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XRCC1 Polymorphism in Breast Cancer: A Case-control Study on Kashmiri Population

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Abstract—*X-ray cross complementing gene-1(XRCC1) is a member of Base Excision repair pathway involved in fixing DNA damage due to various exogenous as well as endogenous insults. At least three SNPs have been described for this gene that has been implicated as a risk factor for many cancers. In this case control study we examined XRCC1 Arg194Trp by PCR-RFLP method in Kashmiri population and studied its association with the breastcancer and its clinicopathological parameters. There was no overall association of breast cancer with the polymorphism. However a positive association was observed between XRCC1 194 polymorphism with BMI and loss of estrogen and Her-2-Neu receptors. Our results suggest that XRCC1 194 polymorphism may not be a risk factor for breast cancer in ethnic Kashmir population. However higher parity and menopause are the associated risk factors for breast cancer.*

Key words*: DNA repair, XRCC1, polymorphism, Breast Cancer, Kashmir*

1. INTRODUCTION

Breast Cancer, like all other cancers is considered to be a genetic disease characterized by genetic lesions, amplifications, deletions, point mutations, loss of heterozygosity, chromosomal rearrangements and over all aneuploidy[1].Breast cancer continues to be the global health burden, with number of cases on ever increasing rise. A more recent study reported that 2013 saw 0.7 million cases of breast cancer with 0.5 million deaths, globally, and this number is expected to double to 3.2 million cases by year 2030 in low and middle income countries, mainly India and china [2]. Today, Breast cancer ranks as the leading cancer in females worldwide, including India, where 41.3% of all cancers in females account for breast and cervical cancers [3]. Similar trend is observed in Kashmir also, with 1797 breast cancer cases reported between 2000-2013, accounting for the 16.83% of all the cancers among women [4]. Regardless of this fact, exact etiology of breast cancer continues to remain hard to pin down and it is believed to be a multi factorial disease arising as a result of the interaction of genetic and environmental factors[5]. Over the past decade or two, significant progress has been made in determining the risk factors for the breast cancer and identification of specific genetic alterations has improved the treatment for many cancer patients through targeted therapies,exemplified by the following two examples: treatment of *ERBB2* over-expressing metastatic breast cancers with Trastuzumab (Herceptin)[6] and use of Vemurafenib in melanoma patients with specific *BRAF* mutation (V600E) [7].

During evolution, mammalian cells have optimized distinct pathways to repair DNAthereby preserving genome integrity by fixing damage to DNA caused by external insults as well as by exposure to mutagenic substances and reduction in mammalian DNA repair capacity is associated with increasing birth defects, cancer and reduced life span.Polymorphisms in several DNA repair genes have been described which influence DNA repair capacity and modulate cancer susceptibility risk by means of gene-environment interactions[8]. One such gene is XRCC1, X-ray cross complementing group-I which corrects defective DNA strand-break repair and sister chromatid exchange following treatment with ionizing radiation and alkylating agents [9]. The human XRCC1 has 17 exons and assigned to chromosome 19q13.2, encodes for a 633aa protein [10]. Genetic polymorphisms in XRCC1 gene could through alteration of protein structure, lead to defective functioning of DNA polymerase β, PARP and ligase III enzymes resulting in defective DNA repair and increased risk of various cancers [11]. In XRCC1 three single nucleotide polymorphisms (Arg194 Trp;Arg280His and Arg399Gln) ,all in the multi – protein interacting regions of the gene have been reported with the result in amino acid changes from Arginine (Arg) to Tryptophan (Trp) at codon 194 (Arg/Trp), from Arginine (Arg) to Histidine (His) at codon 280 (Arg/His) and from Arginine (Arg) to Glutamine (Gln) 399 (Arg/Gln), respectively, thereby weakening the DNA repair capacity[11]

Numerous epidemiologic studies have been conducted to evaluate the associationbetween these XRCC1 polymorphism and breast cancer. These finding have been inconsistent with

the rare allele of each polymorphism (XRCC1 194 Trp allele; 280 His allele and 399 Gln allele) been associated with either an increase or decrease in breast cancer risk depending upon the population or subgroupdue to the differences of the allele frequencies, types of polymorphisms as well as the exposed carcinogens in a studied population .Same is the case with Kashmir valley, whose population is ethnically different from the rest of India with respect to its geography, climate, social and dietary habits. XRCC1 polymorphisms are associated with variable cancer risk, which suggests a possible role in cancer development. Furthermore, in a results of the one of the study suggest that Arg399Gln polymorphism of the XRCC1gene alone may not play a substantial role in the risk of breast cancer among Kashmiri women. To our knowledge, nodata has been reported till date, regarding association of XRCC1 polymorphism with breast cancer susceptibility risk for this population. This study therefore is first investigative attempt in describing the association between XRCC1 polymorphism and breast cancer risk in Kashmir valley.

2. SUBJECTSAND METHODS:

2.1. Sample collection.

About 200 clinically diagnosed breast cancer patients for the study were recruited from Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Kashmir, after procuring the necessary ethical clearance. Complete clinical data of the patients was collected. Staging of the breast cancer into four stages I, II, III, IV was done taking Tumor–node–metastasis (TNM) system of the American Joint Committee on Cancer/ the Union Internationale Contre le Cancer (AJCC/UICC) as reference [12] whereas the Scarff-Bloom-Richardson classification [13] was used to grade the tumors histopathologically as GI, GII and GIII. Also, for the case-control study, about 200 healthy individuals were recruited. The subjects selected as controls were disease free and had no family history of any cancer. About 3ml of the peripheral blood was collected in the EDTA vials, from both patients and healthy controls, and stored at -20^{o}C till further processing. The cases and the controls were gender and age matched, with all the individuals being female.

2.2. DNA Extraction from Whole Blood and PCR.

DNA was isolated from Whole Blood using Phenol-Chloroform method. The integrity and quality of the genomic DNA was checked by gel electrophoresis using 0.8 % agarose gel. Once it was confirmed that the genomic DNA is present and concentration and purity is also desirable, the desired fragment of DNA i.e. Exon 6, codon 194 of XRCC1 gene (**rs ID: 1799782**) was amplified individually by Polymerase Chain Reaction (PCR), using the primer pair Forward 5'GCC CCG TCC CAG GTA3' and Reverse 5' AGC CCC AAG ACC CTT TCA CT3'according to the cycling parameters given in **Table 2.1**. PCR was performed in total volume of 25µl reaction mixture that had 50-150 ng genomic DNA, 0.2mM dNTPs, and 0.4 pmoles/µl of each primer and 1U/µl of Taq polymerase in 1 X PCR buffer.

Table 2.1: PCR cycling parameters

Steps	Temperature (oC)	Time	Number of cycles
1.Initial Denaturation	95	5 min	1
2. Denaturation	95	30 sec	30
3. Annealing	55.5	40 sec	
4. Extension	72	30 sec	
5. Final extension	72	5 min	1

2.3.Single nucleotide polymorphism analysis by PCR-RFLP method.

Arg194Trp polymorphism of the *XRCC1* gene, in the exon 6, codon 194 was checked by the restriction digestion of the 491bp amplicon using 1 unit of restriction enzyme, PvuII (Thermo Scientific, USA).The enzyme detects the restriction site created by the C to T substitution.. The mixture was incubated at 37°C for 24 h. The products were then resolved on 3% agarose gels. DNA molecular weight marker was used to assess the size of the PCR–RFLP products.

2.4. Statistical analysis.

Results were statistically analyzed by the specific statistical test and data was expressed as mean ±SD. Allele and genotype frequencies were compared between groups using the Pearson χ2-test and Fisher exact test. XRCC1 genotype and breast cancer was estimated by calculating odds ratio (OR) and their 95% confidence intervals (95% CI).All p-values are two-sided and considered statistically significant at the 0.05 level. Statistical analysis was carried out using Graph pad prism version 5.0 and SPSS (version 20).

3. RESULTS.

3.1. Clinicopathological findings.

The summary of selected social, demographic, reproductive, clinical and pathological risk factors for the breast cancer, of patient population as well as control subjects, assessed for this case-control study are summarized in **Table 2.2**. Median age (in years) for cases and controls was 53 and 51, respectively. Mean age (in years) of the patients was calculated to be 50.137+ 11.366 and that of controls was 52.121+13.122 while as mean BMI was calculated to be 20.54+1.21 and 20.47+2.13 kg/m2 for cases and controls, respectively. When these parameters were compared for the cases and the control subjects, significant association was observed for menopausal status and parity with the breast cancer risk. Women with number of children more than two was significantly more in controls than in cases. Similarly the number of individuals who have attained menopause was significantly lower in cases than in controls.

Table 2.2: Frequency distribution analysis of the selected Socio-Demographic and Reproductive risk factors in breast cancer cases and control subjects:

Variable	Cases(200)	Controls(200)	X2	OR(95% CI)	p-Value
Age(years)					
<50	66	69			
>50	134	131	0.1	0.93(0.6-1.41)	0.32
Menopausal status					
Pre	113	136			
Post	87	64	5.63	0.6(0.40-0.91)	0.02
Parity					
<2	104	74			
>2	96	126	9.11	1.8(1.23-2.74)	0.003
BMI					
<24.9	69	79	-	1 (reference)	-
25-29.9	73	111	0.01	0.97 (0.61-1.53)	1.0
>30	58	60	0.32	0.85(0.56-1.46)	0.58
Dwelling					
Rural	79	71			
Urban	121	129	0.68	1.18(0.79-1.77)	0.46

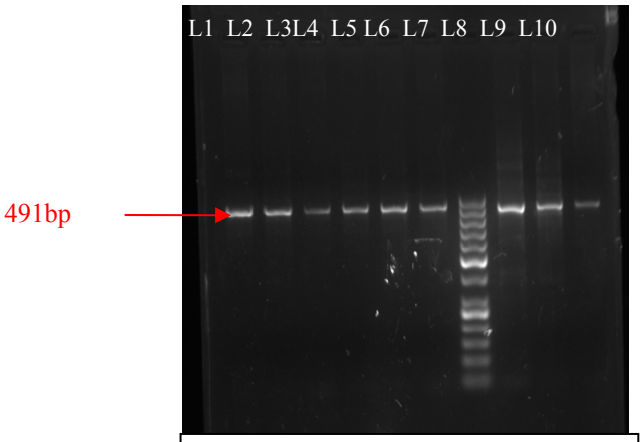

Figure 1: PCR product run on 2.0%agarose gel.Lane 7 :25bp DNA

3.2: XRCC1genotyping:

Genomic DNA from total of 400 blood samples (200 cases and 200 controls) was subjected to PCR amplification as per protocol given in the methods section. PCR amplified desired target encompassing codon 194 SNP in XRCC1 gene after designing a specific primer pair against it and generated 491bp amplicon. After efficient amplifications, 5-6µl of PCR product was analyzed on 2% agarose gel. The amplified product was then visualized under UV exposure, using Ethidium bromide as visualizing agent. The result of PCR is shown in **Figure 1**. A single band of 491 bp was visible which represented exon 6 of the gene. The SNP of the codon 194, due to C to T substitution resulting in substitution of amino acid Arginine with Tryptophan was detected by the PCR-RFLP method. The digestion of this amplicon resulted in the generation of 199bp and 292bp product for homozygous mutant allele 491bp, 199bp and 292bp product for heterozygous mutant allele (C/T) and for the homozygous wild alleles(C/C) ,the PCR products remains undigested (**Figure 2**) .

3.3. Frequency distribution analysis of the genotypes and alleles.

The restriction digestion of PCR amplicon resulted in identification of three Genotypes: Wild Type (C/C), Homozygous Mutant (T/T) and Heterozygous Mutant (C/T).The three genotypes, C/C, C/T and T/T are represented by the frequency of 82.5%, 12% and 5.5% in control samples where as in cases these are represented by the frequency of 78%, 19.5% and 5%, respectively (**Table 2.3**). When the allelic frequencies were compared, wild type Isoleucine was present in 71.8% of cases where as the mutant Valine allele was present in 28.2% of cases as compared to the 61.6% of Isoleucine and 30.83 % of Valine in controls (**Table 2.3).**

Table 2.3: Genotypic and allelic frequency distribution of wild and mutant *XRCC1* alleles; Cases Vs Controls

	Genotype	Cases N=200(%)	Controls N=200(%)	χ^2	OR(95% CI)	p-value
Gene XRCC1 Arg194 Trp	C/C	156(78)	165(82.5)	-	1 (reference)	-
	C/T	39(19.5)	24(12)	3.73	0.58(0.33-1.02)	0.05
	T/T	5(2.5)	11(5.5)	1.84	2.08(0.70-6.12)	0.20
	C	351(87.75)	354(88.5)	-	1 (reference)	-
	T	49(12.25)	46(11.5)	0.11	0.93 (0.60-1.42)	0.83
	C/T+T/T	44	35	0.85	1.24(0.78-1.99)	0.40

OR (odds ratio) calculated at 95% Confidence Interval (CI): p-value (two –sided) Perasonsχ2 chi square test. $p<0.05$ is considered to be significant.

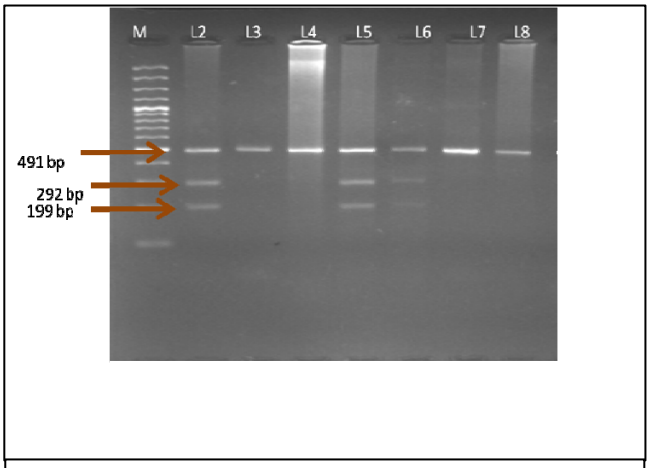

Figure 2:Restriction digestion products resolved on 2% agarose gel.Lane M- 100bp DNA ladder.Lane 2,5,6 – heterozygous condition(Arg/Trp). Lane 3,4,7,8 – Homozygous wild condition(Arg/Arg)

3.4: Co-relation between the *XRCC1*genotype and clinicopathological parameters.

Univariate logistic regression analysis was used to find association, if any between the *XRCC1* genotype and the breast cancer risk factor. **Table 2.4** summarizes this association study. Positive association was found between any of the studied parameter and the *XRCC1*genotype.

Table 2.4: Association between the clinicopathological characteristics of the patient population with the genotype of the codon 194 of XRCC1 gene.

	Genotype			χ^2	p-Value
Variable	C/C	C/T	T/T		
Age(Years)					
<50	45	18	3		
>50	111	21	2	5.91	0.05
Menopausal Status					
Premenopausal	94	16	3		
Postmenopausal	62	23	2	4.72	0.94
Lymph node					
Positive	99	20	2	2.83	0.24
Negative	57	19	3		
BMI					
<24.9	39	20	2		
25-29.9	80	7	2	15.70	0.0003
>30	37	12	1		
Tumor Stage					
I	30	7	1		
II	71	28	1	1.73	0.41
III	55	4	3		
Tumor Grade					
I	29	7	1		
II	80	32	2	1.29	0.52
III	47	0	2		
Estrogen Receptor					
positive	105	37	4		
negative	51	2	1	12.15	0.0022
Progesterone receptor					
Positive	93	30	2		
negative	63	9	3	5.09	0.78
Her-2-Neu receptor					
Positive	14	16	2		
Negative	142	23	3	26.04	0.0001
Dwelling					
Rural	65	12	2		
Urban	91	27	3	1.55	0.46

Bold values indicate statistical significance $p<0.05$, p value (two-sided). Pearson's χ2 test. $p<0.05$ is considered to be significant

4. CONCLUSION AND DISCUSSION:

XRCC1 is an important member of the DNA repair pathways like BER, SSBR and BNHEJ[14-16] . Negative XRCC1 expression has been associated with poor clinical outcome and biologically aggressive tumors in breast cancer patients, the property which has been the groundwork for the treatment for breast cancer by DSB repair pathway blockage employing ATM or DNA-PKCS inhibitors[17]. Multiple SNPs in the XRCC1 genome have been implicated as the risk factor for the breast cancer development, Arg194Trp being one such SNP[18]. The candidates with such substitution polymorphism have been reported to show adverse response to the radiotherapy treatment

Present study deals with examining the association ofArg194Trp SNP of XRCC1, as the risk factor for breast

cancer development as well as its association with the various risk factors for breast cancer.

In our study we found no association of this SNP with increased risk factor for breast cancer.

These findings are in sync with the previous published report by Smith *et al.[*19] that showed only a weak association between the two and metaanalysis carried out by Huang et al., 2009[18]but in contrast with the meta analysis by Zibin Hu et al., [20] that presented Arg194Trp substitution polymorphism to be the biomarker for the cancer susceptibility. Our results are also in contrast to the findings by saniya nissar et al., 2015, that showed the positive association of this polymorphism with colorectal cancer in the same population[21]. A previous study on the same polulation has shown the allele distribution as 68.5%for wild type, 24% for heterozygous mutant and 7.5% for homozygous mutant that do not differ significantly from our observation of the allele frequencies[21].

Comparison of selected physical, social and demographic characteristics between cases andcontrol subjects showed positive association of parity and menopausal status with breast cancer risk. These observations are consistent with the various studies on different populations that reported increasing parity to be associated with marked decrease in breast cancer risk 22-25].Similarly increased exposure of body to reproductive hormones, that is early menarche and late menopause are also considered to be risk factors for breast cancer development[26] and present study is consistent with these findings as it has more frequency of cases in pre-menopausal status as compared to controls.

When XRCC1 genotype was compared with the standard immuno histochemical parameters viz. estrogen receptor status, progesterone receptor status and Her-2- Neu receptor status, a significantly positive association was observed for loss of estrogen receptor and Her-2-Neu receptor a feature that can further contribute to the resistance of these tumors through conventional treatment strategies.

In conclusion, our study observed that XRCC1 genotype is not associated with increase in the risk for breast carcinoma for Kashmir population. Also, our study suggest that the analysis of clinicopathological features, together with genetic polymorphisms need further evaluation as it may contribute to better understanding of the mechanisms of this disease as a result of possible interactions between these genotypes and well-established risk factors for breast carcinoma . The present study, further adds to the finding that gene frequency distribution is based on ethnic differences. However, the study needs to be validated for larger cohorts.

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6. CONFLICT OF INTEREST

Contributing authors declare no competing interests.

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