

## Significance of N-acetyltransferase-2 (NAT-2) gene polymorphism in the patients of cervical cancer

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### ABSTRACT

In concern of cervical cancer through the various screening and health care programmes running in Asian and Western countries, it was established that the prevalence and mortality has declined tremendously. However cervical cancer is still considered as one of the fetal and common cancer in females, followed by breast cancer and lung cancer in India. In the present study, blood samples of confirmed cervical cancer patients through biopsy examination were collected and subjected for genomic DNA isolation. Isolated DNA samples were amplified for detection of NAT-2 gene, subsequently gene polymorphism was detected using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. We observed that NAT-2 gene polymorphism was significantly associated with cervical cancer risk.

Figures : 06

References : 12

Table : 01

KEY WORDS : Amplification, Cervical cancer, Genomic DNA, NAT-2 gene polymorphism

### Introduction

Benign and malignant tumors are common in cervix. Cervix is the site of lesions that include cervical dysplasia, carcinoma *in situ* (Cervical Intraepithelial Neoplasia) now termed as Squamous Intraepithelial lesions (SIL). The most common malignant tumor is known as squamous carcinoma of cervix (SCC). The symptoms of cervical cancer are like: blood-stained vaginal discharge, post-coital bleeding, post menopausal-vaginal bleeding, vaginal bleeding between menstruation or menstruation periods that are longer or heavier than normal, watery vaginal discharge that has a strong odor may contain blood, pelvic pain or pain during sexual activity, low backache<sup>6</sup>. Variant alleles of NAT-2 gene that cause slow or fast metabolism by particular enzymes could be the risk factor for cervical carcinogenesis. The present study was aimed to examine the association of NAT-2 gene polymorphisms

with cervical cancer risk in Indian population. In this study total 75 cervical cancer cases and 75 healthy normal controls was enrolled, polymorphisms were screened by PCR-RFLP method. The finding of present study shows significant association of NAT-2 gene polymorphism with risk of cervical cancer.

**Functions of N-acetyltransferase-2 (NAT-2) genes:** In humans, NAT-2 gene is responsible for N-acetyltransferase activity and several allelic variants of NAT-2, which cause variations in acetylation capacity, have been detected<sup>5</sup>. NAT-2 are key enzymes in the conjugation of certain drugs, has an important role in the metabolism of several carcinogens<sup>2</sup>. Variations in NAT-2 gene among different populations could affect the metabolism and disposition of drugs<sup>4</sup>. In previous studies the polymorphisms of NAT-2 gene have been reported and revealed that the risk of cervical carcinoma was higher in individuals with NAT-2 mutant allele.

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**TABLE-1: Genotype distribution, allelic frequency and association analysis of NAT-2 gene polymorphism with risk of cervical cancer.**

Genotype/Allele		patients (n=75)	Control (n=75)	Odd Ratio (95% CI)	P- value
CC		37(49.33%)	22 (29.33%)	Ref	Ref
CT		21 (28%)	29(38.66%)	0.4306 (0.199- 0.93)	0.032*
TT		17 (22.66%)	24 (32%)	0.421 (0.186-0.951)	0.037*
Recessive model	TT	17	24	0.622 (0.301-1.287)	0.201
	CT+CC	58	51		
Dominant model	CT+TT	38	53	0.426 (0.217 - 0.83)	0.012*
	CC	37	22		
Co-Dominant model	CT	21	29	0.616 (0.31- 1.22)	0.167
	CC+TT	54	46		
Allele					
C		95 (63%)	73 (49%)	0.548 (0.346- 0.87)	0.01*
T		55 (37%)	77 (51%)		

OR: odds ratio, CI: confidence interval, n: number in sample.

Individuals with the NAT-2 slow genotype had a significantly higher risk of cervical cancer as compared with individuals with the NAT-2 fast genotype<sup>1</sup>. NAT-2 gene polymorphism is a genetic susceptibility factor involved in the carcinogenesis of cervical cancer<sup>2</sup>. A point mutation in the NAT-2 gene leads to the recessive trait for the slow acetylators phenotype<sup>10</sup>. Consuming red meat significantly increased colorectal cancer risk for group comprising all NAT-2 fast acetylators<sup>8</sup>.

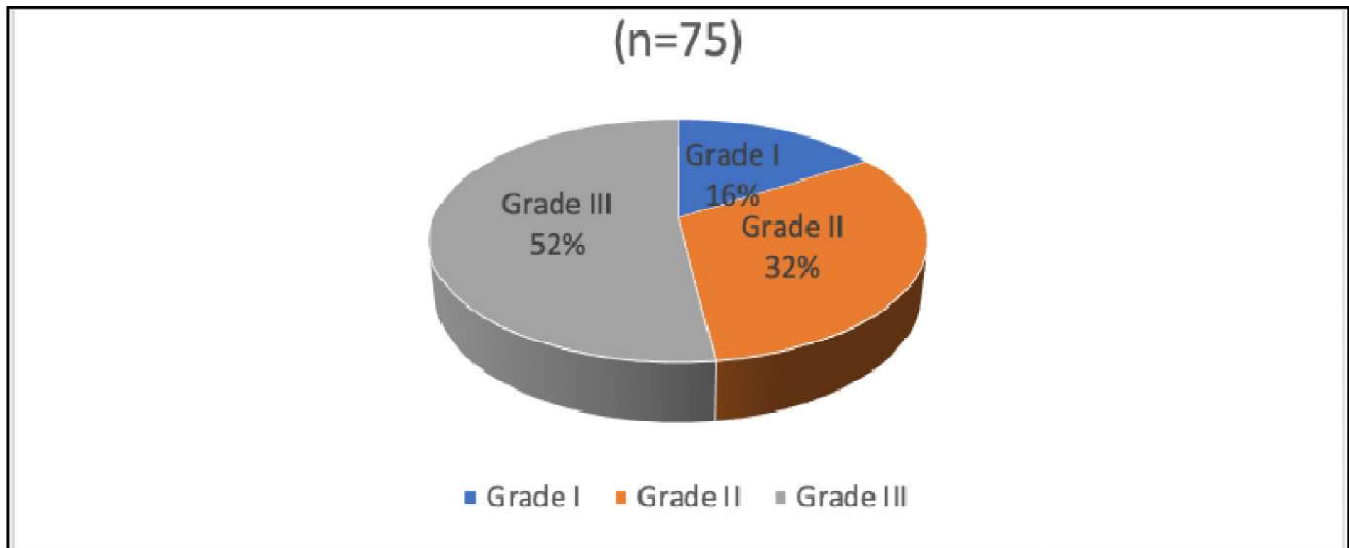
### Materials and Methods

In present study total of 75 patients of cervical cancer were included, those satisfied the selection criteria and a total 75 patients non-malignant lesions of cervical tissue were taken as control after obtaining the human ethical clearance from Institutional Ethical Committee (wide letter no. 116 / Ethical Committee/ S.C.-1/2018 Dated 10/01/2018 issued from Principal office,

Maharani Laxmi Bai Medical College, Jhansi). Inclusion criteria was that those patients those were diagnosed as squamous cell carcinoma, their blood samples were taken. Exclusion criteria were those patients with the history of prior radiation exposure to the site (prior radiotherapy) and history of chemotherapy. The written informed consent was collected from all participating subjects and patients. The relevant clinical history of all patients was collected and clinical history was used for the selection of appropriate patients as per exclusion/ inclusion criteria of the study.

### DNA Isolation and the Genotyping

The genomic DNA was isolated from blood samples of cervical cancer patients and normal control individuals by phenol-chloroform method. Genotyping of the SNPs in NAT-2 was performed by using the polymerase chain reaction-restriction fragment length



**Fig. 1: Distribution of cases (percentage) in different Grade (n = 75)**

polymorphism (PCR-RFLP) assay using a standard protocol.

### Statistical analysis

Chi-square test was applied for comparing genotype and allele frequencies for statistical significance between cervical cancer patients and controls. Observed and expected genotype frequencies of NAT-2 gene polymorphism in controls showed no deviation from Hardy-Weinberg equilibrium. Chi-square test showed that there was significant deviation from Hardy-Weinberg equilibrium for NAT-2 SNP genotypes ( $p=0.032$ ). Odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were determined to assess the strength of association of NAT-2 polymorphism with cervical cancer risk. Statistical significance was set at  $p < 0.05$ .

### Result

In present study a total number of 75 cases of cervical carcinoma were examined. All the cases fell in

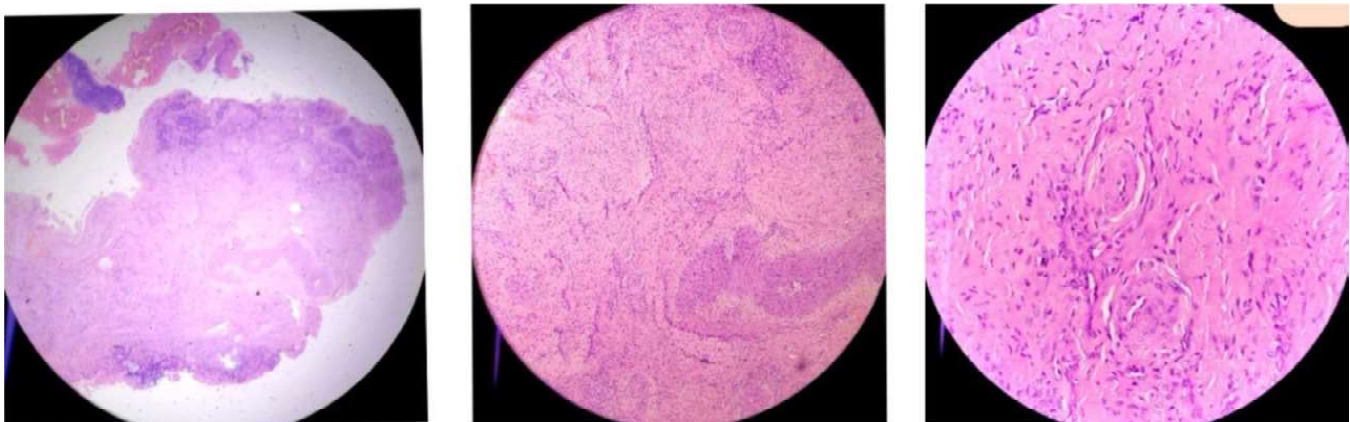
the age range between 18 to 70 years. The median age of cases in the study group was 48 years, while the mean age was 48.69 years.

### Histological grade-wise distribution of cases

All the 75 cases of cervical carcinoma in the study group were routinely processed. These cases were divided into three histological grades based on their morphological features (**Fig. 01**). The maximum number of cases fell into grade III (52%; 39/75, (**Fig. 04**) while 16% (12/75) and 32% (24/75) cases were classified into grade I (**Fig. 02**) and grade II (**Fig.03**) respectively.

### Polymorphism in NAT - 2:

For the detection of NAT-2 polymorphism (C481T, C>T), the primers (forward 5'-GGAACAAATTGGACTTGG-3' reverse 5'-GCAGAGTGATTCATGCTAGA-3') were used to amplify a 180 bp DNA fragment (**Fig.05**). Then, the PCR product was digested with 5 units of *TaqI* (NEB) overnight at



**Fig. 2: Well differentiated Squamous Cell Carcinoma (Grade I, HE stain 5X,10X,40X)**



**Fig.3: Moderately differentiated Squamous Cell Carcinoma (Grade II, HE stain 5X,10X,40X)**

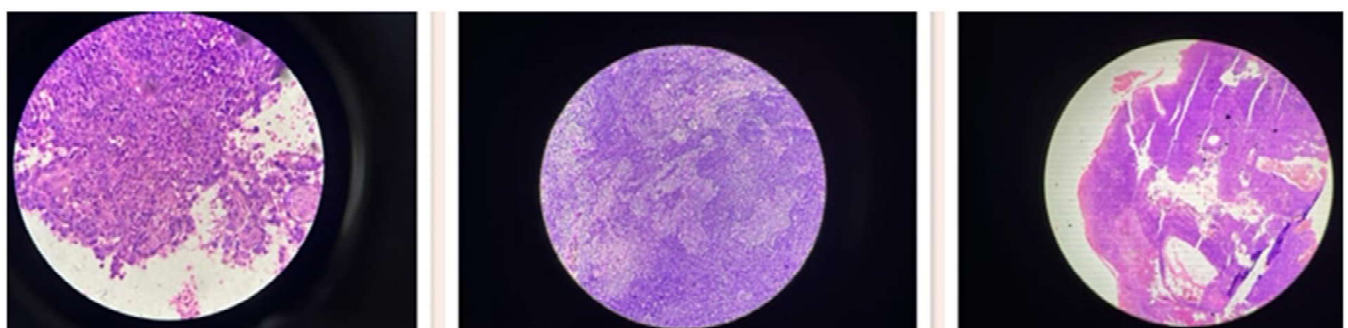
37°C. The wild-type allele (CC) produced one band (180 bp); wild-type/variant allele (CT) produced 106 bp, 74 bp and 180 bp and the variant allele (TT) produces two bands 106 bp and 74 bp bands (**Fig.06**). For *NAT-2 gene*, PCR conditions were initially denaturation at 96°C for 5 minutes followed by 35 cycles at temp. 96°C–for 45 sec., at 56°C for 45 sec., and at 72 °C for 30 seconds and a finally extension step at 72°C for 10 min.

**Association of NAT-2 gene polymorphism with cervical cancer patients:** Since in homozygous wild type CC and alleles frequencies of NAT-2 genes show mutation (SNP) as allele frequencies of CC, CT and TT genotype were resulting in higher occurrence in cervical cancer patients from 49.33%, 28% and 22.66% respectively in patients and 29.33%, 38.66% and 32% in control group respectively (Table 01). The statistical analysis of observed genotypic frequencies show significant association ( $p=0.032$ ). Likewise there was significant difference in allele frequencies between patients and control (OR= 0.548; 95% CI: 0.346-0.87;  $p=0.01$ ). We observed significant association between allele C polymorphism and cervical cancer risk under the dominant (OR=0.426; 95% CI: 0.217-0.83;  $p=0.012$ ). Whereas no significant relationship was found in recessive model (OR=0.622; 95% CI: 0.301–1.287;  $p=0.201$ ) and co-dominant model (OR=0.616; 95% CI: 0.31- 1.22;  $p=0.167$ ). OR: odds ratio, CI: confidence interval, n: number in sample (Table 01).

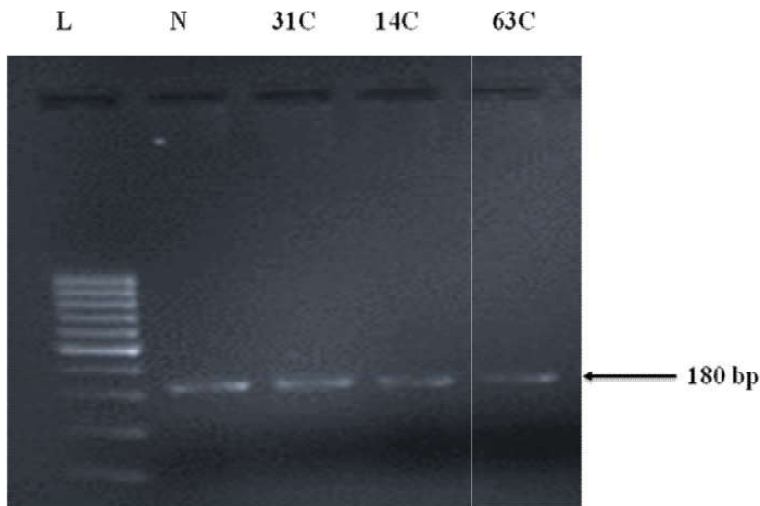
### Discussion

The present study concludes that NAT-2 gene

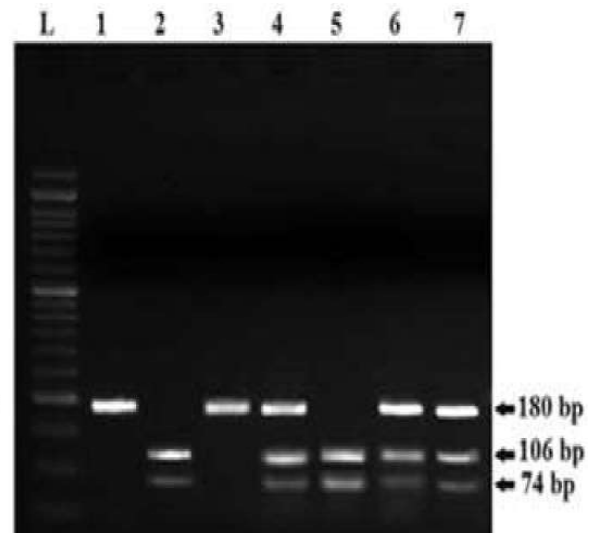
polymorphism were significantly associated with cervical cancer risk as also reported in earlier study performed by different groups. It was found that cases, control and final  $p$  value at confidence interval CI with sample number ( $n=75$ ) showed the different odd ratio revealing that the correlation exists between mutation (SNP) and the number of cervical cancer incidence. The comparative studies coping with different factors have shown satisfactory upto the *threshold* limit in accordance to the  $p$  value. In cancer, expression of protein and functions are found to be influenced by different mutations. Genotypic alterations in NAT-2 gene may play important role in cervical cancer initiation and progression as this contains series of target genes involving various cancer suppressor genes and oncogenes. Damaged cells consisting deregulatory growth mechanism undergone apoptosis<sup>7,9</sup>. Apoptosis process plays a very crucial role in carcinogenesis<sup>12</sup>. Over expression of certain genes is associated with different kinds of cancer development<sup>3</sup>. Based on these result & it is proposed that each population need to evaluate its own genetic profile for cervical cancer risk that may be more helpful for better understanding the racial and geographic differences, as reported earlier for cervical cancer prevalence and death. Further molecular study, might be helpful in development of screening, diagnostic methods for prevention and screening of cervical cancer. However many recent researches do not find any correlation between the gene polymorphism and cancer<sup>11</sup>.



**Fig. 4: Poorly differentiated Squamous Cell Carcinoma (Grade III, HE stain 5X,10X,40X)**



**Fig 5: Amplified NAT-2 Gene in normal (N) and cancer patient (C).**



**Fig 6: Gel picture of PCR-RFLP of NAT-2 Gene amplified fragment.**

### Conclusion

The results of present study suggest that NAT-2 gene polymorphism is significantly associated with high risk patients of cervical cancer. The findings of this study reveal the role of PCR based genotyping methods in predicting the occurrence of cervical cancer as well as defining high risk persons.

**Human Ethical Approval:** Institutional Ethical Committee (wide letter no. 116 / Ethical Committee/ S.C.-1/2018 Dated 10/01/2018 issued from Principal office, MLB Medical College, Jhansi) approved the present study.

**Conflicts of Interest:** Authors have no conflicts of interest.

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