RESEARCH ARTICLE

XRCC3 Thr241Met Gene Polymorphism and Risk of Colorectal Cancer in Kashmir: a Case Control Study

Saniya Nissar[1,4], Aga Syed Sameer[3], Tufail A Lone[2], Nissar A Chowdri[2], Roohi Rasool[1]*

Abstract

XRCC (X-ray cross-complementing group) genes contribute to important DNA repair mechanisms that play roles in the repair of single strand breaks (SSBs) induced by a variety of external and internal factors, including ionizing radiation, alkylating agents and reactive oxygen species. These repair genes have a pivotal role in maintaining genomic stability through different pathways of base excision repair (BER). The aim of this study was to investigate the *XRCC3 Thr241Met* gene polymorphism in colorectal cancer (CRC) in Kashmir. We investigated the genotype distribution of *XRCC3* gene in 120 CRC cases in comparison with 150 healthy subjects and found a significant association between *XRCC3* genotypes and CRC ($p \leq 0.05$). Both heterozygous genotype (Thr/Met) as well as homozygous variant genotype (Met/Met) were moderately associated with elevated risk of CRC [OR=2.53; OR=2.29 respectively]. Also, Thr/Met and Met/Met genotypes demonstrated a significant association with the risk of CRC ($p = 0.003$). This study displayed a significantly elevated risk for CRC in individuals with *XRCC3* Thr/Met and Met/Met Genotype of about 2.5 times that with the Thr/Thr wild genotype.

Keywords: Colorectal cancer - *XRCC3* - polymorphism - PCR-RFLP - Kashmir

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Introduction

Colorectal cancer (CRC) being one of the commonly diagnosed cancer in both men and women accounts for the third most common cancer in men and the second most common cancer in women worldwide (Jemal et al., 2011). CRC is the second leading cause of cancer-related death in many parts of the western world (Gellad and Provenzale, 2010). In Kashmir valley CRC represents the third most common GIT cancer (Sameer AS, 2013) after esophageal and gastric cancer.

DNA repair systems maintain the integrity of the human genome, hence deficiency in the repair capacity due to mutations or polymorphisms in genes involved in DNA repair can lead to genomic instability that, in turn, is related to chromosomal instability syndromes and increased risk of developing various types of cancer (Berwick et al., 2000; Goode et al., 2002; Zienolddiny et al., 2006).

Genetic polymorphisms in homologous recombination repair (HRR) genes (one of the four repair pathway in cell) leading to the insufficient protein have been found to be associated with increased risk of cancer (Smilenov, 2006). The *XRCC3* gene located on chromosome 14q32.3 codes for a protein involved in HRR for double strand breaks of DNA (DBSs) and cross-link repair in mammalian cells (Matullo et al., 2001b; Nassiri et al., 2013). DSBR pathway is responsible for repairing double-strand breaks resulting from exogenous as well as endogenous agents such as ionizing radiation or environmental carcinogens and endogenously generated ROS. They can also be produced when DNA replication encounter DNA single-strand breaks or other types of lesion (Jacobsen et al., 2004). *XRCC3*, which participates in DNA double-strand break via homologous recombinational repair, is a member of an emerging family of Rad-51-related proteins that likely participate in homologous recombinational repair (HRR) in order to maintain chromosome stability (Tebbs et al., 1995). *XRCC3* along with Rad51 and Xrcc2 form the core component of DNA double strand breaks (DSBs) repair by HRR (Nissar et al., 2014).

A common polymorphism in exon 7 of the *XRCC3* gene results in an amino acid substitution at codon 241 (Thr241Met) due to C18067T transition affecting the enzyme function and/or its interaction with other proteins involved in DNA damage and repair (Matullo et al., 2001b). Several studies have been conducted on *XRCC3* gene polymorphism in cancer. Many of them have proved the association between this polymorphism and an increased risk, like in lung (Jacobsen et al., 2004; Ryk et al., 2006), skin (Winsey et al., 2000; Blankenburg et al.,

*[1]Department of Immunology and Molecular Medicine, [2]Department General Surgery, Sher-I-Kashmir Institute of Medical Sciences, Soura, Department Biochemistry, [3]Sher-I-Kashmir Institute of Medical Sciences Associated Medical College, Bemina, [4]University of Kashmir, Hazratbal , Srinagar, Kashmir, INDIA *For correspondence: roohi_wani@yahoo.com*

2005), breast (Smith et al., 2003) and colorectal (Mort et al., 2003, Zhoa et al., 2012) cancer.

In the present work we analysed the potential influence of Thr241Met polymorphism of the *XRCC3* gene on the CRC risk and clinico-pathological parameters in a Kashmiri population.

Materials and Methods

Subjects

This study included 120 consecutive primary colorectal cancer patients. All CRC patients were recruited from Department of Surgery, Sher-I-Kashmir Institute of Medical Science. Tumor types and stages were determined by two experienced pathologists. Blood samples of 150 age and sex matched cases with no signs of any malignancy were collected for controls. The mean age of both patient and control groups was 55 years.

Data on all CRC patients were obtained from personal interviews with patients and or guardians, medical records and pathology reports. The data collected included sex, age, dwelling, tumor location, Dukes Stage, lymph node status. All patients and or guardians were informed about the study and their will to participate in this study was taken on predesigned questionnaire (Available on request). The collection and use of tumor and blood samples for this study were previously approved by the appropriate Institutional Ethics Committee.

DNA extraction and genotype analysis

DNA extraction was performed using Ammonium Acetate Method. One µL of DNA was used as the template for each PCR. Genotype analysis of *XRCC3* gene was carried out by PCR-RFLP using Primers (F: 5'-50-GCCTGGTGGTCATCGACTC-3; reverse, 5'-ACA G GGCTCTGGAAGGCACTGCTCAGCTC ACGCA CC-3') generating a fragment of 136 bp as described previously (Krupa et al., 2011). Briefly, PCR was carried out in a final volume of 25 µL containing 50 ng genomic DNA template, 1X PCR buffer with 2 mM MgCl2, 0.5 µM of each primer, 50 µM dNTPs and 0.5 U DNA polymerase. For PCR amplification, the standard program was used as follows: one initial denaturation step at 94°C for 7 min, followed by 35 denaturation cycles of 1min at 94°C, 1min of annealing at 57°C, and 1 min of extension at 72°C, followed by a final elongation cycle at 72°C for 7 min.

PCR product of 136 bp was digested by 10 U NcoI at 37oC. The homozygous Thr/Thr genotype produced 39 and 97 bp fragments, heterozygous genotype displayed three fragments: 136, 97 and 39 bp and the homozygous Met/Met genotype produced one 136 bp fragment (Figure 1). Restriction fragments were analysed on 3% agarose gels stained with ethidium bromide.

Statistical Analysis

Statistical analysis was performed by using SPSS Software (IBM). Observed frequencies of genotypes in cancer patients were compared to controls using chi-square or Fisher exact tests when expected frequencies were small. The chi-square test was used to verify whether genotype distributions were in Hardy-Weinberg equilibrium. Odds ratio was used to determine association of presence of mutations with various Clinico-epidemiological characteristics. Statistical significance was considered when p≤0.05.

Results

A total of 120 cases and 150 control subjects were included in this study with prior consent. All of the cases presented constipation and bleeding per rectum as their

Table 1. Frequency Distribution Analysis of Selected Demographic and Risk Factors in Colorectal Cancer Cases and Controls

Variable		Cases n=120	Controls n=150	P-Value
Age	>50	57	80	0.3
	≤50	63	70	
Gender				0.7
	Males	70	84	
	Females	50	66	
Smoking Status				
	Ever	63	85	0.5
	Never	57	65	
Dwelling				
	Rural	62	98	0.2
	Urban	38	42	

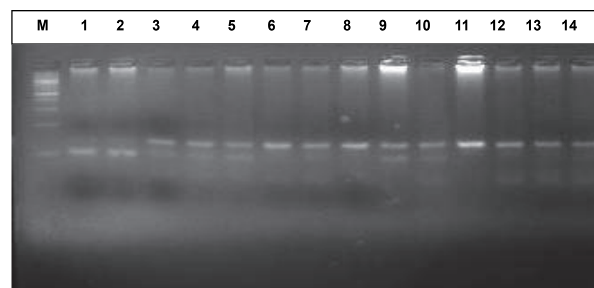

Figure 1. Representative gel of *XRCC3* Thr241Met polymorphism, Showing NQOI Digested Amplicons. The homozygous Thr/Thr genotype is represented by 39 and 97 bp fragments, heterozygous genotype Thr/Met is represented by three fragments: 136, 97 and 39 bp and the homozygous Met/Met genotype is represented by undigested one 136 bp fragment. 39 bp in not visualized in gel, only 136 and 97 bp are used for the distinguishing the three genotypes. Lane M: 100-bp ladder.

Table 2. Genotype Frequencies of *XRCC3* Gene Polymorphism in CRC Cases and Controls

XRCC3 Genotype	CRC Cases (n=120)	Controls (n=150)	OR (95% CI); P¥; Fψ	χ^2; P Value (Overall)
Thr/Thr-(Wild)	72 (60.0%)	118 (78.6%)	1	11.18; 0.003
Thr/Met-(Heterozygous)	34 (28.33%)	22 (14.66%)	2.53 (1.37-4.66);0.002; 0.003	
Met/Met-(Variant)	14 (11.66%)	10 (6.66%)	2.29 (0.96-5.4); 0.05; 0.07	

¥Pearson's P Value; ψFisher Exact P Value; Significant P values are shown in bold

Table 3. Association between *XRCC3* Polymorphism and Clinicopathological Characteristics

Variables		n = 120	Cases (n=120)			χ^2 ; p value
			GG	AG	AA	
		72 (60.0%)	34(28.3%)	14 (11.6%)		
Age group	≤50	63 (52.5%)	41	13	9	4.12; 0.12
	>50	57 (47.5%)	31	21	5	
Gender	Female	50 (41.66%)	30	14	8	1.23; 0.54
	Male	70 (58.33%)	42	20	6	
Dwelling	Rural	62 (51.6%)	37	18	7	0.04; 0.98
	Urban	58 (48.3%)	35	16	7	
Smoking status	Never	57 (47.5%)	29	22	6	5.66; 0.05
	Ever	63 (52.5%)	43	12	8	
Tumor location	Colon	52 (43.3%)	30	13	9	2.94; 0.22
	Rectum	68 (56.6%)	42	21	5	
Nodal status	Involved	78 (65.0%)	50	18	10	3.05; 0.21
	Not Involved	42 (35.0%)	22	16	4	
Tumor grade	WD	90 (75.0%)	52	26	12	1.19; 0.55
	MD+PD	30 (25.0%)	20	8	2	

[a]One was squamous cell carcinoma; Significant P values are shown in bold

chief complaint. Furthermore, out of 120 confirmed cases of CRC, 70 were males and 50 cases were females (M/F ratio=1.4), 62 were rural and 58 were urban, 52 cases had carcinoma in colon and 68 in rectum and 63 were smokers and 57 non-smokers (Table 1). The mean age of patients having confirmed CRC was 55 years. Among control subjects, 84 consisted of males and 66 females (M/F ratio=1.27). No significant gender-or age-related differences were observed between the groups (p>0.05).

Among the CRC cases, we found the frequency of the *XRCC3* genotype to be 60.0 per cent (72/120) for Thr/Thr, 28.33 per cent (34/120) for Thr/Met and 11.66 per cent (14/120) for Met/Met, while the frequency in the general control population was 78.6 per cent (118/150) for Thr/Thr, 14.66 per cent (22/150) for Thr/Met and 6.66 per cent (10/150) for Met/Met. The overall association between the *XRCC3* polymorphism and the CRC cases was found to be significant (p<0.05) (Table 2). Overall both the heterozygous genotype (Thr/Met) as well as homozygous variant genotype (Met/Met) were associated with the elevated risk for CRC [OR=2.53 (95% CI=1.37-4.66); OR=2.29(95% CI=0.96-5.4)] respectively. Also, independent analysis for the Thr/Met and Met/Met genotypes revealed a significant association with the risk of CRC (p<0.05). The overall hazard ratio of the *XRCC3* Met allele in CRC was 2.29. (95% CI=0.96-5.4).

The correlation of *XRCC3* polymorphic status with the clinico-pathological characteristics was also carefully analyzed. We found a significant association of variant allele of *XRCC3* gene with smoking status (p value=0.05) (Table 3).

Discussion

In this study, we have examined whether polymorphism in DNA repair gene *XRCC3*, involved in the double-strand break (DSBR) DNA repair pathways, is implicated in modulating the risk of development of CRC in Kashmiri population in continuation of our previous study on XRCC1 gene polymorphism (Nissar et al., 2013).

Since the *XRCC3* protein is one of five identified paralogs of the RAD51 protein in humans and functions through complex interactions with other proteins to repair DSB and maintain genome integrity in multiple phases of HR. Double strand DNA breaks are the most dangerous DNA damage occurring either directly in cells as the result of endogenous and exogenous insults or as a result of a conversion of single strand breaks (Kowalska-Loth et al., 1998) and can result in amplification or loss of genetic material which can further lead to neoplastic transformation by activation of oncogenes, inactivation of suppressor genes or loss of heterozygosity. It can be result of decrease of HRR fidelity or switch of repair over less correct process of non-homologous end joining (NHEJ).

During HRR, the *XRCC3* along with Xrcc2 interacts with Rad51 and likely contributes to maintain chromosome stability. The HR pathway uses a second intact copy of a homologous chromosome as a template to copy the information lost at the DSB site, resulting in a high-fidelity process and preventing chromosomal aberrations (Christmann et al., 2003). *XRCC3* deficient hamster cells showed a high frequency of multiple centrosomes and abnormal spindle formation. Also cell deficient with *XRCC3* gene product are defective in HR and demonstrate genomic instability (Takata et al., 2001; Griffin, 2002; Deans et al., 2003; Thacker, 2005) suggesting its importance in repair mechanism. Thus polymorphisms of this gene may result in a reduced DNA repair capacity.

Our results show the frequency of the *XRCC3* genotype to be 60.0 per cent (72/120) for Thr/Thr, 28.33 per cent (34/120) for Thr/Met and 11.66 per cent (14/120) for Met/Met, while the frequency in the general control population was 78.6 per cent (118/150) for Thr/Thr,, 14.66 per cent (22/150) for Thr/Met and 6.66 per cent (10/150) for Met/Met. The overall association between the *XRCC3* polymorphism and the CRC cases was found to be significant (p<0.05) (Table 2). Overall both the heterozygous genotype (Thr/Met) as well as homozygous variant genotype (Met/Met) were modestly associated with the elevated risk for CRC [OR=2.53 (95%CI=1.37-4.66); OR=2.29 (95%CI=0.96-5.4) respectively]. Also, individual analysis for the Thr/Met and Met/Met genotypes revealed a significant association with the risk of CRC (p<0.05). The overall hazard ratio of the *XRCC3*

Met allele in CRC was 2.29. (95% CI=0.96-5.4). Our findings are in concordance with those reported by Mort et al. (2003), Krupa et al. (2011) and Importa et al. (2008) and contradictory to those reported by many authors (Tranah et al., 2004; Stern et al., 2005; Yeh et al., 2005; Skjelbred et al., 2006) which show negative association. Also, it has been suggested that variant genotypes of *XRCC3* have decreased repair capacity and thus individuals with this genotypes do not repair double strand DNA breaks efficiently by HRR and are susceptible to risk of CRC (Krupa et al., 2011).

XRCC3 plays a key role in maintaining the genome integrity and substitution of Thr to Met in codon 241 is the most frequent polymorphism in *XRCC3* that may affect the biological implications of the enzyme's function and thus alter DNA repair capacity as well (Matullo et al., 2001; Jin et al., 2005).

Further we also found association of polymorphism with the smoking status which is in agreement with study of Matullo et al. 2001 study who has associated the 241Met polymorphism with 32P-DNA adduct levels, indicating a possible role of the *XRCC3* gene in the repair of bulky DNA adducts. Thus, variations in DNA repair capacity caused by polymorphisms of DNA repair genes may modulate the genotoxic effect of tobacco smoking.

Our results suggest that genetic polymorphism of the *XRCC3* gene may be associated with an individual's susceptibility to colorectal cancer. However, we acknowledge the relatively limited sample size as a limitation of this study as there are number of variables that may affect the outcome of the genotype vis a vis polymorphism of this gene in interaction with the environment.

In conclusion, this study displays a significantly elevated risk for colorectal cancer in individuals with *XRCC3* Thr/Met and Met/Met genotypes, suggesting DSBR DNA repair pathway is modulating the risk of developing CRC in Kashmiri population with almost two and half times than Thr/Thr genotype.

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