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SNP study of XRCC1 gene in local population of Patna, Bihar

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ABSTRACT

Endogenous and exogenous mutagens regularly damage DNA in most cells. Unrepaired damaged DNA can cause apoptosis or uncontrolled cell proliferation, which can lead to cancer. If cell machinery detects DNA damage, a variety of reactions may occur to limit replication in the presence of genetic errors. Alternatively, the damage to the DNA can be repaired, allowing the cell to replicate normally. XRCC1 is a protein involved in base excision repair (BER). Variants in the XRCC1 gene may change protein structure/function or result in alternatively spliced proteins, affecting BER efficiency and individual susceptibility to cancer. SNPs (Single nucleotide polymorphisms) are single base mutations with a frequency of more than 1% in the population. Several studies have found a link between SNP and several types of cancer. Arg194Trp is one of the most researched SNPs in the XRCC1 gene in humans. Many studies showed association of Arg194Trp SNP with increased risk of cancer. No such study has been done in local population of Patna, Bihar. In this study, we studied the genotype of 22 control samples in population of Patna. We found that among 22 samples, 5 samples showed heterozygous Arg194Trp variant while 17 samples showed normal wild type Arg194Arg genotype. No sample with homozygous Trp194Trp was obtained. We have also checked the association of SNP in male and female samples. Further we have studied the association with age groups above 60 years and below 60 years. This study will act as control when studying association between XRCC1 Arg194Trp polymorphism and cancer.

Key words: XRCC1, SNP, Polymorphism, Genotype, RFLP.

INTRODUCTION

DNA repair is essential for survival and vital for the prevention of many diseases, including cancer Lower DNA repair capacity has been linked to an increased risk of cancer in a number of population studies (Wei *et al*, 1996; Cheng *et al*, 1998). XRCC1 is a DNA repair gene that was originally found on human chromosome 19 and was later discovered on the long arm of the chromosome in the region 19ql3.2-13.3. There are 17 exons and 16 introns in this gene. The XRCCI gene is 33 kb in length and encodes the 633-amino-acid XRCC1 protein (69.5 kDa) (Thompson *et al*, 1990). The protein product of this gene is involved in effective repair of DNA single-strand breaks caused by ionising radiation and alkylating agents. In mammalian cells, XRCC1 protein plays a major role in DNA repair of single-strand breaks, via an ability to interact with multiple DNA repair enzyme (Caldecott, 2003; Wilson, 1998; Masson, 1998; Vispeet, 2000; Savas, 2004). It may be involved in DNA processing and recombination in germ cells during meiogenesis. In patients with variable levels of radiosensitivity, a rare microsatellite variation in this gene has been linked to cancer.

There are 590 SNPs in the XRCC1 gene. In XRCC1, there are eight nonsynonymous coding single nucleotide polymorphisms, three of which are common and result in amino acid substitutions at codon 194 (exon 6, base C to T, amino acid Arg to Trp), codon 280 (exon 9, base G to A,amino acid Arg to His), and codon 399 (exon 10, base G to A, amino acid Arg to Gln). XRCC1 Arg194Trp variant show contrasting results in association with cancer. In some study Arg194Trp variant has been associated with increased risk of cancer (Chen *et al* 2002; Xing et.al 2002; Tuimala, 2002). The Trp/Trp genotype of the 194th codon of the XRCC1 gene is a risk genotype for lung cancer in the Chinese population (Chen *et al* 2002). Individuals with the Trp/Trp genotype at the XRCC1 Arg194Trp site showed a 2 fold greater risk of Esophageal Squamous Cell Carcinoma in the Chinese population compared to those with the Arg/Arg genotype (Xing et.al 2002). Metsalo *et al*, observed a statistically significant association between XRCC1 genotypes and breast cancer risk in Finnish smoking women, especially when enzymes in both DNA repair pathways i.e. Base Excision Repair(BER) and Nucleotide Excision Repair (NER) were defective (Metsalo *et al*, 2005). Larger investigations are needed to confirm these findings and elucidate the mechanisms that underpin them. These results vary from population to population. Till date, no such study has been conducted in Population of Patna, Bihar. On the basis of above discussed problems we proposed to carry out SNP genotyping of XRCC1 gene in local population of Patna, Bihar.

MATERIALS AND METHODS

Sample collection:

Blood samples were collected from the local population of Patna, Bihar. Lymphocyte DNA was available for 22 samples. Other samples in which DNA was not obtained were excluded from the study. The final group included 22 samples (age group ranging from 23 to 88 years).

Primer designing:

XRCC1 gene (accession no.: NM_006297) sequence was retrieved from NCBI. Foreword and reverse primer were designed against exon 6 sequence including the 194 codon. The reliability of primers was checked in GENE RUNNER for formation of hairpin loops, dimmers etc. after that *in silico* PCR was done using FASTA PCR tool and the desired product of 138bp was tested.

XRCC1 (Arginine 194 Tryptophan)

Foreword primer:

Length: 21bp; Tm: 67.6ºC; %GC: 57%

Sequence: 5′-GTTTGTGAAGGAGGAGGA-3′

Reverse primer:

Length: 27bp; Tm: 66.7 ºC; %GC: 52%

Sequence: 5′-CGAGTCTAGGTCTCAACCCTACTCACT -3′

Genotyping analyses

In PCR-based restriction fragment length polymorphism (RFLP) experiments, lymphocyte DNA (100 ng) isolated using DNAsure kit (genetix) was utilized as a template. For XRCC1 Arg194Trp genotype determination, a 138 (base pair) bp was amplified using above described primer . After digestion of PCR product with restriction enzyme Pvu II (Fermentas), the XRCC1-194-Arg wild type allele was revealed by the presence of intact 138 bp whereas the 194Trp variant allele was indicated by 75 bp fragment and 63 bp fragment. Genotype analysis was done for all the 22 samples containing genomic DNA. Digested PCR products were verified on 3.5% agarose gel electrophoresis and images were taken in the Gel Documentation system and results were interpreted.

RESULTS AND DISCUSSION

Study and Subjects:

Samples were selected randomly from the common population of Patna, Bihar. 22 DNA samples for XRCC1 genotype were identified for PCR-RFLP analysis. The genomic DNA was extracted from each blood sample as discussed earlier shown in figure 1 which was used as template in PCR based RFLP assay. For XRCC1 Arg194Trp genotype determination, a 138 bp fragment was amplified using the primers described in materials and methods. After digestion of the PCR product with 10U *Pvu*II enzyme, the 194Arg wild type allele was revealed by intact 138 bp fragment while two fragments of 75 bp and 63 bp indicated in the 194Trp variant allele as shown in figure 2, figure 3A and figure 3B.

Genomic DNA isolation:

Genomic DNA was extracted from each blood sample collected randomly from the population of Patna, Bihar. Figure 1 A & B shows genomic DNA of some of the blood samples. In figure 1A sample no. 1, 2 and in figure 1B sample no. 1,2,4,5 DNA was obtained. But no DNA was obtained in sample no. 3 in both figure 1A and figure 1B because of clotted blood. XRCC1 codon 194 (Arg) lies within exon 6 and its mutation to Trp (CGG to TGG) forms a new *Pvu*II site The SNP analysis of XRCC1 codon 194 exon 6 (Arg194Trp) were analyzed by PCR-Restriction Fragment Length Polymorphism (RFLP). 138bp fragment including Arg194Trp allele in XRCC1 was amplified. The restriction enzyme *Pvu*II was used to type Arg194Trp allele.

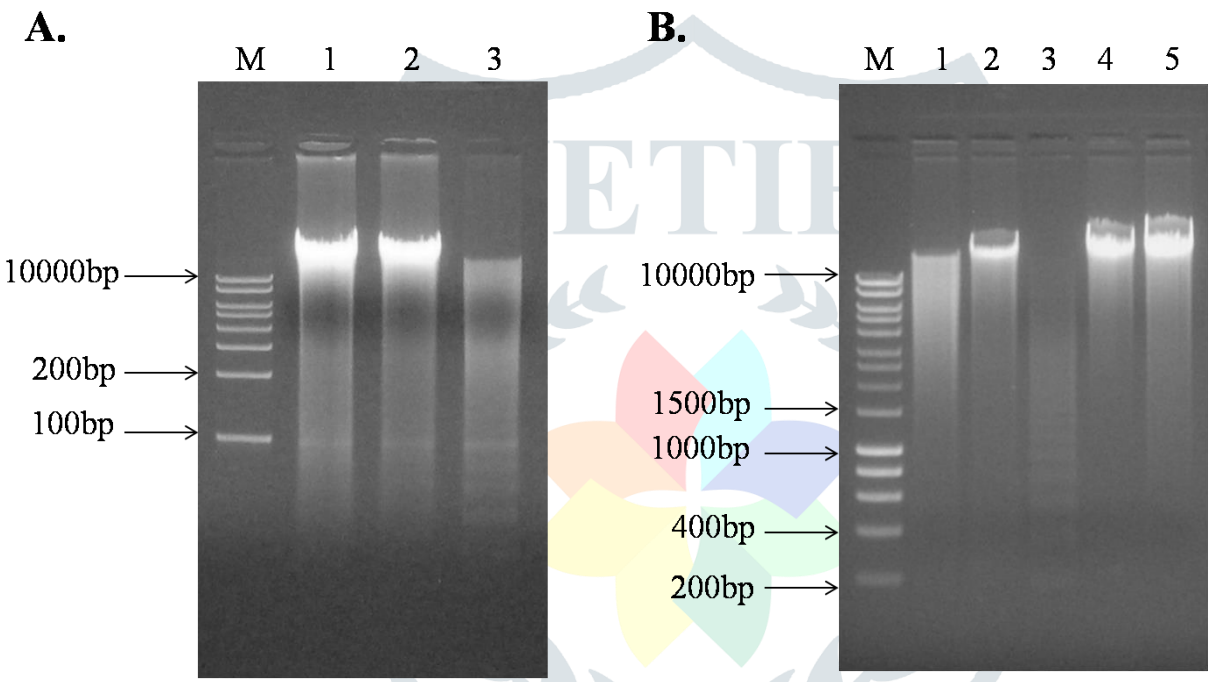

Figure 1: Genomic DNA isolation from 22 blood samples (some of the samples are represented), **A.** M = 1kb ladder, lane 1,2: DNA obtained, lane 3:No DNA obtained. **B.** M = 1kb ladder, lane 1,2,4,5: DNA obtained, lane 3:No DNA obtained.

Genotyping using RFLP:

Isolated genomic DNA were used as a template for the PCR amplification. Further, the PCR products were purified by PCR clean up kit (Genetix). Purified PCR product was digested with 10U *Pvu*II Restriction Enzyme. Figure 2 and figure 3A & 3B shows restriction digestion profile of Exon 6 (E6) 138bp amplicon. In XRCC1, due to SNP at 194th codon, Cytosine gets converted into Thymine thus changing the codon coding for Arginine to Tryptophan. This creates a restriction site for *Pvu*II in this codon. Digestion with *Pvu*II resulted in an undigested product of 138bp in homozygous Arg/Arg genotype, two fragments in homozygous

recessive Trp/Trp genotype (75 bp and 63 bp) and three fragments in heterozygous Arg/Trp condition (138bp, 75 bp and 63 bp).

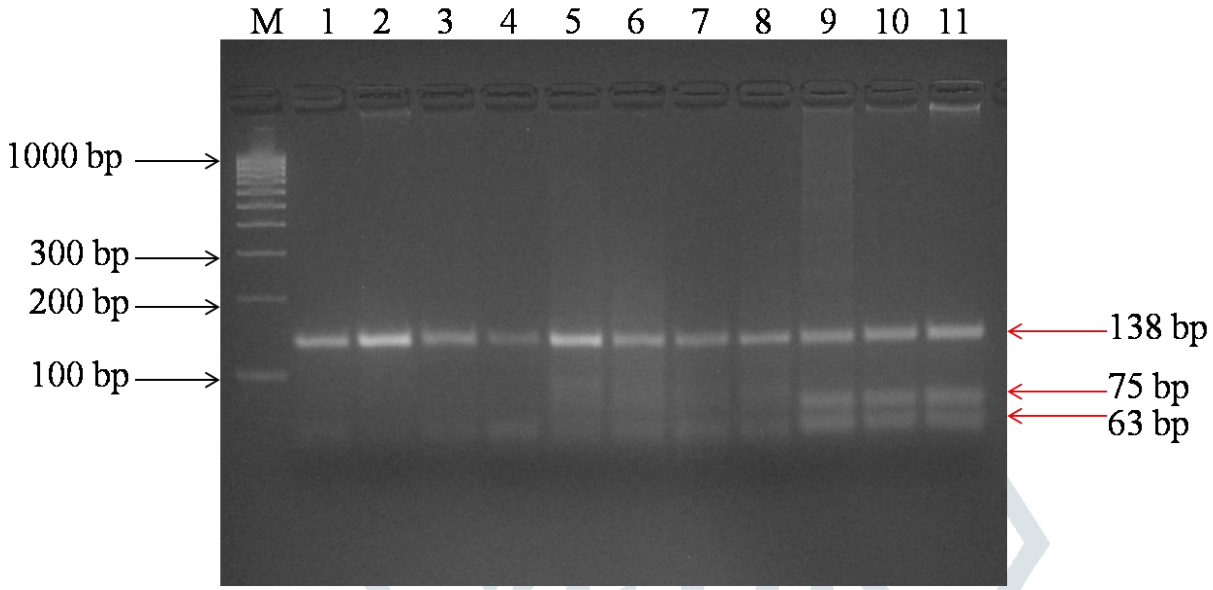

Figure 2: XRCC1 R194W Pvu II Digestion; M = 100bp ladder, lane 1,2,3,4,6,7,8: shows single band of 138bp, lane 9,10,11: shows three bands of 138bp,75bp and 63bp.

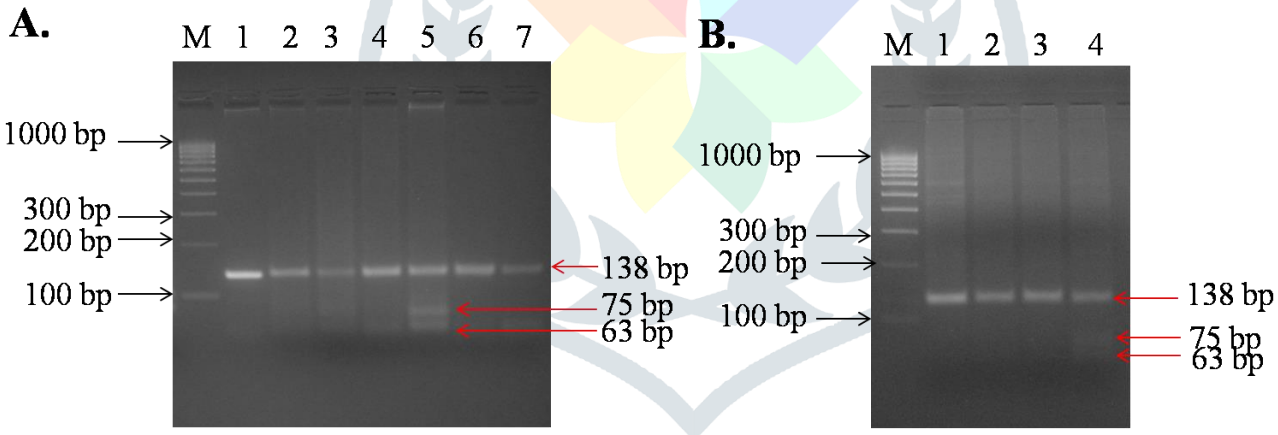

Figure 3: XRCC1 R194W Pvu II Digestion **A**. M = 100bp ladder, lane 1,2,3,4,6,7 : shows single band of 138bp, lane 5: shows three bands of 138bp,75bp and 63bp. **B.** M = 100bp ladder, lane 1,2,3 : shows single band of 138bp, lane 4: shows three bands of 138bp,75bp and 63bp.

5 (22.73%) samples were in heterozygous Arg/Trp condition and 17 (77.27%) were in homozygous Arg/Arg condition shown in figure 2 and figure 3A & 3B. During the analysis there was no homozygous recessive Trp/Trp genotype found as mentioned in table 1 and shown graphically in figure 4A.

Table 1: XRCC 1 Genotypes Control samples

XRCC1 genotype	Number	Percentage (%)
Arg/Arg	17	77.27
Arg/Trp	5	22.73
Trp/Trp	0	0

Among the all DNA samples 13 were male samples and 9 were female. In the overall male samples, 3 (23.07%) samples showed heterozygous Arg/Trp condition having three bands of (138bp, 75 bp and 63 bp) whereas 10 samples showed single band showing homozygous Arg/Arg condition mentioned in table 2 and represented in figure 4B. Among female samples, 2 (22.22%) samples with heterozygous Arg/Trp condition and 7 (77.78%) samples with homozygous Arg/Arg was observed as mentioned in table 3 and figure 4C. This suggests that heterozygous Arg/Trp condition occurs more frequently in males as compared to female in XRCC1 at 194 position.

Table 2: XRCC 1 Genotypes in male samples

XRCC1 genotype	Number	Percentage (%)
Arg/Arg	13	76.93
Arg/Trp	3	23.07
Trp/Trp	0	0

Table 3: XRCC 1 Genotypes in female samples

XRCC1 genotype	Number	Percentage (%)
Arg/Arg	9	77.78
Arg/Trp	2	22.22
Trp/Trp	0	0

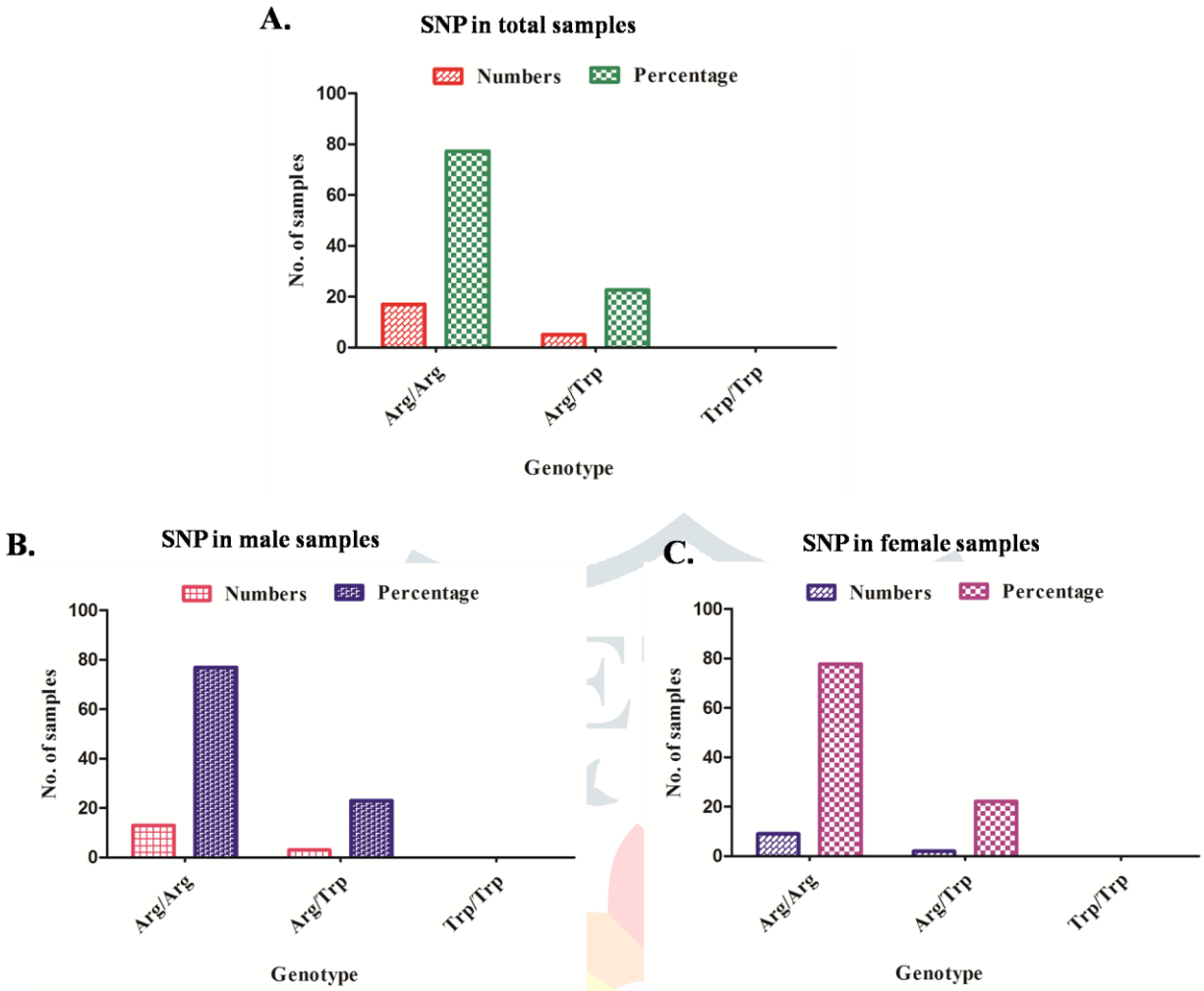

Figure 4: XRCC1 genotypes**: A.** genotyping in total collected samples, **B.** genotyping in total male samples, **C.** genotyping in total female samples**.**

When total samples were observed on the basis of age group we have found the heterozygous Arg/Trp condition in 4 samples above 60 age group whereas 1 sample in below 60 age group. Other 17 samples were normal with homozygous Arg/Arg condition as depicted in figure 5. This suggests that higher age group has the higher chance of polymorphism.

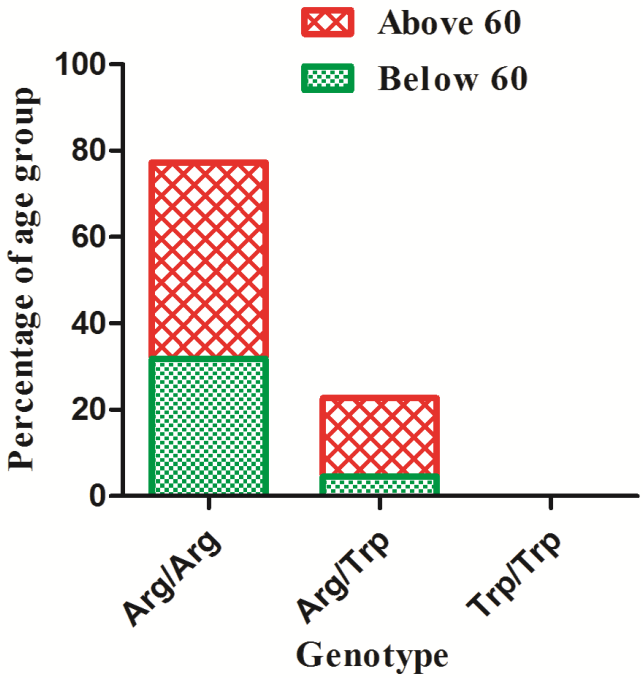

Figure 5: Genotyping on the basis of age group.

CONCLUSION

It is well known that DNA repair is very important in the maintenance of genetic stability, and in protection against the initiation of cancer. XRCC1 is one of the most important DNA repair genes. XRCC1 is known to participate in base exision repair (BER). XRCC1 is a multidomain protein that has no known catalytic activity itself but it recruits DNA polymerase β, DNA ligase III, APE1 and PARP that are needed at the site of DNA damage. (Metsola *et al* 2005).

Three gene polymorphisms resulting in non-conservative amino acid substitutions (Arg194Trp, Arg280His, Arg399Gln) have been identified in XRCC1 gene (Metsola *et al*, 2005). The study was based on XRCC1 Arg194Trp polymorphism located in the area coding for a APE 1 binding site. The main aim of the present study was to study XRCC1 genetic polymorphisms in the population of Patna, Bihar. In the control study we found that 5 (22.73%) samples were in heterozygous Arg/Trp condition and 17 (77.27%) were in homozygous Arg/Arg condition. The heterozygocity was observed more in male samples as compared to female samples. Above 60 age group were more prone to heterozygous Arg/Trp condition as compared to below 60 age group. Homozygous variant genotype was not observed in any sample. 5 Samples having heterozygous SNP genotype may be vulnerable to cancer progression in their life time.

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