ISSN 0095-4527, Cytology and Genetics, 2020, Vol. 54, No. 4, pp. 363–371. © Allerton Press, Inc., 2020.

Genetic Polymorphisms of Three DNA-Repair Genes (*PRKDC*, *XPD*, *XRCC1*) are Related to Colorectal Cancer Susceptibility

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Received February 13, 2019; revised April 20, 2019; accepted May 18, 2020

Abstract—Although the specific causes of colorectal cancer (CRC) are not known, a robust DNA repair capacity may decrease the risk of this malignancy. DNA repair capacity may be reduced by alterations of genes involved in DNA repair process. This may affect susceptibility to carcinogenesis. It is hypothesized that single nucleotide polymorphisms (SNPs) of several DNA repair genes may be a risk factor for CRC susceptibility and prognosis. Using PCR–RFLP method, we conducted a case-control study to genotype 291 patients with CRC and 140 healthy individuals to determine variants in the *PRKDC*, *XPD* and *XRCC1* genes. Results showed that the genotypes of *XRCC1* c.580C>T polymorphism were associated with the risk of CRC. Compared with CC, CT (odds ratio (OR) = 5.35, $P < 0.001$) and CT/TT (OR = 4.74, $P < 0.001$) as well as T allele (OR = 4.95, $P < 0.001$) were overrepresented among the CRC patients. Variant genotype CC (OR = 2.37; $P = 0.042$) and C allele of *XPD* c.2251A>C (OR = 1.37; $P = 0.028$) polymorphism, enhanced the risk of CRC cases. Compared with GG, positive association was also obtained for all genotypes (GT, TT, GT/TT) of *PRKDC* rs7003908; 6721G>T polymorphism with CRC. Moreover, T allele of *PRKDC* demonstrated significant risk for CRC (OR = 5.61; $P < 0.001$). Besides, significant relevance of the *PRKDC* rs7003908; 6721G>T variations to smoking as well as *XPD* c.2251A>C variations to smoking and alcohol consumption in individuals with CRC was observed. Our findings indicated that genetic polymorphisms of *PRKDC*, *XRCC1*, *XPD* genes may influence susceptibility of CRC in the Iranian population.

Keywords: PRKDC, *XPD*, *XRCC1*, colorectal cancer, polymorphisms, cancer susceptibility

DOI: 10.3103/S0095452720040040

INTRODUCTION

DNA repair pathways have a crucial role in maintaining the genome integrity against mutations caused by general DNA replication errors as well as environmental insults. There are multiple DNA repair pathways. Bulky DNA lesions were removed by Nucleotide Excision Repair (NER); damaged bases were repaired by Base excision repair (BER) which links to single strand break (SSB) repair. DNA non-homologous end-joining (NHEJ) as well as Homologous Recombination (HR) pathways repair DNA double strand breaks (DSBs); mismatched base pairs were corrected by Mismatch repair (MMR) [1]. The x-ray repair cross-complementation group 1 (*XRCC1*) mapped to chromosome 19q13.2–13.3 has an essential role in the BER repair pathway. Although no known enzymatic activity has been reported for XRCC1, there are several crucial repair proteins that it can interact well with through its different domains, such as, DNA polymerase β, DNA ligase III, and PARP (ADP-ribose polymerase) in a complex, to coordinate the processes of BER repair pathway. The BER pathway effectively involves in DNA damage repair caused by a wide range of exogenous and endogenous factors, such as ionizing radiation, oxidation, and alkylating agents [2, 3]. Excision repair cross-complementing group 2/xeroderma pigmentosum group D (*ERCC2/XPD*), mapped to chromosome 19q13.3, has a vital role in the NER repair pathway via eliminating bulky DNA adducts caused by xenobiotics and environmental toxins [4]. The DNA-dependent protein kinase catalytic subunit (DNA-PKcs; encoded by *PRKDC*) has a pivotal role in DSBs repair via the NHEJ pathway [5].

The DNA repair capacity may be influenced by single nucleotide polymorphisms (SNPs) of DNA repair genes which, in turn, can be associated with an increase in cancer risk. Amongst the known repair genes' polymorphisms, SNPs in *XRCC1*, *XPD* and *PRKDC* genes were studied most frequently in most cancer types. Three SNPs including p.Arg399Gln

(exon 10, G to A substitution), p.Arg194Trp (exon 6, C to T substitution) and p.Arg280His (exon 9, G to A substitution), more often found in *XRCC1*' conserved sites. It has been identified that these polymorphisms are related to cancer susceptibility [6]. As an example, the *XRCC1* p.Arg399Gln variant has been associated mainly with colorectal, gastric, breast, esophageal, head and neck, and lung cancers [2, 7, 8]. Also, the *XRCC1* c.580C>T (p.Arg194Trp) polymorphism was related to various cancers such as head and neck, skin, colorectal, and gastric tumors [9–11]. Although the functional effects of *XRCC1* polymorphisms have not been revealed yet, it is proposed that *XRCC1* function may be altered by amino acid changes [1]. Furthermore, polymorphism at codon 751 of *XPD* is the most extensively studied SNPs in *XPD* which results in lysine to glutamine substitution. Although the functional effects of *XPD* p.Lys751Gln polymorphism is still unclear, the A to C base substitution leads to reduced DNA repair capacity of the protein [12]. In the past decade, there are a number of molecular epidemiological reports about the relation between *XPD* c.2251A>C (p.Lys751Gln) polymorphism and various types of cancer risk such as lung, breast, head and neck, and colorectal in different populations [12–15]. Moreover, among many polymorphisms in *PRKDC* reported as risk factors for cancers, the intronic polymorphism (rs7003908; 6721G>T) is the most widely studied one. Several studies had investigated the relation between the risk of cancers and *PRKDC* 6721G>T polymorphism. However, the results were not consistent [16, 17].

Colorectal cancer (CRC) has been a grand challenge in global health within the past few decades. Molecular epidemiology has confirmed that genetic susceptibility may have a main role it CRC carcinogenesis. The relation between polymorphisms of several DNA repair genes including *XRCC1*, *XPD* and *PRKDC* and CRC risk has been evaluated in some populations [18–22].

At present, three common polymorphisms including *XRCC1* c.580C>T (p.Arg194Trp), *XPD* c.2251A>C (p.Lys751Gln) and *PRKDC* (rs7003908; 6721G>T) have been found in the Iranian population. But, studies focused on the association between CRC susceptibility and these polymorphisms in this population were few [18, 23, 24]. More importantly, due to different patient population as well as relatively small sample size, the results published from foreign population about these polymorphisms were inconsistent or even contradictory. Therefore, this study was stablished in the Iranian population in order to reveal the relation among the genetic polymorphisms of *XRCC1* c.580C>T (p.Arg194Trp), *XPD* c.2251A>C (p.Lys751Gln) and *PRKDC* (rs7003908; 6721G>T) genes, age, smoking or drinking habit and CRC susceptibility.

MATERIALS AND METHODS

Study Subjects and Sample Collection

This study of colorectal cancer has been conducted from 2014 to 2017. The case group was comprised of 291 patients (153 males and 138 females) with a mean age of 60.93 years and a confirmed invasive adenocarcinoma of the colon through colonoscopy and histology of biopsies. They had no known types of inherited cancer syndrome. The control group was consisted of 140 healthy individuals (67 males and 73 females) with no identified cancer profiles, with a mean age of 59.09 years. All demographic data were recorded by the specialists. Five milliliters of peripheral blood was placed into ethylene diamine tetraacetic acid (EDTA)-coated tubes and stored at –70°C until analyzed.

XRCC1, XPD and PRKDC Genotyping

Extraction of the genomic DNA from whole blood was based on salting out method [25]. Genotyping for *XRCC1* c.580C>T (p.Arg194Trp), *XPD* c.2251A>C (p.Lys751Gln) and *PRKDC* (rs7003908; 6721G>T) polymorphisms was based upon polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay using primers shown in Table1. PCR was conducted in a total volume of 25 μL reaction mixture consisting of 50 ng genomic DNA, 0.5 μM of each primer (GeneScript, Germany), and 12.5 μL 2× *Taq* DNA Polymerase Mix-Mgcl2 1.5 mM (Amplicon, Denmark). The thermal cycling programs conducted were 2 min at 95°C followed by 30 cycles of 95°C for 45 s, annealing temperature for 30 s at 62°C for *XRCC1* c.580C>T and *PRKDC* rs7003908;6721G>T as well as 63°C for *XPD* c.2251A>C, and extension at 72°C for 20 s. A final extension was set at 72°C for 5 min. For confirmation of PCR amplicons of 485, 436 and 368 bp for *XRCC1* c.580C>T, *XPD* c.2251A>C and *PRKDC* 6721G>T were resolved through a 1% agarose gel respectively. In about 10% of the samples, the sequences of PCR products were confirmed utilizing DNA sequencing. Amplicons were then digested with *Pvu*II for *XRCC1* c.580C>T and *PRKDC* rs7003908;6721G>T PCR products as well as *Pst*I for *XPD* c.2251A>C PCR products at 37°C and were separated using 3% agarose gel electrophoresis. The genotypes were determined as listed in Table 1.

Statistical Analysis

Using Chi square (X^2) test, the Hardy-Weinberg equilibrium (HWE) for any deviation from expected allele frequencies was evaluated before association study was performed. χ^2 test was used to compare nonquantitative (allelic and genotypic) variables across groups. To conclude the crude odds ratio (OR) and 95% confidence interval (CI) between each of *XRCC1* c.580C>T (p.Arg194Trp), *XPD* c.2251A>C (p.Lys751Gln) and *PRKDC* (rs7003908; 6721G>T)

Table 1. Primers and PCR-RFLP conditions used for the genes genotyping

Repair system	Gene	SNP	Amino acid change	Exon/intron	PCR product size, bp	Band patterns, bp	Enzyme	Primer sequences 5'-3'	Reference
BER	*XRCC1*	c.580C>T (rs1799782)	p.Arg194Trp	Exon 3	485	C: 485 T: 396, 89	*Pvu*II	F: GCCAGGGCCCCTCCTTCAA R: TACCCTCAGACCCACGAGT	[26]
NER	*XPD*	c.2251A>C (rs13181)	p.Lys751Gln	Exon 23	436	A: 294,142 C: 231,63,142	*Pst*I	F: GCCCGCTCTGGATTATACG R: CTATCATCTCCTGGCCCCC	[27]
DSBs	*PRKDC*	6721G>T (rs7003908)	–	Intron 8	368	G: 368 T: 274,94	*Pvu*II	F: CGGCTGCCAACGTTCTTTCC R: TGCCCTTAGTGGTTCCCTGG	[28]

Table 2. Analysis of polymorphisms and CRC risk estimates

Variable	Cases no., %	Controls no., %	OR*	95% CI	*P*-value	X^2 *P*-value (overall)
XRCC1 c.580C>T (p. Arg194Trp)						
CC	240 (82.5)	134 (95.7)	1.00 (ref)	–	–	
CT	45 (15.5)	6 (4.3)	5.35	2.13–13.38	0 < 0.001	
TT	6 (2.1)	0 (0.0)	N/A[a]	–	0.999	
CT/TT	51 (17.5)	6 (4.3)	4.74	1.98–11.3	0 < 0.001	0.001
C allele	525 (90.2)	274 (97.9)	1.00 (ref)	–	–	
T allele	57 (9.8)	6 (2.1)	4.95	2.11–11.6	0 < 0.001	
XPD c.2251A>C (p. Lys751Gln)						
AA	29 (10.0)	22 (15.7)	1.00 (ref)		–	
AC	197 (67.7)	101 (72.1)	1.21	0.631–2.33	0.56	0.018
CC	65 (22.3)	17 (12.1)	2.37	1.03–5.48	0.042	
AC/CC	262 (90.0)	118 (84.3)	1.68	0.92– 3.05	0.084	
A allele	255 (43.8)	145 (51.8)	1.00 (ref)	–	–	
C allele	327 (56.2)	153 (48.2)	1.37	1.03–1.83	0.028	
PRKDC (rs7003908; 6721G>T)						
GG	54 (18.6)	103 (73.6)	1.00 (ref)	–	–	
GT	155 (53.3)	24 (17.1)	11.5714.06	6.33–21.15	0 < 0.001	0 < 0.001
TT	82 (28.2)	13 (9.3)	14.06	6.75– 29.27	0 < 0.001	
GT/TT	237 (81.4)	37 (26.4)	12.21	7.57–19.7	0 < 0.001	
G allele	262 (45.0)	230 (82.1)	1.00 (ref)	–	–	
T allele	320 (55.0)	50 (17.9)	5.61	3.97–7.94	0 < 0.001	

Abbreviations: OR, odds ratio; Ref, reference.
* Adjusted for age, gender, and study center.
[a] Not available.

polymorphisms and CRC, the logistic regression was considered separately. All analyses were carried out by using SPSS version 22.0 (SPSS Inc, Chicago, Illinois). Major characteristics of study groups were as mean and percent. Statistical significance was set at $P < 0.05$.

RESULTS

In this study, a total of 431 subjects in two groups, control and case, were enrolled. The mean age was 60.93 ± 11.3 years for the colorectal cancer patients. Among those, 153 were male and 138 were female. The mean age of the control group at baseline was 59.09 ± 12.3 years comprising 67 male and 73 female. We found no statistically significant difference with regard to gender and age between cases and controls ($P > 0.05$), indicating a well-matched study population. All single-nucleotide polymorphisms were consistent with Hardy–Weinberg equilibrium for controls.

The genotype distribution of polymorphisms in *XRCC1* c.580C>T (p.Arg194Trp), *XPD* c.2251A>C (p.Lys751Gln) and *PRKDC* (rs7003908; 6721G>T) genes for both groups are shown in Table 2. The frequencies of genotypes including CC, CT, and TT for *XRCC1* polymorphism were 82.5, 15.5, 2.1% and 95.7, 4.3, 0% among cases and controls, respectively. The frequency of *XPD* polymorphism was found to be 10% for AA, 67.7% for AC, and 22.3% for CC in CRC population while among control group the frequency was 15.7% for AA, 72.1% for AC, and 12.1% for CC. The frequencies of genotypes including GG, GT, and TT for *PRKDC* polymorphism were 18.6, 53.3, 28.2% and 73.6, 17.1, 9.3% among cases and controls, respectively.

In this study, the relation of *XRCC1* c.580C>T, *XPD* c.2251A>C and *PRKDC* rs7003908; 6721G>T polymorphisms to colorectal cancer susceptibility was investigated. According to the results listed in Table 2, we set individuals with *XRCC1* CC, *PRKDC* GG and *XPD* AA genotypes as the baseline for statistical analysis of association between CRC and genetic polymorphisms. Based on obtained results, when compared with the CC wild-type, the *XRCC1* CT heterozygous genotype (OR = 5.35; 95% CI, 2.13–13.38) as well as *XRCC1* CT/TT (OR = 4.74; 95% CI, 1.98–11.3) appeared to be significantly relevant to CRC while the *XRCC1* TT homozygote was not significantly

Table 3. Stratification analysis of the genotypes frequencies and risk factors in 291 CRC patients

	Age group		Gender		Smoking status		Alcohol consumption	
	<50	>50	female	male	never	ever	never	ever
				XRCC1 c.580C>T				
CC ($n = 240$)	28	212	116	124	157	83	210	30
CT ($n = 45$)	12	33	18	27	36	9	39	6
TT ($n = 6$)	0	6	4	2	5	1	6	0
P	***0.017***		*0.374*		*0.113*		*0.641*	
				XPD c.2251A>C				
AA ($n = 29$)	6	23	16	13	24	5	26	3
AC ($n = 197$)	26	171	94	103	116	81	166	31
CC ($n = 65$)	8	57	28	107	58	7	63	2
P	*0.511*		*0.540*		***0 < 0.001***		***0.025***	
				PRKDC 6721G>T				
GG ($n = 54$)	7	47	23	31	48	5	48	6
GT ($n = 155$)	15	140	77	78	83	72	132	23
TT ($n = 82$)	18	64	38	44	66	16	75	7
P	***0.033***		*0.651*		***0 < 0.001***		*0.357*	

related to an increased risk of colorectal cancer. Also, the frequencies of heterozygous and homozygote variant genotypes of *PRKDC* rs7003908; 6721G>T polymorphism were not significantly different between the CRC patients and control group. Carriers of the GT *PRKDC* or TT *PRKDC* polymorphisms, had 11.57-fold (95% CI [6.33–21.15], $p < 0.001$) and 14.06-fold (95% CI [6.75–29.27], $p < 0.001$) increased risk to develop CRC, respectively. Moreover, the risk of CRC for individuals carrying GT/TT genotypes was increased, and there was 12.21-fold compared with the individuals carrying GG genotype (Table 2), suggesting that *PRKDC* GT/TT genotype may increase the risk of CRC. In *XPD* c.2251A>C polymorphism, the risk (OR) to develop CRC was significantly increased to 2.37 ($p = 0.042$) in association with the homozygous CC-*XPD* genotype. The risk for CRC was not significantly different for individuals featuring the *XPD* AC heterozygous genotype (OR = 1.21; 95% CI, 0.631–2.33), or the *XPD* AC/CC genotypes (OR = 1.68; 95% CI, 0.92–3.05) (Table 2). Among cases and controls, the allele frequency distributions are shown in Table 2. The allele frequencies of *XRCC1* c.580C>T (p.Arg194Trp), *XPD* c.2251A>C (p.Lys751Gln) and *PRKDC* (rs7003908; 6721G>T) genes polymorphisms were statistically different between CRC patients and normals. The prevalence of the G allele of the *PRKDC* rs7003908;6721G>T polymorphism among the case and control groups was 45 and 82.1% respectively. The T allele frequency of this polymorphism were 55 and 17.9% in patients and healthy individuals respectively. So colorectal cancer risk showed a significant relationship with the T allele (OR = 5.61; 95% CI, 3.97–7.94). The frequencies for the T (*XRCC12* p.Arg194Trp) and C (*XPD* p.Lys751Gln) alleles amongst the CRC were, respectively, 9.8 and 56.2%. In addition, the distribution of these alleles were 2.1 and 48.2% in controls respectively. Therefore, the T allele of the *XRCC1* (p.Arg194Trp) (OR = 4.95; 95% CI, 2.11–11.6) and C allele of the *XPD* (p.Lys751Gln) (OR = 1.37; 95% CI, 1.03–1.83) polymorphisms may increase the risk of CRC (Table 2).

Analysis of *PRKDC*, *XPD* and *XRCC1* genotypes with demographic and risk parameters showed a significant association between the *PRKDC* (rs7003908;6721G>T) variations and smoking in colorectal cancer patients (Table 3). *XPD* (c.2251A>C) variations were also relevant to smoking and alcohol consumption in individuals with CRC (Table 3). No significant association was noted between *XRCC1* genotypes and these two risk parameters (Table 3). Also, significant relationships between the *XRCC1* and *PRKDC* variant genotypes and CRC risk stratified by age factor were shown here.

DISCUSSION

CRC is one of the most commonly diagnosed malignancies worldwide whose development and progression can be associated with a range of factors including environmental and lifestyle. Many environmental factors such as smoking, diet and radiation result in DNA damage. Unrepaired DNA leads to gene mutations, and genomic instability. Therefore, DNA repair genes have a pivotal role in protecting against mutations. Several studies have revealed that the polymorphisms of repair genes can affect the repair capability of them. There is increasing evidence to support the important role of DNA-repair genes'

polymorphisms in CRC susceptibility. For example, the correlation between RAD51, XRCC2, and XRCC3 polymorphisms and CRC was conducted by Krupa R et al. in 100 Polish patients [29]. They reported an increase in risk of CRC when XRCC2 (p.Thr 241 Met) was in combination with XRCC3 (p.Arg188 His). They also showed that the risk of CRC can be decreased when XRCC2 (p.Thr 241 Met) was combined with RAD51 (c.135C>C). Another study investigated the role of the XRCC2 (p.Arg188His) in Turkish population reported that in CRC patients, the frequency of XRCC2 polymorphism was two-fold higher than in control group [30]. Also, Larijani et al. (2018) has reported a novel polymorphism (p.Ser150Arg) in the XRCC2 gene in Iranian population and showed that the 150Arg in XRCC2 is associated with the development of CRC [31].

Here, we established a case-control study to determine the relationship between *XRCC1* c.580C>T (p.Arg194Trp), *XPD* c.2251A>C (p.Lys751Gln) and *PRKDC* (rs7003908; 6721G>T) polymorphisms and the CRC risk in an Iranian population. This investigation included 291 patients with CRC and 140 age and gender matched controls. Many studies with the aim of identifying the role of *PRKDC* (rs7003908;6721G>T), *XPD* (p.Lys751Gln) and *XRCC1* (p.Arg194Trp) polymorphisms on the risk of various cancers have been established. For example, previously, a significant association was found between *XRCC1* (p.Arg194Trp) and *PRKDC* (rs7003908;6721G>T) polymorphisms and prostate cancer [16]. Moreover, results obtained from a meta-analysis assessed the effect of *XPD* (p.Lys751Gln) polymorphism on various cancer types, reported a significantly association between this polymorphism and increased risk for cancers such as esophageal, breast and lung [12].

We revealed that the variant allele (Trp) in heterozygous (Arg/Trp) genotype of XRCC1 gene was related to the increased risk for CRC (OR = 5.35; 95% CI, 2.13–13.38) in an Iranian population, what was consistent with data published by Ye Li. They showed the relationship between *XRCC1* (p.Arg194Trp) polymorphism and an elevated colorectal cancer risk (OR = 1.45, 95% CI 1.12–1.88) [19]. In studies established by Nissar et al. and Abdel Rehman et al., although significant associations between *XRCC1* (p.Arg194Trp) polymorphism and CRC were reported but they showed that individuals inheriting the 194Trp variant allele (in both homozygous (Trp/Trp) and heterozygous (Arg/Trp) conditions) were more likely to develop CRC in Kashmiri and Egyptian populations respectively [10, 32]. Despite our data, few studies suggested that SNP variants in *XRCC1* gene possibly did not have a pivotal role in the CRC development. For example, Muñiz-Mendoza et al. revealed that *XRCC1* (p.Arg194Trp) SNP did not has association with risk of CRC in Mexican population [33]. In another study established in a Malaysian population, no positive relationship was reported between *XRCC1* (p.Arg194Trp) polymorphism and CRC risk [34]. Besides, Mehrzad et al. failed to show any significant relationship between p.Arg194Trp SNP and risk of CRC in Iranian population (north east of Iran) [18].

Here, it was also revealed that variant allele (Gln) in homozygous (Gln/Gln) genotype of *XPD* c.2251A>C (p.Lys751Gln) polymorphism could increase a possible risk of CRC. This observation was consistant with the results reported by Jelonek et al. [21], but contrary results were reported by Sliwinski et al. [35], Skjelbred et al. [36], and Bigler et al. [37]. Only one previous study has experimentally examined the role of the *XPD* c.2251A>C (p.Lys751Gln) polymorphism in CRC in an Iranian population reported no evidence of a significant relationship between this polymorphism and an elevated risk of CRC. In Rezaei et al. study, although compared to control group, the frequency of heterozygous genotype (*XPD* p.Lys 751 Gln) was more in cancer patients, the results were not statistically significant [24].

The *PRKDC* gene had long been investigated about the association with the risk of cancers; however, the results remained controversial [16, 17]. Here, an intronic variant was analyzed in *PRKDC* gene. This variant (rs7003908;6721G>T) not leading to any amino-acid substitution or an out-of-frame mutation. But, it is known that this single-nucleotide polymorphism might affect slightly the *PRKDC* mRNA splicing and, thus, decrease the protein expression level in splicing stages, which can affect DNA repair pathway [38]. Our results demonstrated that individuals with a variant allele (T) in both conditions including heterozygous (GT) genotype (OR = 11.57; 95% CI, 6.33–21.15) and homozygous (TT) genotype (OR = 14.06, 95% CI, 6.75–29.27) had an elevated susceptibility to CRC, when compared to the individuals with GG genotype of *XRCC1* gene, which was inconsistent with the findings published by Saadat et al. [23]. Based on their obtained results, no statistically significant relationship was reported between the variant allele (T) in *PRKDC* gene and risk of CRC in an Iranian population. They suggest that *PRKDC* TT genotype can be an important risk factor for the CRC development among persons with positive FH (family history) [23]. Due to results obtained here, T allele in *PRKDC* may be suggested as a marker for the CRC susceptibility.

Here, the role of environmental risk factors as modulating elements for CRC risk in the presence of various genetic variations in *XRCC1*, *XPD* and *PRKDC* genes in an Iranian population was also investigated. While the role of DNA repair genes (*XRCC1*, *XPD* and *PRKDC*) polymorphisms as risk factors for CRC was considered by Mehrzad et al., Saadat et al., and Rezaei et al. in Iranian population, no study has evaluated the role of interaction between these polymorphisms and environmental risk factors such as alcohol intake and smoking behavior in colorectal carcinogenesis in this population. To the best of authors' knowledge, it is the

first Iranian study that investigated the role of DNA repair genes variants as modulators of the effect of the environmental risk factors on CRC risk. The role of interaction between 194Trp allele and environment in colorectal carcinogenesis was evaluated in other populations. For example, in the United States, the effect of alcohol consumption in the risk of CRC was shown to be modulated by 194Trp allele [20]. Moreover, one meta-analysis published in 2013 suggested that the *XRCC1* p.Arg194Trp polymorphism might be modifier of alcohol consumption and smoking effects on CRC risk in Chinese population in Singapore [39]. However, our results suggested that there was no relationship between effects of *XRCC1* p.Arg194Trp polymorphism in the risk of CRC and alcohol consumption or smoking. In line with our study, no significant role for interaction between *XRCC1* p.194Trp allele and environmental exposures in elevating the risk of CRC was reported by Nissar et al. [32]. Besides, our results showed that p.Lys751Gln *XPD* genetic variations were related to an increase in the risk of colorectal cancer with smoking and alcohol consumption. Consistently, according to results published by Procopciuc et. al., the association between p.Lys751Gln *XPD* genetic variations and smoking was a risk factor for late-onset colorectal cancer [40]. Also, we found that the association between alcohol intake and *PRKDC* rs7003908;6721G>T genetic variations did not increase the risk of CRC. But, individuals positive for *PRKDC* rs7003908; 6721G>T genetic variations, with smoking habit had a higher risk to develop CRC. Furthermore, here, results showed significant associations between the *XRCC1* and *PRKDC* variant genotypes and CRC risk stratified by age factor. Our results were in agreement with those obtained by Datkhile et al. when reported that *PRKDC* gene polymorphisms represented association with an elevated risk of oral cancer when examined by stratifying age factor [41].

This discrepancy between studies established in Iranian population and ours is likely due to lower sample sizes of cases and controls in those studies when compared to our investigation. Also Mehrzad et al. study was restricted to a north east population of Iran [18]. The divergence in single nucleotide polymorphisms results obtained from different population may be due to different levels of carcinogen exposure. Other than polymorphism, environmental exposures or other genetic factors affect cancer formation which are inconsistent among different populations. Moreover, confounding factors including diet, age and gender may attribute to such discrepancies. In addition, the genotyping method may also play important role in contradictory findings among various studies [42]. So further studies incorporating another ethnic population or/and a larger sample size are necessary to confirm the role of these polymorphisms as regards CRC susceptibility in an Iranian population.

CONCLUSIONS

In Conclusions, our evidences showed that T alleles of *XRCC1* c.580C>T and *PRKDC2* (6721G>T) as well as C allele of *XPD* c.2251A>C can be relevant to a higher risk of developing CRC among Iranian population. However, further studies with detailed data on environmental exposure and larger sample size from different ethnicities are needed to confirm these initial encouraging results.

ACKNOWLEDGMENTS

This study was financially supported by the 92/7815 grant from Islamic Azad University (Varamin-Pishva Branch) of Iran.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were approved by the Institutional Ethics Committee and were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

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