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ASSOCIATION OF XRCC1 399GLN AND XRCC3 T241 METH POLYMORPHISM WITH THE RISK OF CERVICAL CANCER IN NORTH INDIAN POPULATION

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ABSTRACT

Association of XRCC1 399Gln and XRCC3 T241 Meth polymorphism with the risk of cervical cancer in North Indian Population. **Objective**: To study the association of polymorphisms in the DNA repair genes of x-ray repair cross-complementing (XRCC) gene 1 399Gln and XRCC3 T241M, to the susceptibility of cervical cancer (CC) in North Indian population. **Material and Methods**: XRCC1 & XRCC3 polymorphisms were characterised in Seventy-one eligible CC patients and Sixty nine healthy controls who had no history of any malignancy by using confronting two pair primers (PCR–CTPP) method, taking DNA from peripheral blood in a case control study. **Results**: Our results revealed that the frequencies of AA genotype of XRCC1 399 polymorphism were significantly higher in the CC patients than in the normal individuals (p 0.001), OR=5.27, 95% CI= (1.934-14.372). While we did not observe any association between the XRCC3 T241M polymorphism and CC risk. **Conclusion**: Our results suggested that, XRCC1 399 gene is an important candidate gene for susceptibility to cervical cancer. Although the sample size was small, the present study indicate a statistical association between cervical cancer and XRCC1 399Gln polymorphism. Future studies are needed that may provide a better understanding of the association between gene polymorphism and cervical carcinoma risk.

KEYWORDS: XRCC1, XRCC3, Gene polymorphism, Cervical cancer.

INTRODUCTION

Cervical cancer is one of the most frequent cancers among women in developing countries.[1] According to statistics globally number of new cases of cervical cancer stand out to be 7.4 per 100,000 annually, while the mortality rate stand out to be 2.3 per 100,000 women per year.[2] Mortality due to cervical cancer is also an indicator of health inequities, as 86% of all deaths due to cervical cancer are in developing, low- and middle-income countries.[3]

Statistical data shows that in India, 122,844 women are diagnosed with cervical cancer and 67,477 die from the disease. Indian women face a 2.5% cumulative lifetime risk and 1.4% cumulative death risk from cervical cancer.[4] It is estimated that about 6.6% of women in the general population may harbour HPV infection. While HPV serotypes 16 and 18 is found to the most common and account for nearly 76.7% of cervical cancer in India. However, only 1% of these women will develop CIN and cervical carcinoma. This indicates that HPV infection is not sufficient to develop cancer, and other cofactors, such as polymorphisms in DNA repair genes, should be considered.

In the view of the above we have studied about the DNA repair genes, Repair gene have been shown to play a key role in maintaining genomic stability and integrity of the cell. It is now thought that an individual's DNA repair capacity is genetically determined, and is the result of a combination of multiple genes that display subtle differences in their activity. In mammalian cells four different DNA repair mechanisms have been identified: base excision repair (BER), nucleotide excision repair (NER), double-strand break repair and mismatch repair.[5] All these DNA repair pathways are finely regulated for the maintenance of genomic integrity and modulation of repair capacity in response to DNA damage.[6] Single nucleotide polymorphisms (SNPs) may cause subtle structural alterations in repair enzymes, and subsequent modulation of cancer susceptibility. A number of SNPs in DNA repair genes have been identified to play a key role in maintaining genomic stability and integrity. Defects in DNA repair pathways are found to be associated with many types of cancer, including cervical carcinoma, Polymorphisms in DNA repair genes are common.

The BER pathway has a primary role in the repair of oxidative base lesions such as 8-hydroxyguanine,

formamidopyrimidines, and 5-hydroxyuracil[9] produced by methylation, oxidation or reduction by ionizing radiation or oxidative damage.

X-ray repair cross complementing group 1 (XRCC1) protein encoded by XRCC1 gene plays a critical role involved in the BER pathway, which interacts with enzymatic components of each stage of DNA strand break repair, including DNA polymerase beta, APE1 (apurinic/apyrimidinic endonuclease 1),[17] PARP-1 (poly [ADP-ribose] polymerase 1), and DNA ligase III.[12] There are more than 60 validated single nucleotide polymorphisms (SNPs) in the XRCC1 gene containing 17 exons and 16 introns on chromosome 19q13.2-13.3, among which codon 399 (exon 10, Arg to Gln) have been studied widely.

The genetic polymorphism of XRCC1 399 results in an arginine to glutamine amino acid substitution. Because amino acid residues at the protein-protein interfaces of multi-protein complexes and residues involved in the active sites play a role in enzyme function, it is possible that XRCC1 polymorphisms lead to alteration of DNA repair capacity.

The XRCC3 proteins are involved in the homologous recombination repair (HRR) pathway which is responsible for DNA double strand break repair(DSB). The XRCC3 is located on chromosome 14q32.[11] XRCC3 acts specifically at the site of a DNA break to assist in the formation of Rad51 foci, most likely by promoting assembly of the Rad51 nucleoprotein filament. The most frequent polymorphism in XRCC3 is the C/T transition from Thr to Met at codon 241 (T241M). In addition, variants of the T241M polymorphism may affect the function of the encoded protein and consequently alter the DNA repair capacity It is a common knowledge that chromosome damage re-sults from non- or misrepaired of (DSBs), with many polymorphisms, such as those of DNA repair genes, hav-ing been associated with increased cancer risk, and a possibly even higher level of chromosome damage. Although it has been reported that associations between the XRCC3 Thr241Met poly- morphism and cervical cancer risk are inconsistent, and the variant XRCC3 241Met allele has been both positively and negatively associated with various cancer.[5] Thus, our hereby was to investigate the polymorphisms and chromosomal damage in women with cervical cancer and healthy controls. We genotyped DNA repair genes XRCC1 399 Gln & XRCC3 241 meth and assessed its contributions to cervical cancer susceptibility, and its association with other epidemiological risk factors.

MATERIAL AND METHOD

This study was a hospital based case-control study histological confirmed primary cervical cancer cases were recruited from the city of Lucknow (Era's Lucknow Medical college & Hospital) Controls were randomly selected from healthy postmenopausal women who requested gynecological examinations. The criteria for selection included no positive findings during examination, no history of cancer. Sexual and reproductive history was obtained using a standardized questionnaire. and each participant signed an informed consent. A total of 71 eligible patients and 69 eligible control women were interviewed, completed the questionnaires, consented to provide blood samples for genotyping. Experiments were undertaken with the understanding and written consent of each subject.

Polymorphisms Analysis

Patients blood samples were collected in EDTA-containing tubes. Genomic. DNA was extracted from peripheral whole blood with the Qiagen extraction kit (Hilden, Germany), according to the manufacturer's protocol. PCR amplifications for these two polymorphisms were performed in a programmable thermal cycler Bio rad.

Genotyping

Genotyping were carried on duplex polymerase chain reaction with the confronting-two-pair primer (PCR-CTPP) method. The amplified DNAs are allele-specific in their sizes, so that the DNA products can be applied directly for electrophoresis without the digestion by a restriction enzyme All primers were added into the same tube.[7] The XRCC1 Arg399Gln polymorphism primers were XRCC1: 5-CCCAAGTACAGCCAGGTC-3 (forward) and 5-CCGCTCCTCTCAGTAGTC-3. (reverse).[8] XRCC3: 5-GCTCGCCTGGTGGTCATC-3 (forward) and 5-GCTTCCGCATCCTGGCTAA-3 (reverse). PCR amplification was carried out to a total volume of 25 µl, containing approximately 100 ng of genomic DNA, 0.5 µl of each primer, 12.5 µl of PCR master mix (2X) (Thermo Scientific, USA) (it contains dream Taq DNA polymerase supplied in 2X Dream Taq green buffer, dATP, dCTP, dGTP, dTTP, 0.4 mM each and 4 mM $MgCl_2$) and 0.5 ul of H_2O. Reaction conditions included initial denaturation at 94ºC for 5 min, followed by 30 cycles at 94ºC for 1 min, at 66ºC for 1 min, at 72ºC for 45s and a final extension at 72ºC for 5 min. PCR products were analyzed on 2% agarose gel electrophoresis while 50 bp DNA Ladder was used as reference.

Statistical Analysis

The data were analyzed by SPSS (Statistical Package for Social Science) version 17.0 on IBM compatible computer. Continuous variables were expressed as mean±standard deviation (SD) while categorical variables were shown as frequencies and percentages. We compared genotype distributions, XRCC1Arg399Gln & XRCC3 241 Meth among cases and controls, Adjusted odds ratios (OR) and their 95% confidence intervals (95% CI) of the association between genotype were calculated. P value were considered statistically significant which had a value which was less than or equal to 0.05.

RESULTS

A total of 71 cases and 69 control were analyzed in the study, the characteristics of the study participants are shown in table (1) There were no significant difference in age between the case and control with the mean age of patients and control were 49.1±13.1, 45.3 ±10.5 There were 7(9.85%), 3(4.34%) smoker in the total population of case and control. HPV infection in carcinoma patients were 88.73% and 45% in control while HPV negative cases were 11.26% and 38% in control. The alleles and genotype frequency of XRCC1-399 Gln and XRCC3 T241M polymorphism are shown in table (2). All genotype distribution of case and control were consistent with Hardy-Weinberg Principle of Equilibrium Binary logistic regression was used to calculate odds ratio for cervical cancer. Our results indicated that the homozygous AA genotype of XRCC1 399 codon was associated with risk of cervical cancer with **(OR =5.27, CI 1.934-14.372, p value=0.001**), while no association was found with XRCC3 241M codon.

Table 1: Clinicopathological characteristic of Case & Control.

Variables	Control n=69	Case n=71
Age (years) (mean ± SD)	45.37 ±10.4	49.1 ± 13.1
Smoking Status		
Smoking	3 (4.34)	7 (9.85)
Non Smoking	66 (95.6)	64 (90.14)
HPV Status		
HPV-Positive	31 (44.92%)	63(88.73%)
HPV-negative	38(55.07%)	8(11.26%)

Table 2: Genotype and allelic frequency distribution of XRCC1- 399 & XRCC3 T241M gene polymorphisms.

		Control (69)		Case (71)				
XRCC1-399		**N**	**Frequency (%)**	**N**	**Frequency (%)**	**OR**	**95% CI**	**p values**
Genotype	GG	22	31.8	11	15.4	1 Ref		
	GA	31	44.9	22	30.9	1.42	(0.573-3.515)	0.499
	AA	11	15.9	29	40.8	5.27	(1.934-14.372)	0.001
	GA+AA	42	60.8	51	71.8	2.43	(1.058-5.575)	0.036
Allele	G	75	54.3	44	30.9	1 Ref		
	A	53	38.4	89	62.6	2.86	(1.729-4.739	<0.001
		Control (69)		**Case (71)**				
XRCC3-241		**N**	**Frequency %**	**N**	**frequency (%)**	**OR**	**95%CI**	**p values**
Genotype	TT	21	30.4	8	11.2	1 Ref		
	CT	31	44.9	10	14	0.846	(0.286-2.499)	0.76
	CC	24	34.7	7	9.85	0.765	(0.23-2.47)	0.65
	CT+CC	55	79.7	17	23.9	0.81	(0.30-2.16)	0.67
Allele	T	73	52.8	26	18.3	1 Ref		
	C	79	57.2	24	16.9	0.85	(0.45-1.6)	0.62

DISCUSSION

The role of genes involved in the DNA repair mechanism on the human cancers is being increasingly recognized. Genetic instability is one of the most common features of human carcinoma and recent evidence suggests that mutations in DNA repair genes are implicated in both initiation and cancer progression. Although infection with high-risk HPV is recognized to be an essential initiating event in cervical tumorigenesis, this alone is not sufficient for progression to invasive cancer. Despite the recent progress in understanding the molecular aspects of cervical cancer, the genetic basis of progression of cervical cancer remains poorly understood.[11] Therefore, identification of other factors in cervical cancer is important to understand its biology.[12]

In this sense, The results of the present study showed polymorphisms in XRCC1 Arg399Gln, were related to the risk in Indian population. These results suggest that polymorphisms may have functional significance in Cervical cancer. Various DNA damage may be induced by normal endogenous metabolic processes or by environmental carcinogens. Most of these alterations, if not repaired, may result in genetic instability, mutagenesis and cell death, Moreover, these DNA damages may destroy genome integrity and induce carcinogenesis. BER is predominant a DNA damage repair pathway for the processing of small base lesions, derived from oxidation and alkylation's damage. XRCC1 gene is seen as an important proteins in the multistep BER pathway, and it was the first mammalian gene to be isolated that affects cellular sensitivity to ionizing radiation.[11]

The XRCC1 protein, complexes with three other DNA repair enzymes involved in the BER pathways, including DNA ligase III, DNA polymerase and poly (ADP-ribose) polymerase PARP,[10,17] Codon 399 polymorphism is present on the COOH-terminal side of the PARP interacting domain, which is present in the BRCT1 domain, that are thought to mediate several protein to protein interactions.[14] Amino acid substitutions found in the BRCT domina and in the DNA polymerase β interacting domain in hamster is reported to disrupt the functionality of XRCC1.[13] Mutations of XRCC1 may increase the risk of cancers by impairing the interaction of XRCC1 with other enzymatic proteins and consequently altering DNA repair activity and subsequently induce the pathway for carcinogenesis of several tumors.[15] Our study showed the polymorphism in XRCC1 Arg399Cln could increase the risk of Cervical cancer which was in line of a previous studies. single nucleotide polymorphisms of DNA repair genes are extensively studied in breast and ovarian cancer. Here, we present evidence that supports that XRCC1 399Gln polymorphism may be associated with cervical cancer.

XRCC3 gene codes for a protein participating in HRR of DSB. It is a member of an emerging family of Rad-51-related proteins that may take part in homologous recombination to repair DSB and maintain chromosome stability),[16] XRCC3 T241M polymorphism has shown to affects the DNA repair capacity of the protein encoded by it, and thus contributes to the development of cancer . It has also been seen that the potential influence of the XRCC3 T241M polymorphism may be affected by gene-gene and gene-environment interactions. We failed at finding a significant association between XRCC3 241Meth and cervical cancer.

[11] These results are in agreement with those found by others Infection with the oncogenic types of HPV has been established as a main cause of cervical. We also found a significant association of HPV infection in cervical cancer cases.

Since carcinogenesis is a very complex process and inter related pathways of DNA damage exist involving multiple genetic mechanisms, therefore inconsistency in results among different populations can be attributed to the fact that polymorphism may have multiple effects on cancer progression, which depend on other genetic factors or environmental exposures. The small sample size plus other confounding factors may attribute to such differences.

Additional work is required to determine whether polymorphisms do indeed lead to a reduced DNA repair capacity in vivo and to identify the effects of these phenotypes in different environmental conditions. The identification of polymorphisms in many genes and the determination of their functional importance in cervical cancer will enable the design of susceptibility–risk models for de-novo and therapy-related disease. In conclusion, our results suggest an increased risk for Cervical cancer in individuals with XRCC1 399 polymorphism suggesting BER repair pathway modulates the risk of developing Cervical cancer in north Indian population.

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