RESEARCH ARTICLE

The *XRCC1* Arg399Gln Gene Polymorphism and Risk of Colorectal Cancer: a Study in Kashmir

Nighat Parveen Khan[1], Arshad Ahmad Pandith[2], Adfar Yousuf[1], Nuzhat Shaheen Khan[3], Mosin Saleem Khan[1], Imtiyaz Ahmad Bhat[2], Zahoor Wani Nazir[1], Khursheed Alam Wani[4], Mahboob Ul Hussain[5], Syed Mudassar[1]*

Abstract

Background: The DNA repair gene *XRCC1* Arg399Gln gene polymorphism has been found to be implicated in the development of various cancers, including colorectal cancer (CRC), in different populations. We aimed to determine any association of this polymorphism with the risk of CRC in Kashmir. Materials and Methods: A total of 120 confirmed cases of CRC and 146 healthy cancer free controls from the Kashmiri population were included in this study. Genotyping was carried out by the polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method. Results: Genotype frequencies of XRCC1 Arg399Gln observed in controls were 34.2%, 42.5% and 23.3% for GG (Arg/Arg), GA (Arg/Gln), AA(Gln/Gln), respectively, and 28.3%, 66.7% and 5% in cases, with an odds ratio (OR)=5.7 and 95% confidence interval (CI) =2.3-14.1 (p=0.0001). No significant association of Arg399Gln SNP with any clinicopathological parameters of CRC was found. Conclusions: We found the protective role of 399Gln allele against risk to the development of CRC. The XRCC1 heterozygote status appears to be a strong risk factor for CRC development in the Kashmiri population.

Keywords: X-ray cross complementing gene - SNP - DNA repair - colorectal cancer - Kashmiri population

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Introduction

Colorectal cancer (CRC) is the major cause of mortality and morbidity in western countries. It is third most common cancer in both men and women (Hisamuddin et al., 2006). As per cancer fact and figures for African-Americans an estimated 16,650 cases and 7,050 deaths from CRC occurred among African Americans in 2011. In Kashmir CRC ranks 4th common cancer among other cancers (Arshad et al. accepted for publication). Kashmiri population has a peculiar dietary habits and mode of living. Food of this region has been found to contain nitrosoamines, which are carcinogenic (Khuroo et al., 1992). Exposure to mutagenic and carcinogenic aromatic amines via environmental factors, well-cooked food and other sources can form DNA adducts in vivo and thus lead to DNA damage which if un corrected can ultimately lead to tumorigenesis. However, integrity of damaged DNA is maintained by DNA repairing enzymes. *XRCC1*, XRCC3, and XPD are polymorphic genes belonging to 3 of the major DNA repair pathways. *XRCC1* [X-ray repair cross complementary] gene located on 19q13.31 chromosome encodes protein involved in base excision repair (BER) and the repair of single-strand breaks. The *XRCC1* gene product plays an important role in the pathway by acting as a scaffold for other DNA repair proteins, such as DNA polymerase (Kubota et al., 1996)and DNA ligase III (Caldecott et al., 1994). The protein also has a BRCA1 C-terminus (BRCT) domain, which is characteristic of proteins involved in the recognition of and response to DNA damage. *XRCC1* interacts with poly (ADP-ribose) polymerase (PARP) by means of the BRCT domain to enable the recognition and subsequent repair of single-strand breaks (Caldecott et al., 1996; Masson et al., 1998).

DNA damage caused by a variety of internal and external factors, including ionizing radiation, alkylating agents, and oxidation, requires repair by the BER pathway.*XRCC1* lacking mutant hamster ovary cell lines are hypersensitive to ionizing radiation, hydrogen peroxide and alkylating agents, and are more susceptible to chromosomal aberrations compared to the parental cells.The importance of *XRCC1* to genetic stability is further indicated by that mice lacking *XRCC1* die in early embryogenesis (Tebbs et al., 1999).

DNA damage or reduced DNA repair capacity is viewed as an important mechanism in genetic instability and carcinogenesis caused by ionizing radiation and environmental chemical agents (Lei et al., 2013).

*[1]Biochemistry Department, [2]Immunology and Molecular Medicine Department, [4]Surgery Department, Sher-I-Kashmir Institute of Medical Sciences, [3]Biochemistry Department, [5]Biotechnology Department, University of Kashmir, Kashmir, India *For correspondence: parveennighat24@gmail.com, syed.mudassar@skims.ac.in*

Polymorphisms in *XRCC1* gene that cause amino acid substitutions may impair the interaction of *XRCC1* with the other enzymatic proteins and consequently alter DNA strand break repair. Genetic polymorphisms in DNA repair genes may influence individual variation in DNA repair capacity, which may be associated with risk of developing cancer (Skjelbred et al., 2006: Wang et al., 2009).

Many studies have reported a link between Arg399Gln *XRCC1* SNP and increased risk of colorectal cancer (Krupa et al., 2004: Mariana et al., 2007) and also with other cancers like prostrate, gastric, breast cancer, Hepatocellular Carcinoma, Glioma (Moullan et al., 2003, Rybicki et al., 2004; Liu et al., 2007; Wei-Hong et al., 2012).These studies have reported that AA genotype is associated with increased risk of colorectal cancer. Also GA genotype was found as a risk factor for progression of tumor growth. In contrast to this view some of the studies have reported protective role of AA allele (Olshan et al., 2002; Seedhouse et al., 2002; Skjelbred et al., 2006).

We aim to conduct a case-control study for *XRCC1* Arg399Gln gene polymorphism and to see its association with the risk of colorectal cancer in Kashmiri population.

Materials and Methods

A total of 120 blood samples of CRC patients were collected in the General surgery ward and clinics at Sher-I-Kashmir Institute of Medical Sciences [SKIMS] between May 2009 and January 2011. All the cases were histologically confirmed to be CRC. A pool of 146 control subjects were recruited from the same hospital and belonged to the same geographic area, ethnic background, and were of matching sex and age group. The controls did not have a previous diagnosis of any type of cancer. A written informed consent was obtained from each recruited subject, and the study was approved by the SKIMS ethical committee.

DNA extraction and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

Genomic DNA was isolated by Phenol chloroform method from whole-blood samples of both cases and controls. Previously reported primers (Chih-Ching et al., 2005) were used for the amplification of the target regions of 615bp of the *XRCC1* Arg399Gln polymorphisms. PCR was carried out in a final volume of 25ul containing 50 ng genomic DNA template, 1× PCR buffer (Biotools, B&M Labs, Madrid, Spain) with 2mmol/L $MgCl_2$, 0.4mmol/L of each primer (Genescript, Piscataway, NJ), 50mmol/L dNTPs (Biotools, B&M Labs), and 1.25U Taq polymerase (Biotools, B&M Labs). For PCR amplification, the standard protocol was used as follows: one initial denaturation step at 94°C for 7 minutes, followed by 35 denaturation cycles of 30 seconds at 94°C, 30 seconds of annealing at 58°C, and 30 seconds of extension at 72°C, followed by a final elongation cycle at 72°C for 5 minutes. For RFLP, the PCR products of *XRCC1* SNP were digested with MspI (Fermentas, Inc., Glen Burnie, MD) 1U at 37°C for 4 hours. Digested PCR product was run on 3% Agarose gel and were visualized using ethidium bromide. GG allele revealed 375bp and 239bp fragments whilst the AA allele was not digested by MspI and thus was visualized by 615bp single product. AG (heterozygote) allele revealed three bands of 615bp, 376 and 239bp (Figure 1).

Data analysis

The cases and controls were compared using the chi-square test for categoric variables, such as sex and smoking status, and other demographic variables.Crude odds ratios (OR) were used as estimates of the relative risk, and 95% confidence intervals (CI) were calculated to estimate the association between certain genotypes or other related risk factors of CRC.

Results

In this study 120 confirmed CRC cases and 146 healthy cancer free controls were included. In cases 63 (53%) were males and 57 (47%) females and the controls consisted of 77 (52.7%) male and 69 (47.26%) females. Of the total cases 54 (45%) were non-smokers and 66 (55%) were smoker. 52 (43%) of the cases were in the age group of less than 50 and 68 (57%) of the cases were in the age group of greater than 50. Among controls 66 (45%) were in less than fifty and 80 (55%) were in greater than fifty age group respectively (Table 1). *XRCC1* genotype distributions of wild AA and variant-type alleles GG among different clinico-pathologic characteristics are shown among the Kashmiri population for the cases. Patients with age group ≥50 years had marginally higher risk (OR=1.5) than cases with ≤50 years of age, but the difference is observed to be

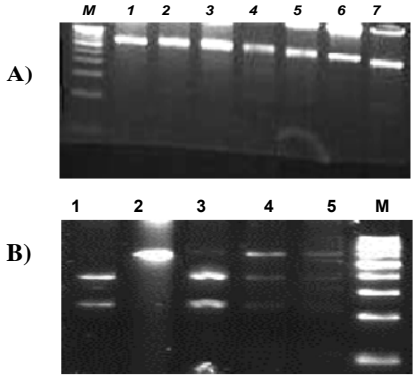

Figure 1. A) Lane M 100bp DNA ladder. Lane 1-7amplified 609 bp PCR product; and **B) Lane M 100 bp DNA Ladder.** Lane 2 AA,1& 3GG, Lane4, 5GA genotype

Table 1. Comparison of Clinico-pathological Characteristics of Controls and Cases

Variables		Cases (n=120)	Controls (n=146)	OR (95%CI)	p value
Age group	<50	52(43%)	66(45%)	0.90(0.6-1.6)	0.75
	≥50	68(57%)	80(55%)		
Gender	Male	63(53%)	77(52.7%)	0.99(0.6-1.6)	0.96
	Female	57(47%)	69(47.26%)		
Smoking status	Never	54(45%)	60(34%)	1.17(0.7-1.8)	0.5
	Ever	66(55%)	86(66%)		
Residence	Rural	64(53.3%)	75(51.36%)	1.00(0.6-1.6)	0.7
	Urban	56(47%)	71(48.63%)		
Tumor site	Colon	72(60%)			
	Rectum	48(40%)			
Grade	WD	77(64%)			
	MD/PD	43(36%)			

Table 2. Association between *XRCC1* 399Arg/Gln Phenotypes and Clinic-pathological Characteristics of Cases

Variables		Cases (n=120)	Arg/Arg (GG)	Arg/Gln (GA)	Gln/Gln (AA)	OR (95%CI)	p value
			34(28.3%)	80(66.7%)	6(5%)		
Age group	<50	52(43%)	14	36	2	Reference	0.8
	≥50	68(57%)	20	44	4	0.9(0.63-1.2)	
Gender	Male	63(53%)	16	37	4	Reference	0.13
	Female	57(47%)	18	43	2	0.9(0.6-1.2)	
Smoking status							
	Never	54(45%)	15	34	3	Reference	0.9
	Ever	66(55%)	19	46	3	1.0(0.75-1.3)	
Residence	Rural	64(53%)	19	47	4	Reference	0.7
	Urban	56(47%)	15	33	2	0.9(0.7-1.2)	
Tumorsite	Colon	72(60%)	21	41	5	Reference	0.4
	Rectum	48(40%)	13	39	1	1.4(1.04-1.9)	
Grade	WD	77(64%)	21	49	5	Reference	0.9
	MD/PD	43(36%)	13	31	1	1.0(0.7-1.4)	

Table 3. Genotype Frequency of *XRCC1* Gene Polymorphism in Cases and Controls

Characteristic		Controls	Cases	OR (95%CI)	p value
XRCC1 polymorphism		N=146	N=120		
Overall	G/G	50	34	Reference	0.5
	A/A+G/A	96	86	1.3(0.6-2.0)	
Age <50 years	G/G	22	14	Reference	0.4
	A/A+G/A	43	38	1.4(0.6-3.1)	
≥50 years	G/G	28	20	Reference	0.5
	A/A+G/A	53	48	1.3(0.7-2.5)	
Male	G/G	31	16	Reference	0.3
	A/A+G/A	57	41	1.4(0.7-2.8)	
Female	G/G	19	18	Reference	0.6
	A/A+G/A	39	45	1.2(0.6-2.5)	
Never smoker	G/G	18	15	Reference	0.7
	A/A+G/A	38	37	1.2(0.5-2.8)	
Ever smoker	G/G	32	19	Reference	0.3
	A/A+G/A	58	49	1.4(0.7-2.7)	
Rural	G/G	26	19	Reference	0.5
	A/A+G/A	56	51	1.2(0.6-2.3)	
Urban	G/G	24	15	Reference	0.4
	A/A+G/A	40	35	1.4(0.56-3.08)	
Allele typed	G	162(55%)	148(62%)		0.15
	A	130(45%)	92(38%)		

statistically insignificant (Table 2).

The lesser frequency of AA genotype and increased frequency of GG/GA genotype in cases clearly show that AA act as protective genotypes while as GG/GA increases the risk of CRC. The distribution of genotype frequency of GG, GA and AA among controls were 34.2%, 42.5% and 23.3%, while in cases it was 28.3%, 66.7% and 5% respectively with (odds ratio OR=1.3 and 95% Confidence Interval: CI=0.6-2.0) (Table 2). The present study shows the higher frequency of GA genotype in cases than in controls (Table 3). The total frequency of allele G and A observed in our study in cases is 0.62 and 0.38 respectively, whereas in controls it is 0.55 and 0.45 respectively. *XRCC1* AA (399Gln) genotype frequency is higher in controls than in cases suggesting its protective role against the development of CRC. We did not find any significant association of any of the clinico-epidemiological characteristics of CRC with *XRCC1* Arg399Gln gene polymorphism (Table 3).

Discussion

XRCC1 is an essential DNA repair gene involved in base excision repair (BER) (Caldecott et al., 2003).The *XRCC1* gene exhibits polymorphic variations, including three common single nucleotide polymorphisms (SNPs) that result in amino acid substitutions in exon 7 (Arg194Trp), exon 9 (Arg280His) and exon 10 (Arg399Gln). These non-conservative amino acid alterations may influence DNA repair capability by altering the protein-protein interactions between *XRCC1* and other BER proteins. The Arg399Gln variant was found to be associated with several phenotypic alterations, including higher levels of sister chromatid exchange (Abdel-Rahman et al., 2000), aflatoxin B1-DNA adducts, glycophorin A mutations (Lunn et al., 1999)and polyphenol DNA adducts (Eric et al., 2000).

In this study carried out for the first time on Kashmiri population we tried to find out any possible association of the three variant alleles of *XRCC1* Arg399Gln gene with the development of CRC. The distribution of genotype frequency observed in our study in controls was 34.2%, 42.5% and 23.3% of GG, GA, AA while as in cases 28.3%, 66.7% and 5% respectively. Our findings suggest that the Arg399Gln polymorphism is associated with the risk of development of CRC. Our findings suggest that the Arg399Gln polymorphism is associated with the risk of development of CRC. No risk could be attributed to subjects with the 399Gln homozygote in the Kashmiri population. Several polymorphic studies have observed a positive association of the *XRCC1* 399 AA allele with various malignant diseases, including cancer of the head and neck (Erich et al., 1999) breast (Duell et al., 2001) lung (Divine et al., 2001) and colon and rectum (Abdel-Rahman et al., 2000).These conclusions corresponded well with the finding that subjects with the 399Gln allele have higher numbers of chromosomal breaks per cell than those with other genotypes (Wang et al., 2003), and that these subjects are more susceptible to chemically induced genetic damage (Li et al., 2003). However Camilla et al. (2006) found 399AA allele to be associated with a reduced risk of high-risk colorectal adenomas.Claire et al. (2002) found that the presence of the *XRCC1* 399Gln allele is protective against the development acute myeloblastic leukemia (Seedhouse et al., 2002: Skjelbred et al., 2006). These studies are in accordance with our study. We found that the presence of the *XRCC1* 399Gln allele is protective against the development of CRC. Allele frequency of Arg and Gln observed in our study in cases is 0.62 and 0.38 respectively, whereas in controls it is 0.55 and 0.45 respectively. A number of studies have reported that the *XRCC1* 399Gln allele has no or an inverse relationship with the risk of neoplasm development (Olshan et al., 2002; Shen et al., 2003; Smith et al., 2003; Hong et al., 2005).

The possible mechanism of the discrepancy that Gln is protective allele and Arg/Gln heterozygous allele is risk allele in the development of CRC could be that the altered protein has modified repair efficiency and increased level of DNA damage that results in apoptosis which ultimately leads to reduced risk of exposure induced cancer (Seedhouse et al., 2002; Jeon et al., 2005)and the possibility of *XRCC1*-independent single-strand break repair (Caldecott et al., 2003).The exact mechanism by

which the *XRCC1* 399Gln allele can have apparently opposite effects on the risk of neoplasm development is not known and should be further studied in detail.

In conclusion our study reports protective role of Gln allele compared to Arg/Gln allele in the risk of development of CRC in Kashmiri population however extensive studies are needed to ascertain this finding.

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