RESEARCH ARTICLE

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Cytochrome *P2E1 (CYP2E1)* Gene Polymorphism as a Potential Prognostic Biomarker in Colorectal Cancer

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Abstract

Background: Colon cancer is the most common type of gastrointestinal cancer. Genetic factors have been shown to have a role in the development of colorectal cancers. The aim of this study was to assess the expression of Cytochrome *P2E1 (CYP2E1*) gene polymorphism as a potential prognostic biomarker in the diagnosis, treatment, and prognosis evaluation of patients with colorectal cancer. **Methods:** in this cross-sectional study, all of our 100 patients with colorectal cancer who underwent surgical operation were included. DNA was extracted from the tumor specimens to assess Cytochrome *P2E1 (CYP2E1)* Gene polymorphism by Conventional-PCR. RFLP-PCR method was used for RsaI polymorphism evaluation. Patients' characteristics were also recorded and their associations with *CYP2E1* were assessed. **Results:** One hundred tumor specimens were assessed. In total, 88 had wild-type, 8 had purely a 96 bp insertion in *CYP2E1*, and 4 were partially mutated by a single allele insertion. Generally, 10% of samples had positive results for the RsaI polymorphism. There were no statistically significant associations between *CYP2E1* gene polymorphism and number of lymph nodes removed during the operation (P = 0.353), number of positive lymph nodes (P = 0.668), tumor specificity including mucinous or non-mucinous (P = 0.053), tumor invasion (P = 0.074), grading (P = 0.898), differentiation (P = 0.941), tumor location (P = 0.42) or staging (P = 0.182). **Conclusion:** There was no association between RsaI type *CYP2E1* polymorphism and colorectal cancer risk. Our study does not support the use of this biomarker to evaluate the prognosis of colon cancer.

Keywords: Colon Cancer- polymorphism- *CYP2E1*- biomarker.

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Introduction

Colon cancer is the most common type of gastrointestinal cancer (Kuipers et al., 2015). It is a multifactorial disease process triggered by genetic factors, environmental exposure (including diet) and inflammatory conditions of the gastrointestinal tract (Dekker et al., 2019). Surgery is currently the definitive treatment. Invasive colorectal cancer is a preventable disease to a great extent. Early detection through screening programs has been the most important factor in the recent reduction in the incidence of colorectal cancer in developed countries (Iversen, 2012; Kuipers et al., 2015; Tsar'kov et al., 2012). Moreover, cytotoxic drugs, biological agents, and radiation therapy have increased the chance of treatment in early-stage disease (stage II and III) and longer survival for patients with higher stages (Cirocchi et al., 2012; Costi et al., 2014; Ihnat et al., 2015; Kagita et al., 2021; Mármol et al., 2017). Many colon cancers are caused by lifestyle-related factors and increase in age and few occur due to inherited genetic disorders (Murphy et al., 2018; Steinke et al., 2013; Syngal et al., 2015).

New research suggests that genetic factors are most linked to colorectal cancer. Hereditary mutation of the APC gene is the cause of familial adenomatous polyposis (FAP) (Brammer et al., 2018). Lynch syndrome is related to inherited mutations in one of the mismatch repair genes, such as hMLH1, hMSH2, hMSH6, hPMS1 and hPMS2. HNPCC accounts for about 6% of all colorectal cancers (Boland et al., 2018; Cohen and Leininger, 2014; Pai et al., 2018).

It has been shown that Hepatic cytochrome *P450*

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2E1 (*CYP2E1*) plays an important role in the metabolic activation of nitrosamines and other carcinogens. Of many known genetic polymorphisms in the *CYP2E1* gene, the RsaI variant of the C-1054T replacement (rs 2031920) and the 96-bp insertion in the Flanking 5 region have attracted considerable attention due to their potential capabilities in developing cancers (Guengerich et al.,1991; Lieber, 1997). Participants with the RsaI c2 allele had a reduced risk of colon cancer in a population-based study in Hawaii (Le et al., 2002), while a statistically significant increase in the risk of colorectal cancer for individuals with the RsaI c2 allele was reported in Hungary and for those homozygous for the c2 allele in China (Gao et al., 2007; Kiss et al., 2000). Moreover, no association was found between RsaI polymorphism and the risk of colorectal cancer in Australia (Butler et al., 2001) and the Netherlands (van der Logt et al., 2006). The RsaI c2 allele is rare in Caucasians and results from Caucasian studies may have been by chance.

Only one case-control study in Hawaii examined the relationship between the 96-bp insertion polymorphism and colorectal cancer (Kiss et al., 2000). This study showed an increased risk of rectal cancer, but not colon cancer, for people with at least one unstable insertion allele. In a recent Japanese study of colorectal adenomas, the 96-bp insertion allele was associated with increased risk of large adenomas (size ≥ 5 mm), but was not associated with small adenomas (below 5 mm), whereas the RsaI c2 allele showed no clear association with large or small adenomas (Morita et al., 2008).

Few studies have evaluated the association between RsaI polymorphism and colorectal cancer risk and their findings are inconsistent. There is still no consensus regarding the role of *CYP2E1* gene polymorphism in the development of colon cancer. Therefore, this study was performed to assess *CYP2E1* gene polymorphisms in patients with colon cancers who underwent the operation at our center.

Materials and Methods

Setting and patients

As a cross-sectional study, all patients with colon cancer who were operated at Firoozgar Hospital, Tehran, Iran from 2014 to 2019 were included. Sampling was performed by the available sampling method. Inclusion criteria were all patients with colorectal cancer who did not receive chemotherapy and their tumor specimen were available at our pathology department. Exclusion criteria were those patients who did not consent to take part in the study or if samples were unavailable due to any reason. Also, patients who underwent neoadjuvant chemotherapy were excluded. Informed written consent was obtained from all patients. The study protocol was approved by the Ethics committee of our university (Ethics Code: IR.IUMS.REC.1398.292). All the study steps were in accordance with the declaration of Helsinki.

Patient's demographic data and tumor-specific characteristics including tumor size, number of excised lymph nodes, number of positive lymph nodes, tumor staging, and grade, tumor invasion and differentiation, tumor site, and definite tumor pathology were recorded from patient file.

Nucleic acid extraction

Specific primers for the *CYP2E1* gene was designed based on genome conserved regions according to Sameer's study (Sameer et al., 2011) using bioinformatics software (Basic Local Alignment Search Tool (BLAST) and CLC workbench v.5). To extract DNA, up to 25 mg of paraffin-embedded cancer tissue was used by a FavorPrep™ FFPE Tissue DNA Extraction Mini Kit (Favorgen, Taiwan) according to protocol. NanoDrop ND-1000 (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used for the evaluation of extracted DNA quality. Then, they were stored at -70°C.

Polymerase chain reaction (PCR)

A conventional polymerase chain reaction (PCR) was performed for *CYP2E1* insertion region (634 or 729 bp) amplification by the forward 5'-GTG ATG GAA GCC TGA AGA ACA-3' and reverse 5'-CTT TGG TGG GGT GAG AAC AG-3' primers based on a previous study. A 50 ul reaction mixture consisted of 25 ul 2× Amplicon MasterMix (Amplicon, Denmark), template DNA or controls corresponds to 0.2–0.5 µM concentration, primers (10 pmol/µl) corresponds to 0.5 mM and sterilized deionized water added to reach the rest of the total volume. A Bio-Rad thermocycler (T100™ Thermal Cycler) was used for the heating program as follow: 5min 95°C, 40 cycles of the 30s 95°C, 30s 58°C, 30s 72°C, and one step 72°C for 10 min. visualization of PCR products under UV was done by a trans-illuminator into a gel electrophoresis system and 1.5% agarose gel stained by Ethidium bromide.

PCR-RFLP

A PCR-RFLP (restriction fragment length polymorphism) was used according to a previous study. The RsaI enzyme was used to evaluate the mutation and enzyme digestion indicated its presence or absence. A conventional PCR was performed for amplification of 413 bp of *CYP2E1* RsaI region by the forward CCAGTCGAGTCTACATTGTCA and reverse TTCATTCTGTCTTCTAACTGG primers. The previous protocol was used for amplification except the 55°C heating for the annealing step. 2U RsaI enzyme (Thermo Scientific, Lithuania) was used for 10 ul PCR products and incubate at 37°C 5 hours. Visualization was performed as mentioned protocol. 352 bp and 61 bp fragments of *CYP2E1* RsaI PCR products were assumed as positive.

Statistical Analysis

SPSS software (SPSS Inc. Chicago, Ill, The USA) version 16 was used for data analysis. Mean and standard deviation was used to express quantitative descriptive findings and frequency and percentage for qualitative data. Independent T-test, ANOVA, and Chi-square were used to analyze normal data and their nonparametric counterparts Mann-Whitney, Kruskal-Wallis and Fisher tests for non-normal ones. $P < 0.05$ was considered statistically significant.

Results

One hundred patients were included in this study (Figure 1). The mean age ± SD of patients was 59.25 ± 13.49 years (27 to 89 years). Of these, 59 (59%) were female and 41 (41%) were male. The mean tumor size ± SD was 5.5 ± 2.7 cm (maximum 17 cm, minimum 1.5 cm). Demographic data of participants and some descriptive characteristics of specimens are presented in Table 1.

In total, 88 had wild-type *CYP2E1*, 8 had a homozygous insertion in *CYP2E1* allele, and 4 had heterozygous 96-bp insertion mutations. Generally, 10% of samples had positive results for the polymorphism RsaI. Tumor pathological characteristics are shown in Table 2 in detail.

There was no meaningful statistical association between age and *CYP2E1* (P=0.296) or RsaI polymorphism (P=0.411). Moreover, there was no meaningful statistical association between gender and *CYP2E1* (P=0.499) or RsaI polymorphism (P=0.155). Additionally, there was no statistically significant difference between mean tumor size and type of *CYP2E1* gene (P = 0.881).

However, there were no statistically significant associations between *CYP2E1* gene expression and number of lymph nodes removed during operation (P = 0.353), number of positive lymph nodes (P = 0.668), tumor specificities including mucinous or non-mucinous type (P = 0.053), tumor invasion (P = 0.074), tumor grade (P = 0.898), tumor differentiation (P = 0.941), tumor location (P = 0.42) or tumor staging (P = 0.182).

Moreover, there was no statistically significant association between the tumor size and RsaI gene expression (P = 0.214). There was a statistically significant difference between the mean number of lymph nodes removed during the operation and RsaI gene expression (P = 0.021), which does not have any clinical value. Meanwhile, there was no significant difference between the mean number of involved lymph nodes and RsaI gene expression (P = 0.214).

Besides, there were no statistically significant associations between RsaI gene expression and tumor specificity including mucinous or non-mucinous type (P = 0.583), tumor invasion (P = 0.444), tumor grade (P = 0.056), tumor differentiation (P = 0.463) or tumor staging (P = 0.056). Despite the fact, there was a statistically significant association between the tumor location and RsaI gene expression (P = 0.005).

Discussion

Nowadays, smoking, obesity, red meat consumption and alcohol, some inflammatory bowel diseases, and hereditary colon polyposis have been identified as risk factors for colorectal cancer, but the definitive mechanism of colorectal cancer is still unclear (Helmy et al., 2020; Johnson et al., 2013). It is known that genetic susceptibility plays an important role in the development of colorectal cancer. There are many genetic polymorphisms identified as colon cancer risk factors, many of which are involved in carcinogenic metabolism, including the glutathione S-transferase family (Helmy et al., 2020; Zhao et al., 2012).

N-nitrosamines found in tobacco and red meat are well-known carcinogens involved in colon cancer.

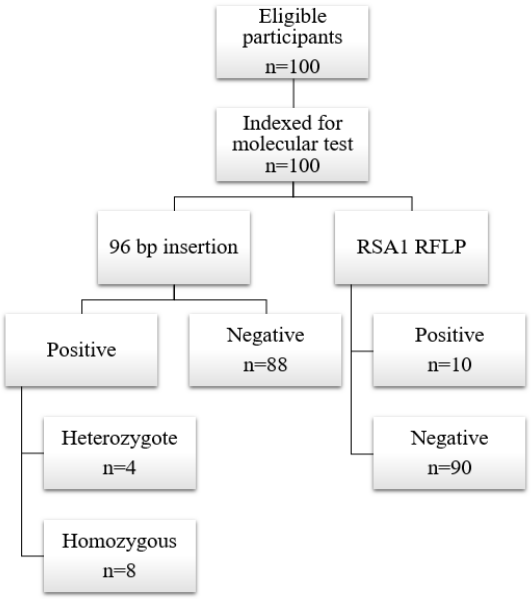

Figure 1. Flow-Diagram Report of Participants through the Study (n=100)

Table 1. Demographic Data of Participants and the Results of Patients *CYP2E1* Insertion, and RsaI Digestion

Variable	Mean ± SD	RsaI Positive		CYP2E1 mutations			
		N=10	p	I/I (n=4)	I/I (n=8)	i/I (n=88)	p
Gender, m/f	41/59	2/8	0.155	2/2	4/4	53/35	0.449
Age (year)	59.25±13.49	9.50±55.90	0.411	14.29±58.75	13.76±52.12	13.40±59.92	0.296
Number of removed Lymph nodes	11.06±7.67	15.82±19.40	0.021	7.93 ± 9.5	4.36 ± 8.25	11.39 ± 7.89	0.353
Number of Involved Lymph Nodes	2.27±3.86	4.66 ± 3.30	0.41	1.5 ± 0.75	1.28 ± 1.25	2.43 ± 4.06	0.668
Tumor size (cm)	5.5 ± 2.72	2.21 ± 6.15	0.214	2.06 ± 5.75	3.07 ± 5.58	2.75 ± 5.48	0.881
Tumor site			0.005				0.182
Recto-sigmoid	70 (70%)	4		4	4	62	
Descending colon	4 (4%)	0		0	0	2	
Ascending & Transverse colon	2 (2%)	0		0	0	4	
Multicentric	24 (24%)	6		0	4	20	

I/I, insertion; i/i, non-insertion; I/i, partial insertion; SD, standard deviation

Table 2. Tumor Characteristics of Studied Patients Regards to *CYP2E1* Insertion, and RsaI Digestion

Variables	Total N	RsaI Positive N=10		*CYP2E1* 96-bp insertion			
		N	p-value	I/I (n=4)	I/i (n=8)	i/i (n=88)	p-value
Tumor grade			0.056				0.898
1	8	0		0	0	8	
2	29	0		2	2	25	
3	45	6		1	5	39	
4	18	4		1	1	16	
Tumor differentiation			0.463				0.941
Poor	16	2		0	3	13	
Intermediate	33	4		1	2	30	
well	51	4		3	3	45	
Invasion			0.444				0.074
Yes	81	9		0	0	69	
No	19	1		4	8	19	
Mucinous type			0.583				0.053
Yes	8	1		2	1	5	
No	92	9		2	7	83	
Tumor staging			0.056				0.182
1	14	1		1	1	12	
2a	39	1		1	3	35	
2b	14	2		0	0	14	
3a	12	1		1	1	10	
3b	13	2		0	1	12	
3c	8	3		1	2	5	

I/I, insertion; i/i, non-insertion; I/i, partial insertion

CYP2E1, as a member of the cytochrome P450 superfamily, has been implicated in the metabolic activation of many carcinogens such as N-nitrosamines and aniline and has been suggested to play a role in host susceptibility to colon cancer (Fang et al., 2017). The other genes like Dihydropyrimidine dehydrogenase (DPYD) polymorphism could be important in colon cancer patients (Salehifar et al., 2019).

There are a number of polymorphisms in the *CYP2E1* gene that might have an impact on susceptibility to colorectal cancer. *CYP2E1* rs2031920 (RsaI) and rs3813867 (PstI) polymorphisms have been reported to affect the transcriptional activity of the *CYP2E1* gene in the 5-flanking promoter region of the *CYP2E1* gene. These two polymorphisms might alter *CYP2E1* enzyme activity and further leading to some changes in host's ability to metabolize carcinogens (Peng et al., 2013). Despite the fact, many studies have been conducted to evaluate the association of *CYP2E1* rs2031920 and rs3813867 polymorphisms with colon cancer risk, but no conclusive findings have been reported (Peng et al., 2013).

We showed that there was no significant association between *CYP2E1* polymorphism and RsaI genotype with colon cancer properties including tumor invasion, staging, grade, differentiation or the number of positive lymph nodes. The only significant finding in this study was the association between tumor location and RsaI gene expression, with lower levels of RsaI gene expression in locally advanced tumors compared to multicentric ones.

Kiss et al., (2007) reported that the c2 allele significantly increased the risk of colorectal cancer, while Gao et al. claimed that only the homozygous c2c2 was associated with colorectal cancer. In other studies, no significant difference was observed (Kiss et al., 2007).

A study by Marito et al., (2008) showed a significant and moderate reduction in the risk of colon cancer in individuals with the RsaI c2 allele and an increased risk of rectal cancer associated with the insertion allele 96. Homozygous individuals for the 96-bp allele were at increased risk for colon cancer.

In the study of Seyed Samir et al., (2011) the *CYP2E1* RsaI genotype frequency rates were 53.5% (46/86) for c1/c1, 17.4% (15/86) for c1/c2 and 29.1% (25/86) for C2/C2 in patients with colorectal cancer. Also, the CsaP2E1 RsaI polymorphism was significantly associated with the risk of colorectal cancer. The overall risk of colorectal cancer was 2.17 times more if homozygous c2/c2 was present (Sameer et al., 2011).

In a meta-analysis, 17 studies with a total of 1782 patients were reviewed. They showed that there was a significant association between *CYP2E1* rs2031920 polymorphism and colon cancer risk in two genetic models. However, there was no significant association between *CYP2E1* rs3813867 polymorphism and colon cancer (Peng et al., 2013). They suggested that *CYP2E1* rs2031920 polymorphism is associated with colon cancer

risk, but the *CYP2E1* rs3813867 polymorphism does not, which is consistent with our study results.

In another meta-analysis by Zhou et al., (2010)10 case-control studies including 4,979 patients and 6,012 controls were evaluated. The results strongly indicated no significant association between *CYP2E1* PstI/RsaI polymorphism and susceptibility to colorectal cancer, which is consistent with the findings of our study (Zhou et al., 2010).

Additionally, Qian et al. in a meta-analysis showed a significant association between *CYP2E1* 96-bp insertion polymorphism and colorectal cancer risk. They proposed further studies with large sample to provide a more accurate estimate of the association of *CYP2E1* T7632A and *CYP2E1* 96-bp insertion polymorphisms with colorectal cancer (Qian et al., 2013).

In conclusion, the overall results of this study showed that there was no association between RsaI type *CYP2E1* polymorphism and colorectal cancer. The disadvantages of this study include low sample size as well as lack of a control group. Therefore, it is suggested to perform more studies with larger sample size and multicentric in different countries including a variety of ethnicities to achieve more accurate findings.

Author Contribution Statement

All authors contributed equally in this study.

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Ethical issue

The study protocol was approved by the Ethics committee of our university (Ethics Code: IR.IUMS.REC.1398.292). All the study steps were in accordance with the declaration of Helsinki.

Availability of data

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

There is no conflict of interests to declare by authors.

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