.11; P = 0.512; risk 3 - OR = 1.00; 95%CI = 0.08-12.19; P = 0.998; risk 4 - OR = 0.20; 95%CI = 0.01-3.89; P = 0.290).

Table 2. Allele and genotype frequencies of both polymorphisms in patient and control groups.

	Patients	Controls
*Pst*I-*CYP2E1*		
Allele frequencies		
c1	0.95	0.93
c2	0.05	0.07
Genotype frequencies		
c1c1	0.89	0.86
c1c2	0.11	0.14
c2c2	0.00	0.00
*Msp*I-*CYP1A1*		
Allele frequencies		
wt	0.84	0.79
m1	0.16	0.21
Genotype frequencies		
wt/wt	0.73	0.65
wt/m1	0.23	0.28
m1/m1	0.04	0.08

Table 3. Distribution of the clinico-histopathological parameters with respect to the *CYP2E1* (*Pst*I) and *CYP1A1* (*Msp*I) polymorphisms.

Polymorphisms		Tumor extension		N involvement	
		T0/T1/T2	T3/T4	N0	N1, N2, N3
*Pst*I-*CYP2E1* genotype	*c1c1* (Ref) [N (%)]	11 (19.3)	38 (66.7)	29 (50.9)	7 (12.3)
	c1c2 and *c2c2* [N (%)]	2 (3.5)	6 (10.5)	20 (35.1)	1 (1.7)
OR (CI95%, P value)		1.00 (Ref)	0.43 (0.06-3.15, 0.408)	1.00 (Ref)	0.29 (0.03-2.89, 0.294)
*Msp*I-*CYP1A1* genotype	*wt/wt* (Ref) [N (%)]	10 (17.5)	3 (5.3)	29 (50.9)	13 (22.8)
	wt/m1 and *m1/m1* [N (%)]	32 (56.1)	12 (21.1)	7 (12.3)	8 (14.0)
OR (CI95%, P value)		1.00 (Ref)	0.79 (0.16-3.87, 0.774)	1.00 (Ref)	3.28 (0.84-12.88, 0,088)

$P \leq 0.05$ was considered to be significant in a multiple-logistic regression model; OR = odds ratio; CI = confidence interval.

DISCUSSION

The results showed that individuals aged 44 and over are more susceptible to SCRC, which corresponded with the data reported by previous studies; this also indicated that SCRC is more common among older people (Diniz and Lacerda-Filho, 2004; INCA, 2015). We also observed that male subjects were less susceptible to SCRC. The literature have showed that the SCRC is most common in women than in men, particularly above 65 years old, indicating the importance of screening this disease in women with advanced age (Kim et al., 2015).

Our study found no association between cancer risk and tobacco usage and alcohol consumption. However, previous studies have associated SCRC with smoking (Chao et al., 2000; Bhattacharya et al., 2014) and alcohol (Seitz et al., 2005; Bhattacharya et al., 2014).

The HWE analysis conducted in our study showed that the *Msp*I-*CYP1A1* gene was in disequilibrium in the control group. Case-control studies analyzing SNPs have shown a departure from HWE in the patient or control groups, or in both groups (Wittke-Thompson et al., 2005).

In our study, the evaluated polymorphisms were not significantly associated with SCRC. We also found no association between polymorphisms and tumor extension and lymph node involvement. A major limitation of this study was the small sample size and low incidence or lack of polymorphic homozygous genotypes which may decrease the statistical power and compromise the analysis and investigation of potential genotypic combinations that influence the risk of SCRC.

Ye and Parry (2002) analyzed United Kingdom Caucasians (82 controls and 41 cases) and reported similar results to ours for *Msp*I-*CYP1A1* polymorphism. Other case-control studies performed in large sample sizes of North-America (Slattery et al., 2004), Australia (Butler et al., 2001), and Spanish (Landi et al., 2005) also did not discover an association between the *Msp*I-*CYP1A1* polymorphisms and SCRC risk. Contrary to our findings, the study conducted by Yoshida et al. (2007) on an Asian population, comprising 66 cases and 121 controls, reported an association between the *Msp*I-*CYP1A1* polymorphism and SCRC. This association also was observed in a study with relatively large sample size (500 cases and 500 controls) in the Hungarian population (Kiss et al., 2007) and confirmed in the meta-analysis by He et al. (2014).

Regarding to *Pst*I-*CYP2E1* polymorphism, previous study performed with Hungarian population successfully established an association between the c2 allele and SCRC susceptibility (Kiss et al., 2007), which differed from the results of our studies. On the other hand, according to our findings, two other studies performed in Australian (Butler et al., 2001) and Spanish (Landi et al., 2005) populations also found no association between this polymorphism and SCRC.

To our knowledge, no other studies evaluated the clinical histopathological parameters of SCRC with respect to the polymorphisms analyzed in this study. Tan et al. (2011) evaluated the association between the *Pst*I-*CYP1A1* polymorphism and the histopathological characteristics of lung cancer; however, no such association was found.

The discrepancy between these studies may be a result of differences in several variables, such as ethnicity and gender of the study subjects, epidemiological factors, and study design. Therefore, further studies are required for a better understanding of the factors involved in SCRC etiology.

CONCLUSION

Our study show that individuals aged 44 years and above are more susceptible to SCRC, while men are less susceptible. Polymorphisms in the *CYP2E1* (*Pst*I) and *CYP1A1* (*Msp*I) genes are not associated with SCRC in the evaluated Brazilian population.

Conflicts of interest

The authors declare no conflict of interest.

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