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The Ability of Polymorphisms in DNA Repair Enzymes to Predict Clinical Outcome in Colorectal Cancer Patients

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Abstract

Background: Genomic polymorphisms of DNA repair enzymes-excision repair cross complementation group 1 (ERCC1), excision repair cross complementation group 2 (ERCC2), and X-ray repair cross complementation group 1 (XRCC1) correlate with survival and therapeutic responses in colorectal cancer (CRC) patients. Therefore, the present study examined the frequency of ERCC1 C118T, ERCC2 Lys751Gln, and XRCC1 Arg399Gln polymorphisms and their prognostic and predictive values in CRC patients.

Method: In this retrospective study, a total of 143 CRC patients were evaluated for these polymorphisms by PCR-RFLP.

Results: The majority of the patients showed heterozygous C/T (56%) compared to wild type C/C (29%) and variant T/T (15%) genotypes for ERCC1 C118T polymorphism. ERCC2 Lys751Gln polymorphism showed wild type A/A (44%), heterozygous A/C (40%), and variant C/C genotypes (16%). The frequency of XRCC1 Arg399Gln polymorphism was 48% (wild type G/G), 42% (heterozygous G/A), and 10% (variant A/A). The relapse-free survival (RFS) significantly decreased in patients with ERCC1 118 C/C wild type genotype in the subgroups of patients with advanced stage and colon cancer; however, variant T/T genotype correlated with reduced overall survival (OS) in patients treated with combined drug 5-FU/Oxaliplatin. Taken together, in CRC patients and patients treated with 5-FU/Oxaliplatin, ERCC2 Lys751Gln A/A wild type genotype led to significantly unfavorable clinical outcomes. However, XRCC1 Arg399Gln polymorphism did not show any significant association with prognosis. Additionally, on analyzing combined effect of ERCC1 and ERCC2 polymorphisms, a significant reduced OS in patients with both unfavorable genotypes (ERCC1: C/C and ERCC2: A/A) was found. Furthermore, in the subgroup of patients treated with 5-FU/Oxaliplatin, RFS and OS significantly decreased in patients with both unfavorable genotypes (ERCC1: T/T and ERCC2: A/A).

Conclusion: The significant relationship of ERCC1 C118T and ERCC2 Lys751Gln polymorphisms with prognosis and treatment response reflects the vital role of these molecules as prognostic and predictive markers in patients with CRC. Additionally, the combined evaluation of ERCC1 and ERCC2 polymorphisms might identify high risk CRC patients with poor prognosis.

Keywords: ERCC1, ERCC2, XRCC1, Polymorphism, Colorectal cancer, Prognosis

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Introduction

Over the past half-century, fluoropyrimidines have constituted the backbone of chemotherapeutic regimens in colorectal cancer (CRC). However, the introduction of the third generation drug, oxaliplatin, has undoubtedly been useful for patients with both early and advanced stage disease. Currently, the combination of oxaliplatin with fluorouracil (FOLFOX) or capecitabine (XELOX) has become a fundamental component of chemotherapeutic regimens in the standard adjuvant treatment of CRC.[1]

Two major DNA repair pathways, namely nucleotide excision repair (NER) and base excision repair (BER), are involved in the repair of damage caused by oxaliplatin. The major NER mediators, excision repair cross complementation group 1 (ERCC1) and excision repair cross complementation group 2 (ERCC2, also known as xeroderma pigmentosum group D -XPD), play a decisive role in repairing platinum-DNA adducts produced by oxaliplatin.[2] ERCC1 acts as an endonuclease and plays a crucial role in repairing platinum-induced DNA damage. ERCC2 is one of the core genes involved in transcription-coupled NER pathway, essential for transcription initiation, nucleotide excision repair, cell cycle control, and apoptosis.[3] Moreover, X-ray repair cross complementation group 1 (XRCC1), a major DNA repair gene in the BER pathway, is also involved in the repair of specific base damages caused by oxaliplatin.[4] XRCC1 acts as a scaffold protein facilitating the recruitment of DNA repair enzymes and a loading platform for the repair process.[5] Defects in BER and NER pathways may impair DNA repair capacity, leading to the accumulation of DNA damage, carcinogenesis and, possibly, reduction in chemotherapeutic sensitivity.[6]

Single nucleotide polymorphism (SNP) of genes involved in the NER pathway affects DNA repair capacity, thereby influencing the prognosis of malignant diseases.[7] Literature survey revealed the association between polymorphisms in DNA repair genes (ERCC1, ERCC2 and XRCC1) and cancer susceptibility, prognosis, and therapeutic outcomes in patients treated with oxaliplatin in CRC. One common C/T polymorphism at codon 118 (Asn118Asn) was identified in the ERCC1. This polymorphism converted a common codon AAC to AAT, resulting in the same amino acid asparagine;[8] however, the increase in the number of T allele led to higher ERCC1 mRNA levels.[9] Further, several ERCC2 polymorphisms are identified in the coding regions, amongst them, one of the most common SNPs occurring is at codon 751 of XPD due to lysine to glutamine substitution (Lys751Gln/A2251C). Certain studies proposed that ERCC1 C118T and ERCC2 Lys751Gln polymorphisms predicted responses as well as survival to platinum-based chemotherapy in CRC patients.[10,11] Additionally, the most extensively investigated XRCC1 Arg399Gln polymorphism on exon 10 leading to G→A amino acid substitution (Arg399Gln) possibly changed BER activity and as a result, the phenotype of the XRCC1 protein, resulting in deficient DNA repair capacity.[12] Many studies reported the association between XRCC1 Arg399Gln polymorphism and risk of CRC; however, few studies investigated the association between XRCC1 Arg399Gln polymorphism and survival in CRC. The results of these studies remain controversial.

The changes caused by gene polymorphism of DNA repair enzymes might impact the therapeutic efficacy and susceptibility to cancer;[3,13] therefore, it is necessary to explore the effect of these enzymes, particularly ERCC1 C118T, ERCC2 Lys751Gln, and XRCC1 Arg399Gln gene polymorphisms in CRC patients. Although these enzymes play a pivotal role in DNA repair, they are not currently recommended for clinical practice due to inconsistent results.[2] Accordingly, the present study aimed to examine the frequencies of ERCC1 C118T, ERCC2 Lys751Gln, and XRCC1 Arg399Gln polymorphisms in CRC patients. We further evaluated the prognostic and predictive values of these patients.

Materials and Methods

Patients

In this retrospective study, we enrolled a total of 143 untreated patients with histologically confirmed CRC at Gujarat Cancer and Research

Institute, Ahmedabad, between 2007 and 2014. The case files maintained at the medical record department of the institute provided the detailed clinical history (age, gender, anatomic site, disease stage, and hisopathological findings). We performed pathologic staging according to TNM classification with World Health Organization (WHO) Grading System. Primary treatment offered to all patients was surgery or surgery followed by adjuvant chemotherapy and/or radiotherapy. Out of 143, 113 patients underwent chemotherapeutic regimen. The main chemotherapeutic treatment included 5-fluorouracil (5-FU) and leucovorin (LV), oral capecitabine, or 5-FU in combination with oxaliplatin (OX). The follow-up on patients continued for a minimum period of 36 months or until death within that period. We obtained complete follow-up details from 114 CRC patients and included them for overall survival (OS) analysis; based on this analysis, 28 patients died within the follow-up period. Of 114 patients, we did not include 13 patients for relapse-free survival (RFS) analysis as they died due to persistent disease. Therefore, 101 patients underwent RFS. Survival analysis was also performed in the subgroups of patients with early and advanced stages of the disease and the subgroups of colon and rectal cancers according to tumor site. To evaluate the predictive efficacy of the studied polymorphisms on survival according to adjuvant treatment, we subgrouped patients into those treated with 5-FU alone and those treated with combined 5-FU+OX irrespective of RT. Regarding adjuvant treatment, out of 101 patients, 83 underwent RFS, and out of 114 patients, 94 underwent OS analysis. We considered the patients treated with adjuvant 5-FU based therapy as single drug group and those treated with adjuvant 5-FU+OX based therapy as combined drug group. The patient and tumor characteristics are shown in table 1.

Table 1. Patient and tumor characteristics

Characteristics	N (%)
Age (Range: 20-86 years)	
Median: 52 years	
<52	68 (48)
>52	75 (52)
Gender	
Female	58 (41)
Male	85 (59)
Anatomic site	
Colon	69 (48)
Rectum	74 (52)
Lymph node status	
Absent	90 (63)
Present	53 (37)
TNM stage	
I	24 (16)
II	64 (45)
III	51 (36)
IV	04 (03)
Tumor differentiation	
Well	29 (20)
Moderate	94 (66)
Poor	20 (14)
Histological type	
Adenocarcinoma	103 (72)
Mucinous/Signet ring cell adenocarcinoma	40 (28)
Treatment (N=143)	
Surgery alone	24 (17)
Surgery+Chemotherapy	67 (47)
Surgery+Chemotherapy+Radiotherapy	46 (32)
Surgery+Radiotherapy	06 (04)
Chemotherapeutic treatment (N=113)	
5-FU alone (5-FU intravenous or oral capecitabine)	66 (58)
5-FU+oxaliplatin [5-FU+OX (FOLFOX or CAPOX)].	47 (42)
Recurrence/Metastasis (N=101)	
Absent	82 (81)
Present	19 (19)
Disease status (N=114)	
Alive	86 (75)
Dead	28 (25)
Adjuvant treatment (RFS: N=83)	
Single drug: 5-FU	55 (66)
Combined drug: 5-FU+OX	28 (34)
Adjuvant treatment (OS: N=94)	
Single drug: 5-FU	60 (64)
Combined drug: 5-FU+OX	34 (36)

RFS: Relapse-free survival; OS: Overall survival.

Sample collection

Prior to primary tumor tissue collection, patients underwent surgery at the Department of Surgical Oncology signed written informed consent. The study was approved by Institutional Scientific and Ethics Review committees (Institutional Review Committee approval no.: IRC/2019/P-22 and Ethics approval no.: EC-O-132-2014). To detect ERCC1, ERCC2, and XRCC1 polymorphisms, we collected primary tumor tissue samples on ice directly from the

operation theatre. A pathologist selected and divided tumor tissues into two portions, one subjected to routine histopathological evaluation and the other immediately snap frozen in liquid nitrogen and preserved at -80°C until DNA extraction.

Polymorphism study of DNA repair enzymes by Polymerase chain reaction (PCR)- Restriction fragment length polymorphism (RFLP)

We extracted DNA samples from the frozen tumor tissues by phenol-chloroform extraction method and quantified them by both agarose gel electrophoresis and spectrophotometry. For polymorphism study, we performed PCR analysis in a total volume of 50 µl using PCR core kit (Qiagen, USA) with 0.1 µg of genomic DNA added per reaction. PCR was performed in a ProFlex PCR system (Applied Biosystems, Life Technologies Corporation, USA) based on the following conditions. Initial denaturation at 94°C for 3 minutes followed by 35 cycles of amplification (denaturation at 95°C for 1 minute; annealing: ERCC1-55.7°C for 45 seconds, ERCC2- 60°C for 30 seconds, XRCC1- 54°C for 30 seconds; extension at 72°C for 1 minute) and final extension at 72°C for 10 minutes. Next, specific restriction enzymes digested the PCR products. Table 2 shows the employed primers, restriction enzymes, and incubation period for each polymorphism. We separated the digested products on 2.5% ethidium bromide-stained agarose gel. After that, we examined the genotypes of the DNA samples for each polymorphism through visualizing the gel on UV transilluminator; finally, gel documentation system (Alpha Innotech, USA) captured the images.

Statistical analysis

We statistically analyzed the data using the statistical package for social sciences (SPSS) software version 17 (SPSS Inc., USA). We primarily tested the distribution of genotypes in patients for the Hardy-Weinberg equilibrium (HWE) by a goodness-of-fit chi-square (x^2) test to compare the observed genotype frequencies to the expected ones. We further calculated RFS and OS using Kaplan-Meier estimates and the difference in survival curve was calculated using Log rank test. Significance level was P value ≤0.05.

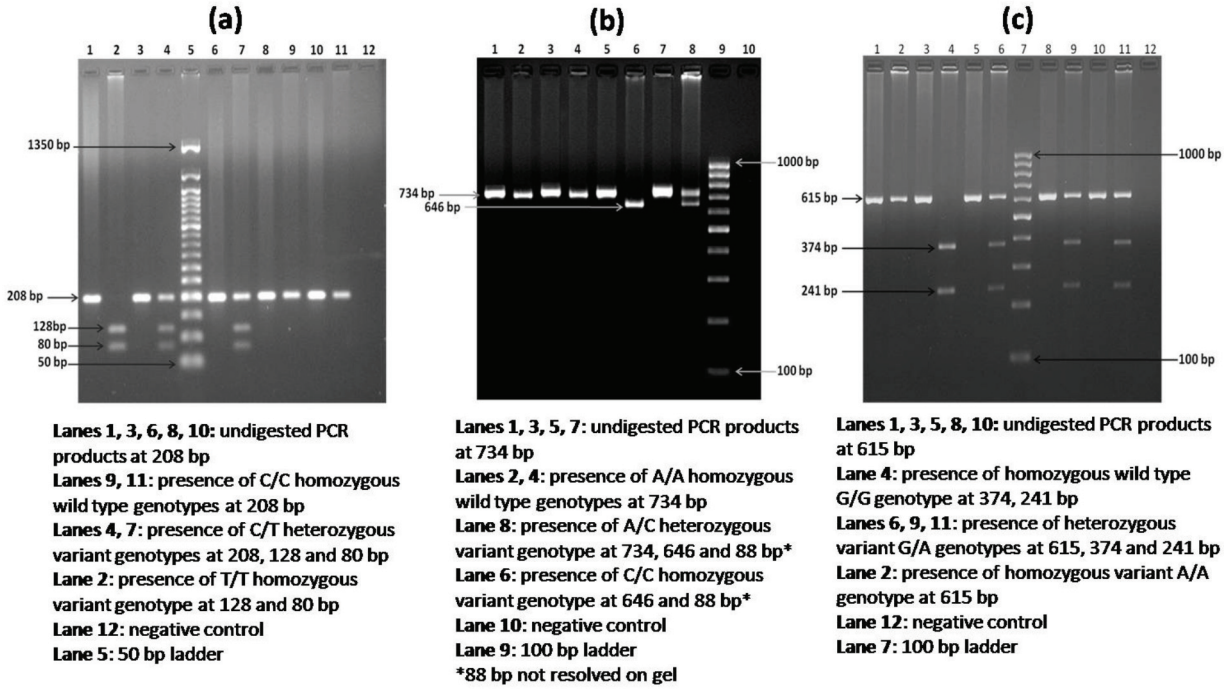

Figure 1. Gel images are representations for polymorphisms of (a) ERCC1 C118T (b) ERCC2 Lys751Gln, and (c) XRCC1 Arg399Gln in colorectal patients.

Results

Frequency of polymorphisms in DNA repair enzymes

We identified three types of genotypes for each ERCC1 C118T, ERCC2 Lys751Gln, and XRCC1 Arg399Gln SNPs in CRC patients (Table 3). The genotype distribution of each polymorphism followed HWE in CRC patients (Table 3). Figure 1 shows the representative gel images for each polymorphism.

For survival analysis with regard to each studied polymorphism, we evaluated the data among three individual genotypes as well as wild type vs combined variant type.

Correlation between ERCC1 C118T polymorphism and prognosis

ERCC1 C118T polymorphism did not show any significant association with RFS or OS in all patients and in the subgroups of early stage disease and rectal cancer. However, in advanced stage patients, RFS significantly decreased in patients with wild type C/C genotype (56%, 5/9) compared to those with combined variant genotypes (C/T+T/T) (24%, 6/25; *P*=0.039; Figure 2a). Additionally, in colon cancer subgroup, patients with wild type C/C genotype (37%, 3/8) showed a significantly reduced RFS compared to those with combined C/T+T/T genotypes (11%, 4/37) (*P*=0.035; Figure 2b). On the contrary, in patients treated with combined drug 5-FU+OX, OS significantly decreased in patients with T/T genotype (67%, 4/6) in comparison with C/C (50%, 5/10) and C/T genotypes (17%, 3/18; *P*=0.043; Figure 2c).

Correlation between ERCC2 Lys751Gln polymorphism and prognosis

In all patients, RFS had a declining trend in patients with wild type A/A genotype (28%, 11/39) compared to combined variant genotypes (A/C+C/C) (13%, 8/62; *P*=0.052; Figure 3a). Moreover, OS was significantly reduced in patients with A/A wild type genotype (36%, 17/47) compared with A/C (18%, 9/49) or C/C genotypes (11%, 2/18; *P*=0.037; Figure 3b); and also with combined variant genotypes (A/C+C/C) (16%, 11/67; *P*=0.012; Figure 3c). Additionally, in patients with colon cancer, wild type A/A genotype 36% (9/25) correlated with decreased OS in comparison with A/C (17%, 3/18) or C/C (0%, 0/8) genotypes (*P*=0.091; Figure 3g). Moreover, colon cancer patients with A/A genotype also correlated with a significant reduced OS as compared to those with combined A/C+C/C genotypes (11%, 3/26) (*P*=0.043; Figure 3f). However, according to the disease stage, OS had a decreasing trend with A/A genotype as compared to combined A/C+C/C genotypes in both early (*P*=0.073; Figure 3d) and advanced stage diseases (*P*=0.097; Figure 3e).

Concerning adjuvant treatment, in patients treated with combined 5-FU+OX therapy, OS was significantly reduced with A/A genotype as compared to A/C or C/C genotypes (*P*=0.013;

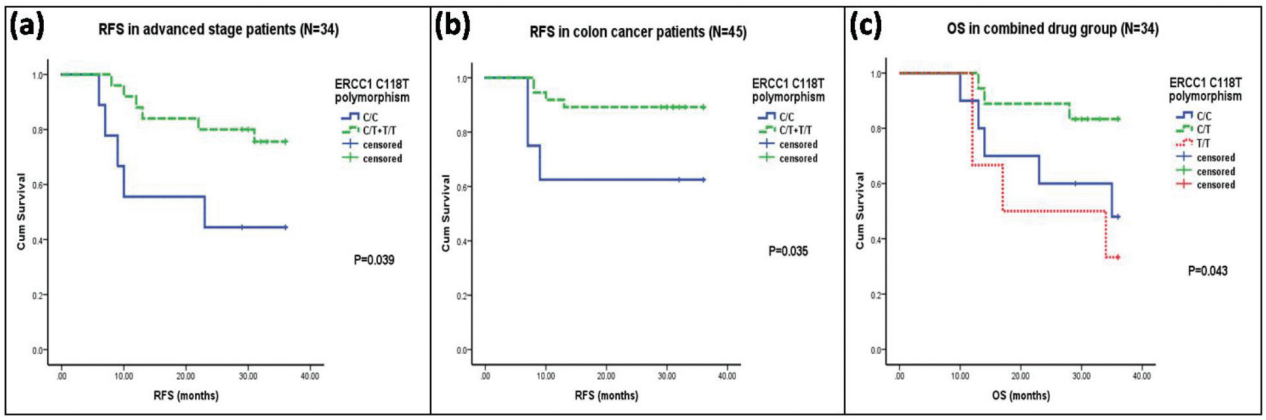

Figure 2. Kaplan-Meier survival curves for ERCC1 C118T polymorphism(a) RFS in advanced stage patients (b) RFS in colon cancer patients (c) OS in patients treated with the combined drug 5-FU/Oxaliplatin.
RFS: Relapse-free survival; OS: Overall survival.

Table 2. Primer sequences, restriction enzymes and incubation period for the polymorphism study

Polymorphism	Primer sequences	Restriction enzyme	Incubation period
ERCC1 C118T	Forward: 5' GCA GAG CTC ACC TGA GGA AC 3' Reverse: 5' GAG GTG CAA GAA GAG GTG GA 3'	BsrDI (New England Biolabs Inc., USA) (2 units/reaction)	65°C for 4 hrs
ERCC2 Lys751Gln	Forward: 5' CCT CTC CCT TTC CTC TGT TC 3' Reverse: 5' CAG GTG AGG GGG ACA TCT 3'	PstI (Roche Diagnostics GmbH, Germany) (10 units/reaction)	37°C overnight
XRCC1 Arg399Gln	Forward: 5' TTG TGC TTT CTC TGT GTC CA 3' Reverse: 5' TCC TCC AGC CTT TTC TGA TA 3'	MspI (New England Biolabs Inc., USA) (10 units/reaction)	37°C overnight

Figure 4c); and also with variant (A/C+C/C) genotypes (*P*=0.003; Figure 4d). Moreover, in this group, patients with wild type A/A genotype had a declining RFS trend as compared to A/C or C/C genotypes (*P*=0.061; Figure 4a) and a significantly reduced RFS compared to combined variant (A/C+C/C) genotypes (*P*=0.019; Figure 4b). Additionally, the trend of disease relapse was higher in patients with wild type A/A genotype (42%) treated with combined drug in comparison to those with A/A genotype (29%) treated with single drug (*P*=0.067). Similarly, higher incidence

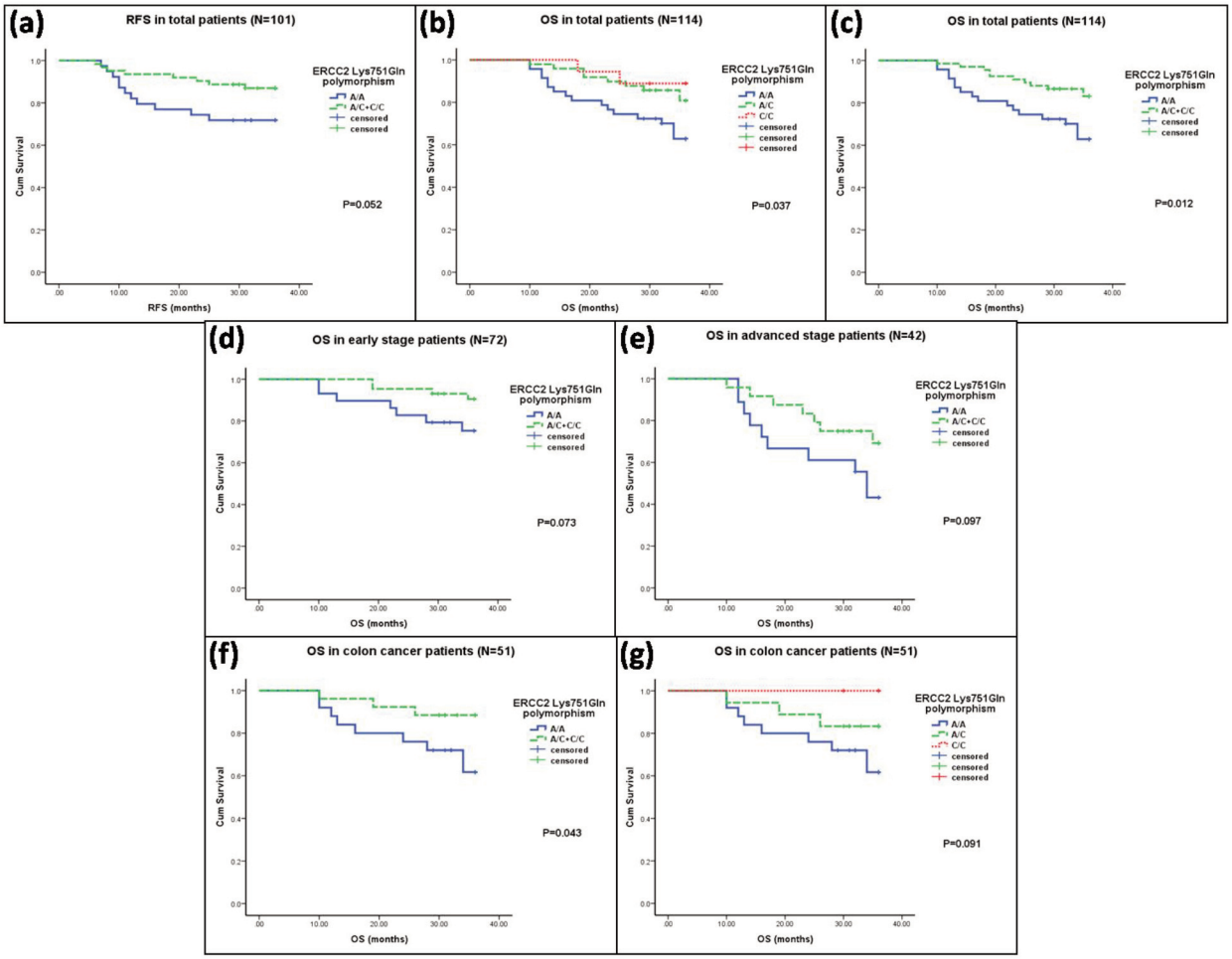

Figure 3. Kaplan-Meier survival curves for ERCC2 Lys751Gln polymorphism (a) RFS in total patients (b) & (c) OS in total patients (d) OS in early stage patients (e) OS in advanced stage patients (f) & (g) OS in colon cancer patients.
RFS: Relapse-free survival; OS: Overall survival

Table 3. Frequency of ERCC1 C118T, ERCC2 Lys751Gln, XRCC1 Arg399Gln polymorphisms

SNPs	Homozygous wild type N (%)	Heterozygous variant N (%)	Homozygous variant N (%)	HWE
ERCC1 C118T	C/C	C/T	T/T	X^2=2.946
	42 (29)	80 (56)	21 (15)	*P*=0.086
ERCC2 Lys751Gln	A/A	A/C	C/C	X^2=2.611
	63 (44)	57 (40)	23 (16)	*P*=0.106
XRCC1 Arg399Gln	G/G	G/A	A/A	X^2=0.105
	68 (48)	60 (42)	15 (10)	*P*=0.744

of death occurred in patients with A/A genotype (59%) treated with combined drug as compared to those with A/A genotype (23%) treated with single drug (*P*=0.063) (Table 4).

Correlation between XRCC1 Arg399Gln polymorphism and prognosis

XRCC1 Arg399Gln polymorphism did not emerge as a significant prognostic marker in total patients or in any of the subgroups. It also did not show any predictive values in the subgroups of patients treated with single drug or combined

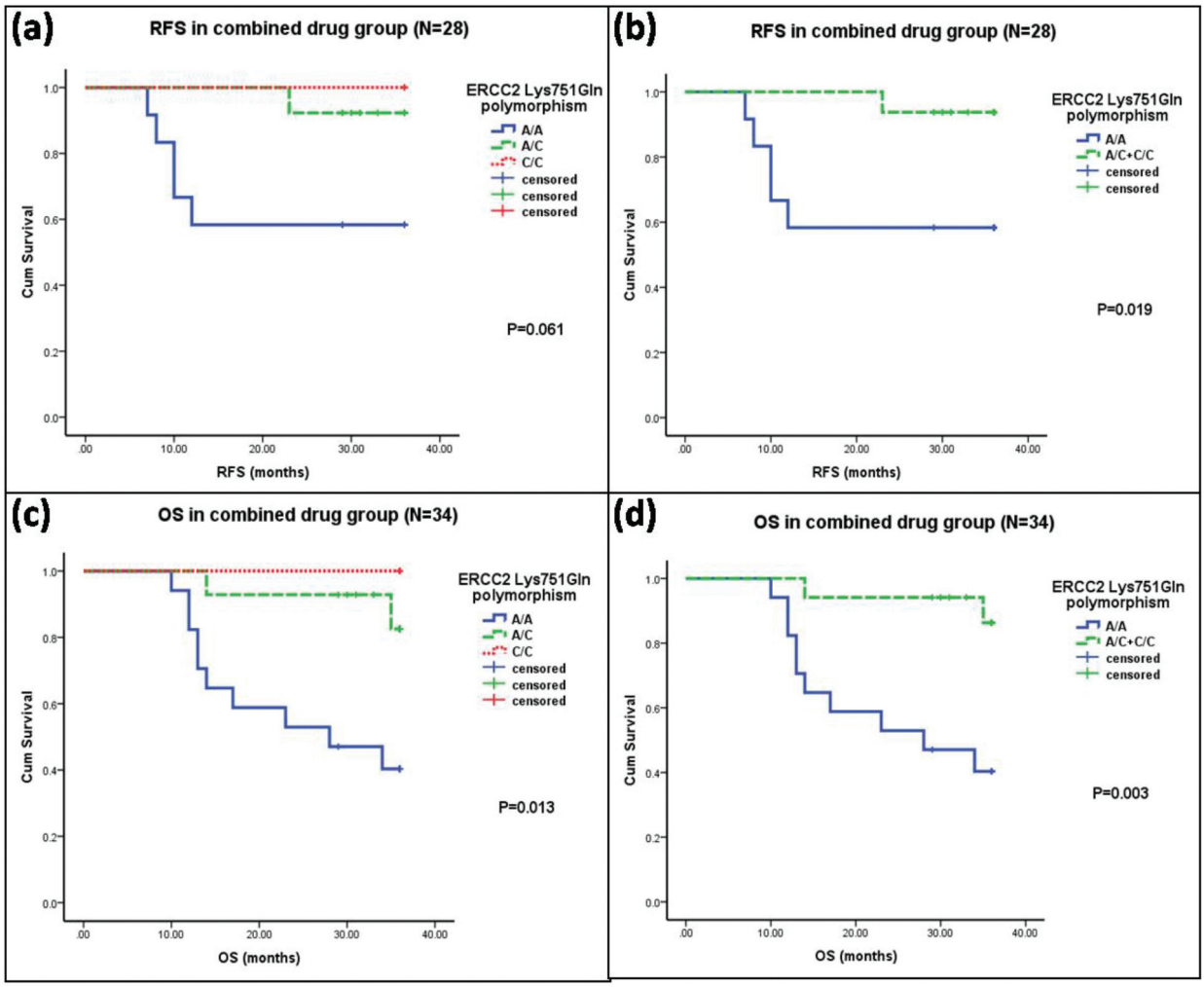

Figure 4. Kaplan-Meier survival curves for ERCC2 Lys751Gln polymorphism (a) & (b) RFS, (c) & (d) OS in patients treated with combined drug 5-FU/Oxaliplatin.
RFS: Relapse-free survival; OS: Overall survival

Table 4. Correlation between ERCC2 Lys751Gln polymorphism and survival in patients treated with 5-FU/Oxaliplatin

ERCC2 Lys751Gln polymorphism	N	No. recurrence N (%)	Recurrence N (%)	Log rank	df	*P*	Log rank statistics
Among three genotypes							
Single drug: 5-FU (N=55)							
A/A	21	15 (71)	06 (29)	0.386	1	0.534	Log rank=3.344
A/C+C/C	34	27 (79)	07 (21)				df=1
Combined drug: 5-FU+OX (N=28)							***P*=0.067**
A/A	12	07 (58)	05 (42)	5.530	1	**0.019**	
A/C+C/C	16	15 (94)	01 (06)				
Wild type vs variant type							
Single drug: 5-FU (N=60)							
A/A	22	17 (77)	05 (23)	0.035	1	0.852	Log rank=3.468
A/C+C/C	38	29 (76)	09 (24)				df=1
Combined drug: 5-FU+OX (N=34)							***P*=0.063**
A/A	17	7 (41)	10 (59)	8.601	1	**0.003**	
A/C+C/C	17	15 (88)	02 (12)				

drug (data not shown).

The combination of ERCC1 C118T and ERCC2 Lys751Gln polymorphisms and prognosis

We analyzed the combined effect of ERCC1 and ERCC2 polymorphisms on the prognosis of CRC patients. The patients were divided into two categories, namely total CRC patients and the subgroup of patients treated with combined 5-FU+oxaliplatin based therapy. In both categories, we classified the patients into three groups: both ERCC1 and ERCC2 unfavorable genotypes (group 1), one unfavorable genotype (group 2), and both favorable genotypes (group 3), (Table 5). In the first category, OS significantly decreased in patients with both unfavorable genotypes as compared to those with any one unfavorable and those with both favorable genotypes (*P*=0.041; Figure 5a). In the second category, RFS (*P*=0.049; Figure 5b) and OS (*P*<0.001; Figure 5c) were significantly reduced in patients with both unfavorable genotypes as compared to the other groups.

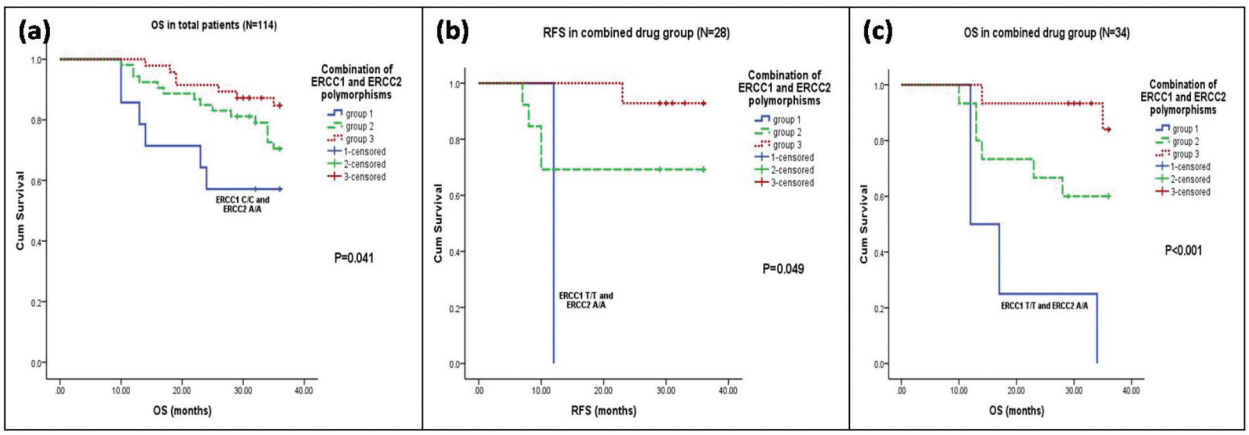

Figure 5. Kaplan-Meier survival curves for combination of ERCC1 and ERCC2 polymorphisms. (a) OS in total patients (b) RFS and (c) OS in patients treated with combined drug 5-FU/Oxaliplatin.

RFS: Relapse-free survival; OS: Overall survival

Table 5. Classification of unfavorable and favorable genotypes for combination of ERCC1 C118T and ERCC2 Lys751Gln polymorphisms

Genotypes	Unfavorable		Favorable	
	ERCC1 C118T	ERCC2 Lys751Gln	ERCC1 C118T	ERCC2 Lys751Gln
Category 1 (total patients)	C/C	A/A	C/T or T/T	A/C or C/C
Category 2 (combined drug group)	T/T	A/A	C/C or C/T	A/C or C/C

Discussion

The current study assessed the possible role of DNA repair enzymes involved in the action mechanism of oxaliplatin (ERCC1, ERCC2, XRCC1) in CRC patients. We observed that ERCC1 C118T and ERCC2 Lys751Gln polymorphisms could be potential predictive and prognostic biomarkers in CRC patients.

ERCC1 C118T polymorphism showed the predominance of heterozygous variant C/T genotype (56%) as compared to wild type C/C (29%) and variant T/T (15%) genotypes in CRC patients. Similarly, Viguier et al. showed the high incidence of C/T genotype (45%) as compared to C/C (22%) and T/T (33%) genotypes in advanced CRC.[8] Also, epithelial ovarian cancer patients had a preponderance of C/T genotype (46.1%).[14] On the other hand, there exist several reports on the high incidence of C/C genotype in CRC patients.[15,16]

In the present study, ERCC1 C118T polymorphism was unable to predict the survival in total patients; however, the subgroups of patients with advanced stage and colon cancer showed unfavorable RFS with C/C genotype as compared to variant T allele carriers. A trend towards high ERCC1 mRNA expression occurred as the number of T allele increased,[9] possibly resulting in high ERCC1 protein expression. Therefore, it could be postulated that C allele is associated with lower ERCC1 expression as compared to T allele. Thus, C/C genotype may lead to poor DNA repair capacity which could result in more biologically aggressive tumors due to their susceptibility to greater genetic aberrations over time, resulting in early recurrence and worse outcomes.[17] In accordance with the present results, Pare et al. showed that C/C genotype was able to significantly predict poor progression-free survival (PFS) and OS as compared to C/T or T/T genotypes in advanced CRC patients receiving 5-FU/oxaliplatin chemotherapy.[18] In contrast, current results showed the association between T/T genotype and worse outcomes in the subgroups of patients treated with adjuvant 5-FU+OX based combination therapy. This may be explained by the fact that ERCC1 C118T polymorphism could influence ERCC1 mRNA and protein expression, thereby affecting the sensitivity to platinum-based chemotherapies.[19] Higher ERCC1 protein expression could resist oxaliplatin-based therapy by repairing the platinum mediated DNA adduct formation, leading to poor survival. In line with the present results, one meta-analysis by Ma et al. showed that T allele correlated with reduced responsiveness to oxaliplatin-based chemotherapy in Asians and gastric cancer patients.[17] In this regard, compared to patients with C/T and T/T genotypes, patients with C/C genotype responded significantly better to FOLFOX4 in metastatic CRC,[20] and survival significantly improved in CRC patients treated with oxaliplatin-based adjuvant chemotherapy.[21] On the other hand, ERCC1 118 genotypes were not significantly correlated with the clinical outcomes in gastric cancer and advanced CRC patients treated with oxaliplatin-based chemotherapy,[22-25] and osteosarcoma patients.[26]

The present study reported 44% (A/A), 40% (A/C), and 16% (C/C) frequencies for ERCC2 Lys751Gln polymorphism in CRC patients. Le Morvan et al. observed similar frequencies in CRC.[27] However, several other studies in CRC showed the predominance of ERCC2 751 A/A genotype ranging from 73 to 92%.[28-30]

Regarding the prognostic role of ERCC2 Lys751Gln polymorphism, the present study found that in all patients, wild type A/A genotype predicted early relapse and death as compared to variant C allele carriers. Similarly, the subgroups of CRC patients with early stage, advanced stage and colon cancer further confirmed wild type A/A genotype as a worse prognostic factor regarding

OS prediction. In line with the present results, a study on DNA repair polymorphisms in patients with head and neck cancer reported that the polymorphic variants (Lys/Gln and Gln/Gln) of XPD 751 were associated with better survival and response to chemotherapy as compared to Lys/Lys genotypes.[31] Huang et al. reported that Taiwanese CRC patients with combined ERCC1 codon118 T/T and XPD codon751 A/A genotypes ran a significantly higher risk of regional recurrence compared with those without these two genotypes.[28] On the contrary, Kumamoto et al. showed that ERCC2 751 A/A genotype correlated with longer median PFS as compared to ERCC2 751 A/C genotype in CRC patients.[29] Dong et al. reported a comparable finding in CRC.[30] On the other hand, ERCC2 751 SNP and survival had no significant association regarding advanced CRC,[24] advanced NSCLC,[32,33] and osteosarcoma patients.[26]

Dai et al. proposed that the defects in BER and NER pathways might impair DNA repair capacity, resulting in the accumulation of DNA damage, carcinogenesis, and reduction in chemotherapeutic sensitivity.[34] Additionally, a recent report demonstrated that most of the anticancer agents were targeted to induce DNA damage. This overwhelms the cellular DNA repair capacity and leads to apoptosis, particularly in rapidly dividing cancer cells. Therefore, treatment efficacy is influenced by the DNA repair capacity of cancer cells; moreover, the differences in treatment response may be affected by the inherited variations of genes encoding DNA repair enzymes.[35] Furthermore, a number of SNPs in DNA repair genes possibly modulate gene expression and contribute to inter-individual variations of DNA repair capacity, ultimately affecting cancer susceptibility, prognosis, and therapeutic outcomes.[34] In this regard, Gan et al. suggested that variant C allele of ERCC2 Lys751Gln polymorphism correlated with its reduced activity; therefore, it led to reduced DNA repair activity in cancer cells.[7] Based on the above reports, it can be suggested that the wild type A/A genotype of ERCC2 Lys751Gln polymorphism probably leads to better DNA repair capacity; this, in turn, might reduce the sensitivity of tumor cells to chemo- and radiotherapy, thereby affecting therapeutic efficacy and possibly ensuing poor survival.

Additionally, ERCC2 wild type A/A genotype was associated with unfavorable prognosis in the subgroup of patients treated with combined 5-FU+OX based therapy. Similarly, in CRC, XPD 751 variant Gln/Gln genotype had significantly higher rates of response to 5-FU/OX chemotherapy and increased the survival in a Chinese population.[7] Lamas et al. also showed that in mCRC patients treated with mFOLFOX6, Lys/Gln significantly correlated with a favorable PFS.[36] On the contrary, patients with variant genotypes (Lys/Gln and Gln/Gln) had a shorter median event-free survival (EFS) and OS compared with Lys/Lys genotype in CRC patients treated with first line oxaliplatin-based therapy.[27] Also, ERCC2 751 variant genotypes (A/C and C/C) correlated with significantly increased risks of progression in CRC patients treated with FOLFOX4 first-line therapy.[11] The present study further showed that wild type A/A genotype had better clinical outcomes in patients treated with single 5-FU based therapy; however, variant genotypes (A/C+C/C) showed better outcomes in patients treated with 5-FU+OX based combination therapy. In accordance with this, Bradbury et al. observed that in esophageal cancer patients with variant C alleles of XPD 751, PFS and OS significantly improved in patients treated with platinum-based therapy; however, in patients who did not receive platinum therapy, variant C alleles of XPD 751 had a significantly worse survival.[37] The plausible reason for the present results might be as follows. Compared to 5-FU single agent, when treated with combined 5-FU+OX based therapy, patients with A/A genotype may develop weakened chemosensitivity due to the increase in the repair of oxaliplatin-induced DNA damage, hence worse clinical outcomes. On the other hand, variant C allele is associated with reduced DNA repair capacity; therefore, carriers of C allele might possibly develop higher chemosensitivity and better outcomes, especially when treated with more effective combined 5-

FU+OX therapy as compared to single agent 5-FU.

Interestingly, the present study analyzed the combined effect of ERCC1 and ERCC2 polymorphisms in all CRC patients and in those treated with combined drug. It was shown that patients with both unfavorable genotypes had reduced clinical outcomes. This suggests that the combined effect of both ERCC1 and ERCC2 polymorphisms may have adverse disease outcomes due to the reduced therapeutic efficacy.

Furthermore, the frequencies of XRCC1 Arg399Gln polymorphism in current study were 48% (G/G), 42% (G/A), and 10% (A/A) in CRC patients, which is in line with Ruzzo et al. who observed 49% of G/G, 43% of G/A and 8% of G/G genotypes in advanced CRC.[11] Stoehlmacher et al. observed similar results in CRC patients.[38] On the other hand, Arg/Gln (53.33%) was more predominant than Arg/Arg (28%) and Gln/Gln (18.67%) in sporadic CRC.[39] Chua et al. reported analogous frequencies, indicating the predominance of G/A heterozygotes (53%) in metastatic CRC.[40]

XRCC1Arg399Gln polymorphism had no significant association with RFS or OS in the present study. It also failed to emerge as a predictor of response to 5-FU/OX based treatment. Similarly, Siewchaisakul et al. reported no correlation between XRCC1 Arg399Gln polymorphism and survival in CRC patients.[41] Other studies on CRC patients treated with 5-FU/oxaliplatin based therapy failed to detect the significant prognostic impact of XRCC1-399 polymorphism in both advanced and metastatic settings.[11,38,40] However, Zaanan et al. reported a trend towards longer disease-free survival (DFS) concerning the variant A/A genotype of XRCC1 399 as compared to (G/G+G/A) genotypes in colon cancer patients treated with adjuvant oxaliplatin-based therapy.[42] In addition to CRC, variant genotypes (G/A+A/A) had a statistically significant better survival compared with the wild-type genotype (G/G) in NSCLC patients treated with platinum-based therapy.[43] In contrast, Liu et al. demonstrated a significantly worse survival with XRCC1-399 Gln/Gln genotype in gastric cancer patients receiving oxaliplatin-based therapy.[44] On the other hand, XRCC1 Arg399Gln polymorphism did not correlate with response to chemotherapy in advanced gastric cancer patients[45] and in advanced NSCLC.[46]

Conclusion

ERCC1 C118T polymorphism has emerged as a valuable prognostic marker for patients with advanced disease stage and CRC, and a predictive marker for selecting better treatment options. Moreover, ERCC2 Lys751Gln A/A wild type genotype could be a useful biomarker for predicting poor prognosis and reduced treatment response for CRC patients. Additionally, the concomitant effect of ERCC1 and ERCC2 polymorphisms might have prognostic and predictive values in CRC patients.

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Conflict of Interest

None declared.

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