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ORIGINAL RESEARCH

Association of *XRCC1*, *XRCC2* and *XRCC3* Gene Polymorphism with Esophageal Cancer Risk

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Aim: The X-ray repair cross-complementing (*XRCC)* gene polymorphisms influence esophageal carcinogenesis by altering the DNA repair capacity. The present study was designed to screen five single nucleotide polymorphisms (SNPs) of *XRCC* genes for their susceptibility to esophageal cancer (EC) risk. There is no previous report on these polymorphisms for EC from India, where EC frequency is high.

Methods: The present study included 497 subjects (213 EC patients and 284 healthy controls). The polymorphisms were screened using the PCR-RFLP method and allele and genotype distribution were compared using chi-square test. Association analysis was done by haplotype analysis and linkage disequilibrium (LD) analysis. Gene–gene interactions were identified using multifactor dimensionality reduction (MDR). The risk was calculated using binary logistic regression.

Results: For *XRCC1* p.Arg399Gln, a decreased risk for EC was associated with the AA genotype [OR (95% CI): 0.53 (0.3–0.95), p=0.03] even after adjusting for various covariates [OR (95% CI): 0.49 (0.26–0.9), p=0.024] and with the recessive model [OR (95% CI): 0.49 (0.27–0.8), p=0.016]. The GA genotype of p.Arg280His was associated with an increased risk for EC [OR (95% CI): 1.7 (1.0–2.82), p= 0.045] after adjustments. The two *XRCC1* polymorphisms, p.Arg399Gln and p.Arg194Trp were in slight LD among EC patients ($\acute{D}$=0.845, r^2=0.042). *XRCC2* and *XRCC3* polymorphisms were not associated with EC risk.

Conclusion: *XRCC1* p.Arg399Gln plays a protective role in the development of the EC. The study is the first report from India, providing baseline data about genetic polymorphisms in DNA repair genes *XRCC1, XRCC2* and *XRCC3* modulating overall EC risk.

Keywords: *XRCC1*, *XRCC2*, *XRCC3*, polymorphisms, esophageal cancer

Introduction

Esophageal cancer (EC) ranks as the sixth leading cause of death from cancer constituting 7% of all gastrointestinal cancers.[1] In India, EC is the fourth most common cause of deaths related to cancer and the second most common cancer among men and fourth among women.[2] Esophageal carcinogenesis is a complex multistep process with poor prognosis where environmental, geographic and genetic factors appear to play a major role.[3] Cancer is a disease characterized by the failure of DNA repair mechanisms. XRCC1 protein as a part of Base excision repair (BER) pathway plays an efficient role in repairing DNA single-strand breaks.[4] It is encoded by the *XRCC1* gene located on 19q13.2, comprising of 17 exons and 16 introns.[5] Two *XRCC1* polymorphisms, p.Arg194Trp (exon 6) and p. Arg280His (exon 9) affect the function of the protein.[6] *XRCC1* p.Arg399Gln polymorphism in exon 10[7] has been associated with breast,[8] lung[9] and head and neck cancers.[10]

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XRCC2 (chromosome location 7q36.1) plays a major role in Homologous recombination repair (HRR) pathway.[11] *XRCC2* p.Arg188His polymorphism located in exon3[12] has been associated with cancers like pancreatic,[13] ovarian,[14] oral[15] and upper aerodigestive tract cancers.[16]

XRCC3 (chromosome location 14q32.3) encodes a 346 amino acid polypeptide[17] that participates in DNA double-strand break repair. Variation in expression of *XRCC3* has been reported in various cancers, like gastric, breast, lung, skin and colorectal.[18–21] The most common polymorphism in *XRCC3* p.Thr241Met in exon 7 can influence the ability to repair DNA.[21]

Allelic variants of *XRCC1, XRCC2*, and *XRCC3* are associated with risk of different types of cancer among different populations all over the world.[22–24] In the world's second-most populous country India, the large and diverse ethnic subpopulations[25] need to be studied to understand the genetic basis of several region-specific complex disorders. To the best of our knowledge, studies related to these DNA-repair genes are very few for esophageal cancer in India (Table 1). The state of Punjab, India, has very high age-adjusted rate (AAR) for EC; it ranks second in males after prostate cancer and fourth after breast, ovarian and cervical cancer in females in this region.[26,27]

Therefore, the present study was carried out to explore the role of five polymorphisms of *XRCC* genes; *XRCC1* (p.Arg399Gln, p.Arg194Trp, p.Arg280His), *XRCC2* (p.Arg188His) and *XRCC3* (p.Thr241Met) in the risk of EC in the population of Punjab, India.

Materials and Methods

Study Population and Sample Collection

The present case-control study was conducted after due ethical clearance according to the tenets of the Declaration of Helsinki by the institutional ethics committee of Guru Nanak Dev University, Amritsar. The study sample included 497 subjects: 213 (125 females and 88 males) esophageal cancer patients without a history of any other cancer, any treatment before sample collection and pre-operative from Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar. Complete information including age, gender, lifestyle, diet, family history, and environmental factors were recorded on a proforma after written informed consent from all the subjects. Two hundred eighty-four (187 females and 97 males), age and gender-matched unrelated healthy controls from the same geographical region were also included in the study (Table 2).

Collection of Blood samples and genotyping: Intravenous blood sample was collected in EDTA from each subject. Genomic DNA from blood was collected using standard Phenol chloroform method.[28] Previously published primer sequences for *XRCC1* p.Arg399Gln,[29] *XRCC1* p.Arg194Trp,[24] *XRCC1* p.Arg280His,[22] *XRCC2* p.Arg188His[30] and *XRCC3* p.Thr241Met[23] polymorphisms were used to amplify the target region. The total volume of the PCR reaction mixture was 15µL containing the reagents: 50ng of template DNA, 10pmol of each primer (Sigma, St. Louis, MO, USA), 10X PCR buffer with 1.5 mM $MgCl_2$, 50 µM of dNTPs mix and 0.6 units of Taq polymerase (Bangalore GeNei).

The PCR products were digested with appropriate restriction endonucleases using manufacturer recommendations (NEB, UK) and analyzed on ethidium bromide stained agarose gel to obtain genotypes based on the size of fragment obtained (Table 3). To reassure of the quality of the result 10% of the samples were re-genotyped.

Statistical Analysis

Statistical analysis was done using SPSS software version 20 (SPSS, Chicago, IL, USA). The analysis of categorical variables was done using the chi-square test and the continuous independent variables were expressed as mean ± standard deviation using the Student's *t*-test. Hardy-Weinberg equilibrium was tested using the chi-square test. Binary logistic regression analysis was done to measure the risk of EC with genotypes of selected polymorphisms and the values were adjusted for the possible confounding effect of age, gender, diet, smoking and alcohol consumption. Odds ratios (ORs), p-values and 95% confidence interval (CI) were calculated as the measure of the strength of association of the SNPs and esophageal cancer. Haplotypes and linkage disequilibrium patterns were studied using Haploview software. Furthermore, multifactor dimensionality reduction (MDR) factor analysis version 2 was used to calculate the effect of Gene–gene interactions on esophageal cancer.

Results

The detailed characteristics of 497 subjects (213 EC patients and 284 controls) are given in Table 2 and the allele and genotype frequencies are summarised in Table 4 and Table 5. All the studied subjects were residents of Punjab, India. The endogamous caste groups were Jatt Sikh, Khatri and Scheduled Caste. The subjects were ethnic Punjabi with Scythian and Caucasian racial admixture.[31] In the present study 5 polymorphisms; *XRCC1* (p.Arg399Gln,

Table 1 Studies Showing Association of *XRCC1*, *XRCC2* and *XRCC3* (p.Thr241Met) Polymorphism in Different Cancers in Populations from Different Geographical Regions of India

Authors	Population of Region (State)	SNPs	Disease	Number of Subjects	Results
GIT Cancers Present Study	North India (Punjab)	*XRCC1* p.Arg399Gln, p.Arg194Trp and p.Arg280His *XRCC2* p.Arg188His *XRCC3* p.Thr241Met	Esophageal Cancer	497	AA genotype of XRCC1 p.Arg399Gln associated with reduced ESCC risk
[34]*	North India (Punjab/ Chandigarh)	*XRCC1* p.Arg399Gln	Esophageal Squamous Cell Carcinoma	280	AA genotype associated with reduced ESCC risk
[43]*	North India (Uttar pradesh)	*XRCC1* p.Arg399Gln p.Arg194His	Gallbladder Cancer	377	GA and AA genotype of p.Arg399Gln associated with decreased risk. No association of p.Arg194Trp polymorphism
[50]	North India (Kashmir)	*XRCC1* p.Arg399Gln	Colorectal Cancer	260	Association of GA genotype with increased cancer risk
[44]*	North India (Kashmir)	*XRCC1* p.Arg399Gln	Colorectal Cancer	266	Association of AA genotype with decreased risk
[52]	Eastern India (West Bengal)	*XRCC1*- p.Arg399Gln, p. Arg194Trp and p.Arg280His	Gastric Cancer	152	GA and GG genotype of p.Arg399Gln associated with increased cancer risk No association of p.Arg194Trp and p.Arg280His polymorphism
[72]*	South India	*XRCC1* p.Arg194Trp	Gastric Cancer	600	No association
Cancers of Other Sites	Eastern India	*XRCC1* p.Arg399Gln and p. Arg194Trp *XRCC3* p.Thr241Met	Oral submucous fibrosis (OSF)	188	No association of *XRCC1* p.Arg399Gln and p.Arg194trp polymorphism CT genotype of *XRCC3* p.Thr241Met associated with increased risk of OSF
[46]*	North India	*XRCC1* p.Arg194Trp, p. Arg280His and p.Arg399Gln	Hepatocellular Carcinoma	407	TT of p.Arg280His associated with increased risk. AA genotype of p.Arg399Gln associated with decreased risk
[90]	North eastern India	*XRCC1* p.Arg194Trp *XRCC3* p.Thr241Met	Breast Cancer	998	Arg/Trp and Trp/Trp genotype of *XRCC1* p.Arg194Trp associated with an increased risk No association of *XRCC3* p.Thr241Met
[101]	Western India (Maharashtra)	*XRCC1*- p.Arg399Gln, p. Arg194His and p.Arg280His *XRCC2*- p.Arg188His *XRCC3*- p.Thr241Met	Breast Cancer	350	No association of *XRCC1* (p.Arg399Gln, p.Arg194His and p.Arg280His), *XRCC2* (p.Arg188His) and *XRCC3* (p.Thr241Met)with breast cancer
[55]	South India	*XRCC1* p.Arg194Trp, p. Arg280His and p.Arg399Gln	Cervical Cancer	250	No association
[51]	North India	*XRCC1* p.Arg399Gln	Prostate Cancer	450	Association of AA genotype with increased risk

(*Continued*)

Table 1 (Continued).

Authors	Population of Region (State)	SNPs	Disease	Number of Subjects	Results
[57]	South India	*XRCC1* p.Arg194Trp, p. Arg280His and p.Arg399Gln	Lymphoblastic Leukemia	234	Association of Arg/Gln and Gln/Gln genotype of p. Arg399Gln with increased risk. No association for p.Arg280His and p.Arg194Trp polymorphism
[58]	South India	*XRCC1* p.Arg399Gln	Acute Myeloid Leukemia	422	Association of GA- genotype with increased risk of AML
[102]	Middle India (Madhya Pradesh)	*XRCC1* p.Arg194Trp, p. Arg280His and p.Arg399Gln	Sickle Cell Anemia	500	GA and AA genotype of p.Arg280His associated with an increased risk. No association of *XRCC1* p.Arg194Trp and p. Arg399Gln polymorphisms
[45]*	North India	*XRCC1* p.Arg194Trp, p. Arg280His and p.Arg399Gln	Head and Neck Cancer	556	AA genotype of p.Arg399Gln and TT genotype of p. Arg194Trp associated with reduced risk. No association of p.Arg280Hispolymorphism.

Note: *Studies with similar results as present study.

p.Arg280His, p.Arg194Trp), *XRCC2* (p.Arg188His) and *XRCC3* (p.Thr241Met) were studied for the association with risk of esophageal cancer (EC). For *XRCC1* p.Arg280His polymorphism, A allele was associated with an increased risk of EC (OR=1.60, 95% CI= 1.02–2.52, p=0.04). According to the Bivariate logistic regression for stratification, genotypes GA and GG+GA (OR=1.8,95% CI =1.1–2.9, p=0.025) provide 2 fold increased risk of EC after adjusting the confounding effect of age, gender, diet pattern, smoking and alcohol consumption. For *XRCC1*p.Arg399Gln polymorphism, AA genotype (OR= 0.53, 95% CI=0.3–0.95, p=0.03) was associated with 50% decreased the risk of EC. This association persisted and became stronger (OR=0.5, 95% CI=0.3–0.9, p=0.024) after adjustments to age, sex, diet pattern, smoking and alcohol consumption. Smoking and Alcohol intake was reported only by male subjects. Smoking was not reported by many subjects probably because of prevalent socio-religious beliefs in the region prohibit smoking and tobacco intake and alcohol intake. No significant difference in allele (p=0.54) and genotype (p=0.52) frequencies of *XRCC1* p.Arg194Trp polymorphism was observed. No association with EC risk was observed for *XRCC2* p.Arg188His and *XRCC3* p.Thr241Met polymorphism (p=0.53) in the subjects.

Genetic model analysis of *XRCC1* p.Arg399Gln and *XRCC3*p.Thr241Met (Table 6) revealed a decreased risk of EC under the recessive model AA vs GG+GA (OR=0.55, 95% CI=0.32–0.95, p=0.027) for *XRCC1*p.Arg399Gln polymorphism which became more significant after adjustment with binomial logistic regression (OR=0.49, 95% CI=0.27–0.88, p=0.016). For p.Thr241Met polymorphism no genotype combination was associated with EC.

Haplotype and Linkage Disequilibrium Analysis

The haplotype frequencies of three polymorphisms of *XRCC1* were examined for their association with EC risk (Table 7). Based on the measures of linkage disequilibrium (LD), the two polymorphisms of *XRCC1*gene, p.Arg399Gln and p.Arg194Trp, were in slight LD among EC patients ($\acute{D}$=0.845, r^2=0.042) (Figure 1A). However, control subjects were not in LD for any of the polymorphism (Figure 1B). The haplotype GGT (p.Arg399Gln, p.Arg280His, and p. Arg194Trp) was predominant in EC cases as compared to controls, but the difference was statistically non-significant (p=0.1). The possible genotype combinations of three studied polymorphisms of *XRCC1* were also examined (Table 8). The combinations comprising the AA genotype (p.Arg399Gln) occurred significantly more often in controls than patients, with AA-CC-CC combination associated with significantly decreased risk of EC (OR=0.5, 95% CI=0.29–0.91, p=0.020).

Table 2 Clinical Characteristics of the Esophageal Cancer Patients and the Controls

Characteristics	Cases (n=213)	Controls (n=284)	Odds Ratio (95% CI)	p-value
Age				
Below 55	94	144		0.15
55 or above	119	140		
Age(years)	55.7±12.4	54.4±11		0.22
Sex				
Females	125	187		0.07
Males	88	97		
Habitat				
Rural	178	241	1.14	0.70
Urban	35	43	(0.67–1.70)	
Diet				
Vegetarians	108	142	0.97	0.87
Non-vegetarians	105	142	(0.68–1.40)	
Smoking				
No	181	279	9.83	**<0.0001**
Yes	32	5	(3.7–25.72)	
Drinking				
No	153	233	1.79	**0.007**
Yes	60	51	(1.17–2.14)	
Family history				
No	192	284		
Yes	21	0		
Type of cancer				
SCC	187	–		
ADC	26	–		
Clinical Stage				
(I)	10	–		
(II)	67	–		
(III)	122	–		
IV	14	–		

Notes: p-value less than 0.05 taken as statistically significant (bold values).
Abbreviation: SCC, squamous cell carcinoma, ADC, adenocarcinoma.

Interaction Analysis (MDR)

Table 9 summarises the MDR analysis evaluating the results of all possible combinations of the studied polymorphisms. According to MDR analysis, the best MDR model included all the five studied polymorphisms *XRCC1* (p.Arg399Gln, p. Arg194Trp, p.Arg280His), *XRCC2* (p.Arg188His) and *XRCC3* (p.Thr241Met). The model had a testing balance accuracy of 0.4648 and a training balance accuracy of 0.5905 with a maximum cross-validation consistency of 10/10. However, the model was not statistically significant (p=0.9). Figure 2 depicts the interaction map of all genes, based on entropy measures between individual genes showing a redundancy effect.

Discussion

In India, the esophagus is a major cancer site (NCRP 2016) with a majority being Squamous Cell Carcinoma (SCC) and a comparatively less frequency of adenocarcinoma patients.[32,33] Similarly, the predominant type of EC in our study was SCC (88%) with only 12% adenocarcinoma patients.

In India, the inter-individual differences in susceptibility to cancer due to the genetic polymorphisms in *XRCC1* previously found.[34] As DNA-repair gene polymorphisms play a very important role in carcinogenesis, we carried out this case-control study to evaluate whether *XRCC1* (p.Arg399Gln, p.Arg194Trp and p.Arg280His), *XRCC2* (p.Arg188His), and *XRCC3* (p.Thr241Met) gene polymorphisms modulate the risk of esophageal cancer. The varied results reported world over for these polymorphisms has been attributed to variations in DNA-repair gene polymorphisms amongst different ethnicities.[35–39] The population of Punjab, North India are an Indo-Aryan ethnic group with Caucasian, Indoscythian racial admixture. The main caste groups prevalent in Punjab are Banias (merchant and money-lender), Brahmins (traditionally priestly but now involved in various occupations), Jatt Sikhs, which constitute the largest part of Sikh community (mainly agriculturist), and Scheduled caste (various occupations).[40] In the present study, about 60% of the subjects belonged to Jatt Sikh Caste group but the subgroup analysis based on castes was not done due to unequal distribution of the sa mples.

The *XRCC1*p.Arg399Gln polymorphism is involved in various protein–protein interactions[41] and higher sister chromatid exchanges and DNA adducts.[42] In the present study, we found that A allele and the AA genotype of *XRCC1*p.Arg399Gln polymorphism was associated with a decreased risk of esophageal cancer. Furthermore, a reduced risk was observed in the recessive model (GG +GAvsAA). When compared with previously reported studies from India, the results of our study were consistent with some studies, especially from North India but contrary to others (Table 1). Very few studies relating the XRCC1 Arg399Gln polymorphism with esophageal cancer risk are available from which only one is from India. Among previous reports from North India on EC; a study of Chandigarh region[34] found association with a decreased risk of *XRCC1* p.Arg399Gln, another study from Uttar Pradesh[43] also reported Arg/Gln (p=0.03, OR= 0.62) and

Table 3 Details of Amplification and Digestion Conditions Used for Genotyping of *XRCC1*, *XRCC2* and *XRCC3* Polymorphisms

Polymorphism (Ref SNP)	Location	Nucleotide Change	Annealing Temperature	Amplicon Size (bp)	Restriction Enzyme	Size of Digested Fragments
XRCC1 p.Arg194Trp (rs1799782)	Exon 6	C>T	59°C	491	*MspI*	CC(292,174) CT(313,292,174) TT(313,174)
p.Arg280His (rs25489)	Exon 9	G>A	66°C	861	*RsaI*	GG(597,201,63) GA(660,597,201,63) AA(660,201)
p.Arg399Gln (rs25487)	Exon 10	G>A	59°C	615	*MspI*	GG(374,241) GA(615,374,241) AA(615)
XRCC2 p.Arg188His (rs3218536)	Exon 3	G>A	59°C	290	*HphI*	GG(290) GA(290,148,142) AA(148,142)
XRCC3 p.Thr241Met (rs1625895)	Exon 7	C>T	62°C	552	*NlaIII*	CC(313,239) CT(313,239,208,10) TT (239,208,105)

Abbreviation: SNP, single nucleotide polymorphism.

Table 4 Allele and Genotype Frequencies of *XRCC1*, *XRCC2* and *XRCC3* Gene Polymorphisms and Their Association with Esophageal Cancer Risk

Gene	SNP	Allele	Allele Frequency		Odds Ratio (95% CI)	p value	Genotype	Cases n (%)	Controls n(%)	Chi Square (χ^2)	p value*
			Cases (n%)	Controls (n%)							
XRCC1	rs25489 p.Arg280His	G A	382(89.7) 44(10.3)	530(93.3) 38(6.7)	Reference 1.60 (1.02–2.51)	**0.04**	GG GA AA	171(80.3) 40(18.8) 2(0.9)	246(86.6) 38(13.4) 0(0)	4.9	0.08
	rs25487 p.Arg399Gln	G A	283(66.5) 143(33.5)	344(60.6) 224(39.4)	Reference 0.78 (0.6–1.0)	0.06	GG GA AA	92(43.2) 99(46.5) 22(10.3)	109(38.4) 126(44.4) 49(17.2)	5.51	0.06
	rs1799782 p.Arg194Trp	C T	394(92.5) 32(7.5)	531(93.5) 37(6.5)	Reference 1.16 (0.71–1.92)	0.54	CC CT TT	181(85) 32(15) 0(0)	247(87) 37(13) 0	0.41	0.81
XRCC2	rs3218536 p.Arg188His	G A	388(91.1) 38(8.9)	515(90.7) 52((9.3)	Reference 0.97 (0.62–1.51)	0.89	GG GA AA	175(82) 38(18) 0(0)	232(81.7) 51(18) 1(0.3)	0.75	0.68
XRCC3	rs1625895 p.Thr241Met	C T	334(78.4) 92(21.6)	449(79) 119(21)	Reference 1.03 (0.76–1.43)	0.80	CC CT TT	129(60.6) 76(35.7) 8(3.7)	178(62.7) 93(32.7) 13(4.6)	0.59	0.74

Notes: Wild type genotype was taken as reference. *Distribution of overall genotypic frequencies between cases and controls done by χ^2 test (df, 2). P-value less than 0.05 taken as statistically significant (bold values).
Abbreviation: SNP, single nucleotide polymorphism.

Gln/Gln (p=0.003, OR=0.37) genotype to be associated with a decreased risk of gall bladder cancer; a study from Kashmir on colorectal cancer[44] also reported a protective role of AA genotype. Similar results were reported in three other studies from North India on head and neck cancer,[45] hepatocellular carcinoma[46] and lung cancer.[47]

Table 5 Distributions of *XRCC1, XRCC2* and *XRCC3* Polymorphisms Genotypes and Development of Esophageal Cancer

Gene	SNP	Genotype	Cases n(%)	Controls n(%)	Unadjusted OR(CI)(p)	AOR* (CI) (p value)	AOR** (CI) (p value)
XRCC1	rs25489 p.Arg280His	GG GA AA GA+AA	171(80.3) 40(18.8) 2(0.9)	246(86.6) 38(13.4) 0(0)	Reference 1.5(0.9–2.4) p=0.09 NC 1.6(0.9–2.5) p=0.06	Reference 1.6(1.0–2.6) p=0.06 NC 1.6(1.0–2.6) **p=0.04**	Reference 1.7 (1.0–2.8) p=**0.04** NC 1.8(1.1–2.9) p=**0.02**
	rs25487 p.Arg399Gln	GG GA AA GA+AA	92(43.2) 99(46.5) 22(10.3)	109(38.4) 126(44.4) 49(17.2)	Reference 0.9(0.6–1.3) p=0.7 0.5(0.3–0.9) p=**0.03** 0.8(0.6–1.2) p=0.28	Reference 0.9(0.6–1.4) p=0.7 0.5(0.3–0.9) p=**0.03** 0.8(0.6–1.2) p=0.27	Reference 1.0(0.7–1.5) p=0.9 0.5(0.3–0.9)p=**0.024** 0.9(0.6–1.3) p=0.4
	rs1799782 p.Arg194Trp	CC CT TT	181(85) 32(15) 0(0)	247(87) 37(13) 0	Reference 1.2(0.7–1.9) p=0.52 NC	Reference 1.2(0.7–2.1) p=0.4 NC	Reference 1.1(0.7–2.0) p= 0.5 NC
XRCC2	rs3218536 p.Arg188His	GG GA AA GA+AA	175(82) 38(18) 0(0)	232(81.7) 51(18) 1(0.3)	Reference 0.9(0.6–1.6) p=0.95 NC 0.9(0.6–1.5) p=0.89	Reference 1.0(0.6–1.6) p=0.9 NC 0.99(0.6–1.6) p=0.9	Reference 0.9(0.6–1.5) p=0.8 NC 0.9(0.5–1.5) p=0.7
XRCC3	rs1625895 p.Thr241Met	CC CT TT CT+TT	129(60.6) 76(35.7) 8(3.7)	178(62.7) 93(32.7) 13(4.6)	Reference 1.1(0.7–1.6) p=0.53 0.8(0.3–2.1) p=0.72 1.09(0.7–1.6) p=0.63	Reference 1.1(0.7–1.6) p=0.6 0.8(0.3–2.2) p=0.8 1.06(0.7–1.5) p=0.75	Reference 1.1(0.7–1.6) p=0.6 0.8(0.3–2.3) p=0.8 1.06(0.7–1.6) p=0.77

Notes: Wild type genotype is taken as reference, p<0.05 taken as statistically significant (bold values). AOR* Adjusted Odds Ratio for age, gender and diet pattern using Binary logistic regression. AOR** Adjusted Odds Ratio for age, gender, diet pattern, cigarette smoking and alcohol consumption using Binary logistic regression analysis.

Table 6 Association Analysis of *XRCC1* p.Arg399Gln and *XRCC3*p.Thr241Met Polymorphisms

SNPs	Comparison	Unadjusted OR (CI) (p value)	AOR* (CI) (p value)	AOR** (CI) (p value)
XRCC1 p.Arg399Gln	Dominant model (AA+GAvsGG) Codominant model(AAvsGA/GGvsGA) Recessive model (GG+GAvsAA)	0.82(0.57–1.22) p=0.280 0.78 (0.60 −1.01) p=0.062 0.55(0.32–0.95) p=**0.027**	0.82(0.56–1.18) p=0.27 1.16(0.71–1.64) p=0.56 0.53(0.30–0.95) p=**0.02**	0.92(0.59–1.31) p=0.46 1.23(0.82–1.82) p=0.29 0.49(0.27–0.83) p=**0.02**
XRCC3 p.Thr241Met	Dominant model (TT+CTvsCC) Codominant model(TTvsCT/TTvsCC) Recessive model (CC+CTvsTT)	1.09(0.76–1.58) p=0.63 1.04 (0.76 −1.42) p=0.80 0.81(0.33–1.92) p=0.65	1.06(0.73–1.5) p=0.75 1.14(0.75–1.62) p=0.62 0.84(0.34–2.08) p=0.69	1.06(0.71–1.63) p=0.77 1.13(0.74–1.65) p=0.61 0.82(0.33–2.05) p=0.64

Notes: Wild type genotype is taken as reference, p<0.05 taken as statistically significant (bold values). AOR* Adjusted Odds Ratio for age, gender and diet pattern using binary logistic regression. AOR** Adjusted Odds Ratio for age, gender, diet pattern, cigarette smoking and alcohol consumption using binary logistic regression.

Contrary to the results of the present study, some previous studies from North India have reported an increased risk with AA genotype of *XRCC1*p.Arg399Gln polymorphism in lung cancer,[48] head and neck cancer,[49] colorectal cancer[50] and prostate cancer.[51] Similarly, some studies from South India reported an association of increased risk with the AA genotype in gastric cancer,[52] colorectal cancer,[29] lung cancer,[53,54] cervical cancer,[55] Naso-pharyngeal cancer,[56] acute lymphoblastic leukemia[57] and acute myeloid leukemia.[58] However, few studies from South India reported no association of *XRCC1*p.Arg399Gln polymorphism with any of cancer (Table 1).

Results of the present study on *XRCC1* p.Arg399Gln polymorphism is in agreement with the studies from different parts of the globe like esophageal cancer in Han Chinese,[59] colorectal adenocarcinoma in Norwegian population,[60] gall-bladder cancer,[61] and non-melanoma skin cancers.[62] In contrast, some previous studies have reported the association of Arg399Gln polymorphism with an increased risk of esophageal,[63] stomach and oral cancers,[64] colorectal cancers in Korean, Egyptian and Japanese populations,[65–67] lung cancer[68] and breast cancer.[22] However, three studies did not find any association between p.Arg399Gln polymorphism

Table 7 Haplotype Frequencies of *XRCC1* Gene Polymorphisms and Their Association with the Risk of Esophageal Cancer

Haplotype *XRCC1* p.Arg399Gln-p.Arg280His-p.Arg194Trp	Studied Population % (n)(497)	Cases N	Controls	χ^2 (p value)*	Odds Ratio (OR)(95% CI) p value**
G-G-C	0.501(249)	0.508(108)	0.496(141)	0.1 (p=0.7)	Reference
A-G-C	0.347(174)	0.314(67)	0.372(107)	3.6 (p=**0.05**)	0.82(0.6–1.2) p=0.3
G-G-T	0.075(37)	0.096(20)	0.058(17)	5.0(p=**0.02**)	1.5(0.7–3.1) p= 0.1
G-A-C	0.055(27)	0.060(13)	0.052(14)	0.3(p=0.5)	1.2(0.5–2.7) p= 0.6
A-A-C	0.014(7)	0.015(3)	0.014(4)	0.02(p=0.8)	0.9(0.2–4.4) p=0.9
A-G-T	0.008(4)	0.007(2)	0.008(2)	0.07(p=0.7)	1.3(0.2–9.4) p=0.8
LD Measures	Esophageal Cancer Patients				
	$\grave{D}$	r^2		$\grave{D}$	
p.Arg399Gln-p.Arg194Trp	0.845	0.042		0.596	
p.Arg280His-p.Arg194Trp	1.0	0.009		0.969	
p.Arg399Gln-p.Arg280His	0.188	0.001		0.633	

Notes: P* Comparison for esophageal cancer patients and controls for each haplotype. P** Comparison for esophageal cancer patients and controls in comparison to the wild type genotype taken as reference, P<0.05 taken as statistically significant (bold values).

and cancer of the esophagus,[69] gall bladder[70] and breast.[71] We did not observe any significant association of *XRCC1*p. Arg194Trp polymorphism with EC risk in the present study. The results were similar to two previous Indian studies.[52,72] Among international studies, no association of *XRCC1* p. Arg194Trp has been reported with EC risk in the population of North Carolina,[73] gastric cancer in Korean population[74] and breast cancer in Caucasian women.[75] On the contrary, Trp allele has been reported to be associated with an increased risk of gastric cancer in the Chinese population.[76] XRCC1 is a protein involved in Base excision repair and efficient repair of DNA single-strand breaks. In the present study, we observed a lower risk of esophageal cancer associated with p.Arg399Gln polymorphism of *XRCC1*. A relationship between polymorphism in *XRCC1*Arg399Gln and increased rate of apoptosis has been reported in patients of ulcerative colitis[77] and in schizophrenia patients.[78] The alterations to the DNA repair system

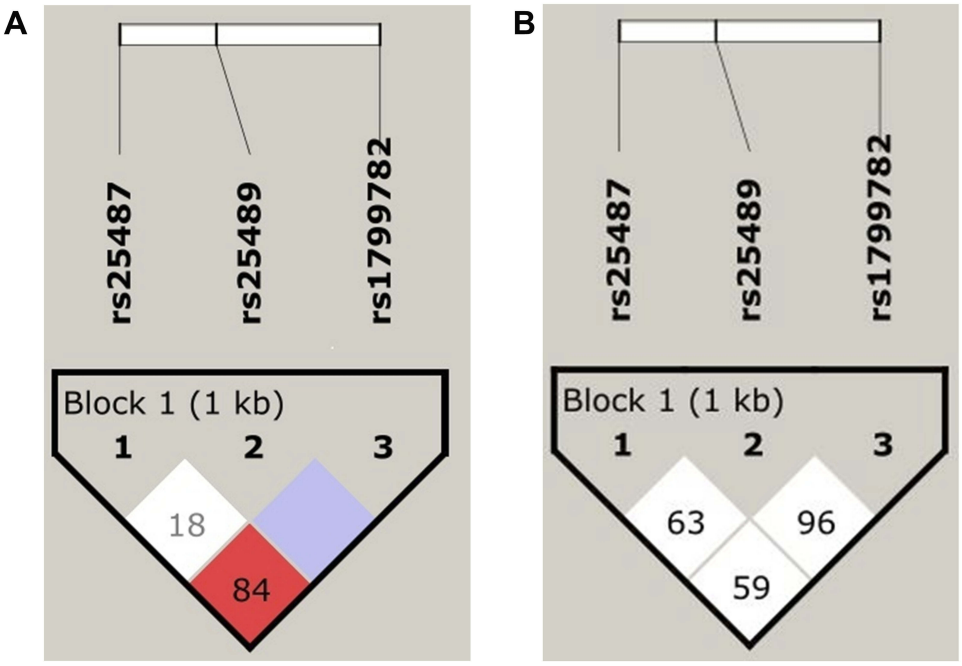

Figure 1 LD plot showing the position of the three *XRCC1* polymorphisms and pair-wise D' values observed in the study population with respect to (**A**) Esophageal Cancer (**B**) Controls.

Table 8 Association of *XRCC1* Genotype Combinations with Esophageal Cancer

Genotype Combination*	Patients	Controls	Odds Ratio (95% CI)	p value
AA-CC-CC	19	45	0.5(0.3–0.9)	**0.02**
AA-nn-CC	21	47	0.5(0.3–0.9)	**0.03**
AA-CC-nn	20	47	0.5(0.3–0.9)	**0.02**
nn-CT-CC	28	34	1.1(0.6–1.9)	0.69
An-Tn-CC	18	20	1.2(0.6–2.4)	0.56
An-nn-Cn	121	175	0.8(0.6–1.2)	0.28
nn-Tn-Cn	32	37	1.2(0.7–1.9)	0.52
Gn-Tn-Cn	30	35	1.2(0.7–1.9)	0.56

Notes: *Genotypes of *XRCC1* p.Arg399Gln, p.Arg194Trp and p.Arg280His. n: any allele. p value <0.05 is taken as statistically significant (bold values)

could not only suppress it to accomplish the repair process, but it would also become more vulnerable to carry apoptosis and help in wiping out the damaged cells. The increased rates of the apoptosis results into the elimination of potential premalignant cells and hence, the *XRCC1* Gln399 may play a protective role in esophageal cancer risk.

The A allele of *XRCC1*p.Arg280His was associated with an increased risk of esophageal cancer in the present study. Reported studies in different populations and different cancers have contradictory results for this polymorphism. From the North Indian population, p.Arg280His has been associated with an increased risk of hepatocellular carcinoma[46] but no association has been reported with SCC head and neck.[45] From a South Indian population, no significant association with breast cancer risk was reported.[22] A meta-analysis within the Asian population has reported an association of p.Arg280His polymorphism with bladder cancer risk.[79] Another meta-analysis reported the heterozygote and the dominant model to be associated with prostate cancer risk.[80] A allele was associated with an increased risk of adenomas in the Norwegian population.[60] A study on the Chinese population reported no association between p.Arg280His polymorphism and ESCC.[81]

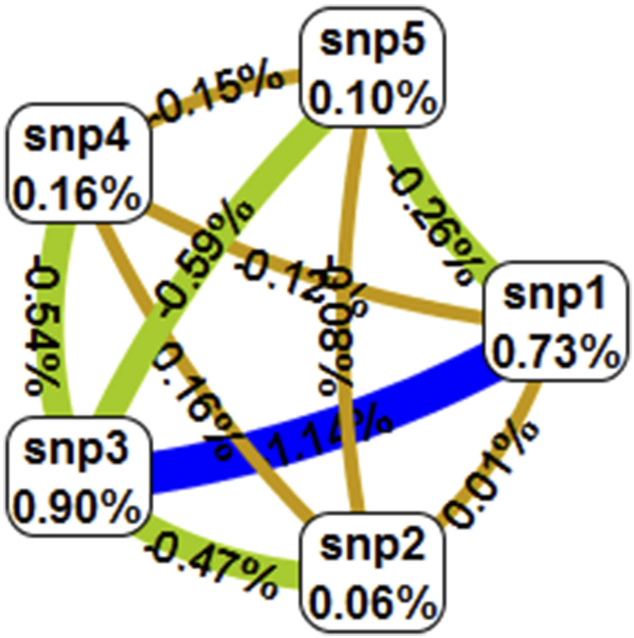

Figure 2 Gene–Gene interaction map for esophageal cancer risk: Values inside nodes indicate information gain (IG) of individual attributes or main effects, whereas values between nodes show IG of pairwise combinations of attributes or interaction effects. Positive entropy (red) indicates strong interaction (not found), (yellow) weak interaction while negative entropy (green) indicates redundancy and blue shows no interactions.
Notes: SNP1: XRCC1 p.Arg399Gln, SNP2: XRCC1 p.Arg194Trp, SNP3: XRCC1 p.Arg280His, SNP4: XRCC2 Arg188His, SNP5: XRCC2 p.Thr241Met.

XRCC2, an essential protein in the HRR pathway is required for the formation of RAD51 focus, with involvement in tumor progression.[13] No association of *XRCC2* p.Arg188His with EC has been observed in the present study, which is the first report from India for esophageal cancer risk. In previous reports from India, no association of *XRCC2* p.Arg188His polymorphism was reported with nasopharyngeal carcinoma[47] whereas another study found an association of GA genotype with increased risk of SCC head and neck.[49] The contradictory reports from different parts of the world have shown that *XRCC2* p.Arg188His polymorphism was associated with a significantly increased risk of pharyngeal cancer[16] and breast cancer[12] but not with bladder cancer,[82]

Table 9 Multifactor Dimensionality Reduction (MDR) Analysis

Locus Number	Model	Training Balance Accuracy	Testing Balance Accuracy	Cross Validation Consistency	p value*
1	SNP1	0.5367	0.4965	7/10	0.37
2	SNP1-SNP3	0.5494	0.4538	4/10	0.99
3	SNP1-SNP3-SNP5	0.5621	0.4547	5/10	0.98
4	SNP1-SNP2-SNP3-SNP5	0.5764	0.4289:	6/10	0.98
5	SNP1-SNP2-SNP3-SNP4-SNP5	0.5905	0.4648	10/10	0.98

Notes: SNP1: XRCC1 p.Arg399Gln, SNP2: XRCC1 p.Arg194Trp, SNP3: XRCC1 p.Arg280His, SNP4: XRCC2 Arg188His, SNP5: XRCC2 p.Thr241Met. *p values were based on 1000 permutations.

colorectal adenoma,[83] skin cancer,[84] thyroid cancer[85,86] and breast cancer.[87] Contrary to this, a protective role of GA and AA genotype in Caucasian breast cancer females of Cyprus has been reported.[88]

XRCC3 gene mainly repairs using the HR pathway and in vitro studies revealed high sensitivity to DNA damaging agents in cells with *XRCC3* gene knockouts.[89] No association of *XRCC3* p.Thr241Met polymorphism with esophageal cancer has been observed in the present study. A previous study from the north-eastern region of India has reported no association of this polymorphism with breast cancer.[90] From Indian subcontinent, two populations practicing consanguineous marriages reported an association with increased risk, for colorectal cancer in Kashmiri population[50] and breast cancer in Pakistani population.[91] For *XRCC3* p.Thr241Met polymorphism, no association was reported with gastric cancer in Italian population[92] and with colorectal cancer in the West Algerian population[93] but an association with an increased risk was reported for lung cancer in an Italian population,[23] oral SCC in Thai population,[94] and gastric cancer in the Chinese population.[95] On the other hand, a protective role of CT and TT genotype has been reported in colorectal cancer in Polish population[96] and Basal cell carcinoma of the skin in population from Hungary, Romania and Slovakia.[97]

The haplotype analysis in the present study shows an association of haplotype GGT of *XRCC1* gene (Arg399-Arg280-194Trp of p.Arg399Gln, p.Arg280His, and p. Arg194Trp polymorphisms) with a risk of EC, however, the association was not statistically significant (Table 7). Among previous reports from India, a study from Andhra Pradesh (South India) also found no association of any haplotype with Chronic Myeloid Leukemia.[98] However, a study from West Bengal (East India) reported CAG haplotype (Arg194-His280-Arg399) to have reduced risk against gastric cancer.[52] A study from Uttar Pradesh (North India) reported CGA and CAG haplotype of *XRCC1* (Arg194-His280-Arg399) to be associated with prostate cancer and haplotype CGA to be associated with bladder cancer.[38]

Post radiotherapy in a cancer patient, radiation injury may develop months to years later after treatment, with numerous individual manifestations. The *XRCC1* codon 399 Arg/Arg genotype has been associated with increased risk of acute radiation dermatitis in nasopharyngeal carcinoma patients treated with intensity-modulated radiation therapy.[99] Hence a patient with the variant AA genotype of *XRCC1*p.Arg399Gln polymorphism might be at lower risk for radiation injury. The present study indicates a protective role of the *XRCC1* p.Arg399Gln towards the development of EC. Therefore, XRCC gene polymorphisms can also be predicting factor in therapeutic decisions for individualized therapy in EC. Some of the results are at variance from reporting studies in Indian Sub-continent and other Asian countries. These might be due to the ethnic variations in the population residing in Punjab, Northwest India, other states of India and the rest of Asia. Inhabitants of Punjab and Northwestern states of India have an admixture of Indo-Scythian and Caucasian racial elements with more similarity with the central Asian populations as compared to populations in Middle India, South India or eastern parts of India.[25,100]

Conclusion

The present study being the first report from India, providing baseline data about five genetic polymorphisms in three DNA repair genes *XRCC1, XRCC2* and *XRCC3* modulating overall esophageal cancer susceptibility in ethnic Punjabi Indian subjects. The future utility of this data in screening the population for EC risk is the major strength of the study. The results have also been adjusted for various confounding factors. The limitation is a lack of stratification of subjects on the basis of endogamous caste groups of Punjab due to small cross-sectional sample in individual caste group. A possible interaction between XRCC polymorphisms and environmental factors on cancer risk was investigated in several studies. It is of great interest to evaluate gene–environment interactions to examine the exact roles of genetic polymorphisms in cancer. However, lacking the original data limited our further evaluation of potential gene–environment interactions. The results suggest a role of *XRCC* gene polymorphisms in esophageal cancer risk and a need to confirm our findings with higher sample size in different ethnic groups inhabiting different geographical areas of India.

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Disclosure

The authors declare that they have no competing interests.

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