

Role of NAT-1 gene polymorphism in the etiology and progression of cervical cancer

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ABSTRACT

Cervical cancer (or carcinoma of cervix) incidences show higher occurrence rate in women population residing in poor socio-economic and unhygienic conditions especially in rural areas. However implication of various effective screening and health programmes in western countries, the incidence of invasive cervical cancer has declined greatly. In spite of that, still today cervical cancer remains the most common cancer in women, next to breast and lung cancer, therefore a leading cause of death in India. Gene polymorphisms modify risk of developing cancer like urinary bladder, colorectal, breast cancer etc. The effect of NAT-1 gene polymorphism on cancer risk varies with organ involved, reflecting tissue specific expression of these genes. Strong associations exist with cause and increased risk for development of cervical cancer in individuals carrying specific alleles among the population.

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KEY WORDS : Acetylators, Cervical cancer, HPV, Pap (papanicolaou) smear.

Introduction

Cervix cancer is the most prevalent in women which affect's over 25,00000 lives and annually 5,00000 newly cases are diagnosed in the world. It is nearly about 80% of cases occurred in low-developing countries. In 99 % of diagnosed cases Human papilloma virus (HPV) is the most prevalent viral infection of the reproductive system, which has been related to nearly all cases of cervical cancer. Cervical cancer is associated with nearly 5% of the death among women suffering from cervical cancer⁵. Cervical cancer is the most prevalent cancer among Indian women with the age group of 15 to 44 years. In recent past year approximately 132,000 women in India are diagnosed with cervical cancer each year, and out of them nearly 74,000 die. However, implementation of recently diagnostic techniques in histological examination of biopsy and Pap (papanicolaou) smear screening programmes

conducted at larger scale, have greatly decreased the mortality of cervical cancer patients.

Although Pap smear test is not highly sensitive due to its certain limitation. This restricts the present screening attempts that require further diagnostic confirmation using biomarkers. Cervical cancer and invasive cervical neoplasia are known to be largely induced by simultaneous infection of HPV¹. Based on their correlation with invasive cervical cancer, about two third of HPV strains are associated with cervical neoplasia, which have been categorized into high- and low-risk groups¹³. Symptoms of cervical cancer have been reported as blood stained discharge per vaginum, post-coital bleeding and low backache¹¹. The associated risk factors are like women having multiple sexual partners, high-risk male partner who previously had a spouse who had cervical cancer and persistent HPV infection⁴, lower socio-economic status, multiple

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pregnancies, promiscuity, exposure to carcinogens, smoking, OCP usage and immuno deficiency make lesions more likely to progress. While a negligible rate among nuns and virgins are reported⁴.

In humans, NAT-1 gene is responsible for **N-acetyltransferase** activity. Several allelic variants of NAT-1 gene which cause variations in acetylation capacity, have been detected¹⁰. O-acetylation of the N-hydroxyarylamine yields an acetoxy arylamine derivative which breaks down spontaneously to a highly reactive **arylnitrenium ion**, the ultimate metabolite is responsible for mutagenic and carcinogenic lesions⁸. Variations in the N-acetyltransferase (NAT-1) gene among different populations could affect the metabolism and disposition of drugs⁹. Therefore, it is required to investigate the role of NAT-1 genotypes and/or phenotypes together with other genetic susceptibility factors, biomarkers and carcinogen exposure to understand the role of NAT-1 acetylation polymorphisms in cancer risk⁷. Such analysis of the allelic profile of populations in different geographic locations may help to understand the incidence of cervical cancer in India. Hence, present study was conducted for the analysis of allelic profile of populations that will help to understand the incidence of cervical cancer much better.

Materials and Methods

In present study, a total of 75 cases of cervical cancer were included, those satisfied the selection criteria and a total 75 non-malignant lesions cases of cervix tissue were taken as control after obtaining the 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 cases which 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/ Patients. The relevant clinical history of all cases was collected and clinical history was used for the selection of appropriate cases as per exclusion/ inclusion criteria of the study.

DNA Isolation and the Genotyping

The genomic DNA was isolated from blood sample of cervical cancer cases and normal control person by phenol-chloroform method. Genotyping of the SNPs in NAT-1 was performed by using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. PCR reactions were performed in a 25 µl reaction mixture containing 1 µl genomic DNA,

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10X PCR buffer 2.5 µl, 2.5 µl dNTP, 0.5 µl of each primer, and 1 µl Taq DNA polymerase. For the detection of NAT - 1 polymorphism (C559T, C>T), the primers (forward 5'- TCAGGTGCCTTGTGTCTTCCGT-3' reverse 5'-TAGTAAATCATTTTGTTCCT-3') were used to amplify a 330 bp DNA fragment. Then PCR product was digested with 5 units of *TaqI* (NEB) overnight at 37°C. The wild-type allele (CC) produced one band (330 bp); wild-type/ variant allele (CT) produced 182 bp, 148 bp and 330 bp and the variant allele (TT) produced two bands 182 bp and 148 bp bands. For NAT-1, PCR conditions include initial denaturation at 96°C for 5 min followed by 35 cycles at 96°C—for 45 seconds, at 56°C for 45 seconds, and at 72° C for 30s and a final extension step at 72°C for 10 minutes. After PCR reaction, 10 µl of each PCR product was digested with restriction enzymes at 37°C for overnight. The digested PCR products were visualized on a 2% agarose gel electrophoresis using ethidium bromide.

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-1 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-1 SNP genotypes ($p = 0.027$). Odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were determined to assess the strength of association of NAT-1 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 maximum number of cervical cancer cases *i.e.* 49.33% (37/75) were in the age group of 45-60 years whereas 20% (15/75) of cases were < 45 years of age and 30.67% (23/75) cases were > 60 years of age. The median age of cases in the study group was 48 years, while the mean age was 48.69 years (Figs. 1 & 2).

(I) Association of NAT-1 gene polymorphism with cervical cancer cases:

Since in homozygous wild type CC and alleles frequencies of NAT-1 gene show mutation (SNP) as allele frequencies of CC,CT and TT genotype were resulting in higher occurrence in cervical cancer cases from 33.33% , 41.33% and 25.33% respectively in cases and 16%, 50.66% and 33.33% in control group respectively. The statistical analysis of observed

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

Genotype/Allele		Cases (n=75)	Control (n=75)	Odd Ratio (95% CI)	p value
CC		25(33.33%)	12 (16%)	Ref	Ref
CT		31 (41.33%)	38(50.66%)	0.391 (0.169- 0.903)	0.027*
TT		19 (25.33%)	25 (33.33%)	0.364 (0.146- 0.907)	0.03*
Recessive model	TT	19	25	0.678 (0.334 - 1.37)	0.283
	CT+CC	56	50		
Dominant model	CT+TT	50	63	0.381 (0.174-0.832)	0.015*
	CC	25	12		
Co-Dominant model	CT	31	38	0.686 (0.359- 1.30)	0.252
	CC+TT	44	37		
Allele					
C	81 (54%)	62 (41%)	0.6 (0.38- 0.94)	0.028*	
T	69 (46%)	88 (59%)			

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

genotypic frequencies show significant association ($p=0.027$). Likewise there was significant difference in allele frequencies between cases and control (OR= 0.6; 95% CI: 0.38-0.94; $p=0.028$). We observed significant association between allele C polymorphism and cervical cancer risk under the dominant (OR =0.381; 95% CI: 0.174-0.832; $p=0.015$). No significant relationship was found as under tabulated recessive model (OR=0.678; 95% CI: 0.334–1.37; $p=0.283$) and co-dominant model (OR=0.686; 95% CI: 0.359- 1.30; $p=0.252$).

Discussion

In present scenario cervical cancer is known as one of the most preventable and treatable forms of cancer in wide arena at global level. The present studies have to meet the following criteria including NAT-1 polymorphisms and cancer risk case-control studies

available genotype frequency for computing odds ratios (ORs) with 95% confidence intervals (CIs), case population, outcome comparison and other important parameters. Abnormal and redundant cells are eliminated by process of apoptosis³. This process of apoptosis includes mitochondrial and death-receptor pathway. These both are propagated *via* a cas-pase cascade which leads to activation of apoptosis process^{12,14}. During carcinogenesis, apoptosis is evaded by different mechanisms: caspase activity failure, improper death receptors signaling, disbalance between anti apoptotic and proapoptotic proteins present^{5,17}.

The result shows that there is correlation between higher incidence of mutation and cervical cancer patients. In respect to p values mentioned in Table-1 show the recessive model 0.283, Dominant model 0.015 while Co- dominant model show 0.252 with Odd Ratio

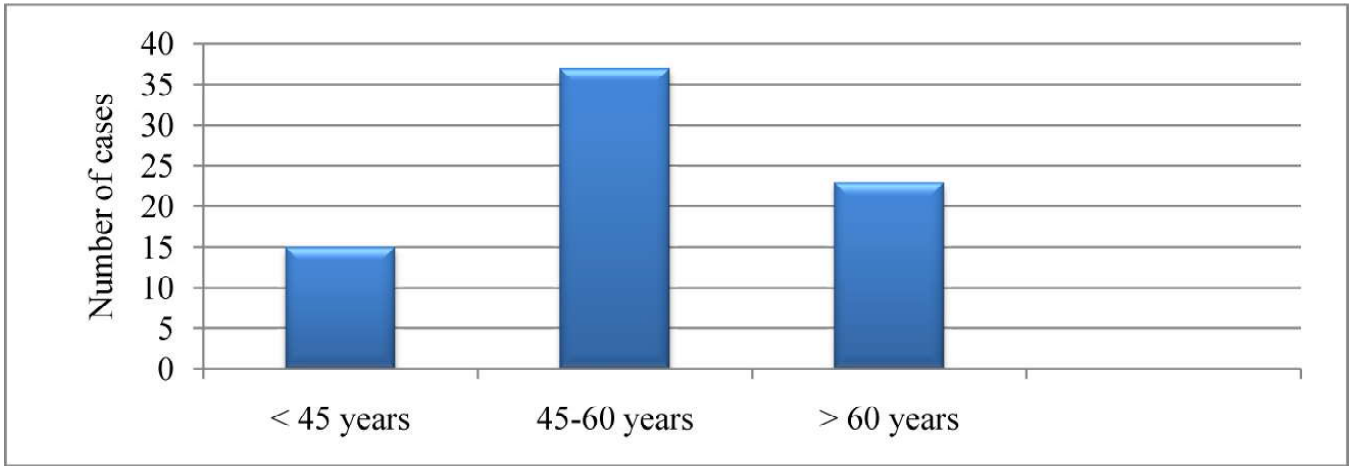


Fig. 1 : Depicting different age of patients under study.

of 95% CI. While allele frequency with C and T separately show comparatively case 54% and 46 %; control 41%, 59% and *p* value 0.028. Finally 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 exist 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-1 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. Further advance study, along with findings of present study, might be useful in development of better technique for prevention, screening, diagnosis of cancer

of cervix.

Conclusion

Finally, the present study concludes that NAT-1 gene polymorphism were significantly associated with cervical cancer risk. It has been revealing significant association of NAT-1 gene polymorphism with high risk of cervical cancer in Indian population. The genetic risk factors identification can be useful in predicting the occurrence of cervical cancer and defining high risk persons. Therefore we have proposed that each particular population is required to evaluate its own genetic profile for cervical cancer risk that may be helpful for better understanding of racial and geographic differences reported for cervical cancer prevalence and death. The findings will be useful for future prospects in health improvement at each level by prevention and final mitigation in short time.

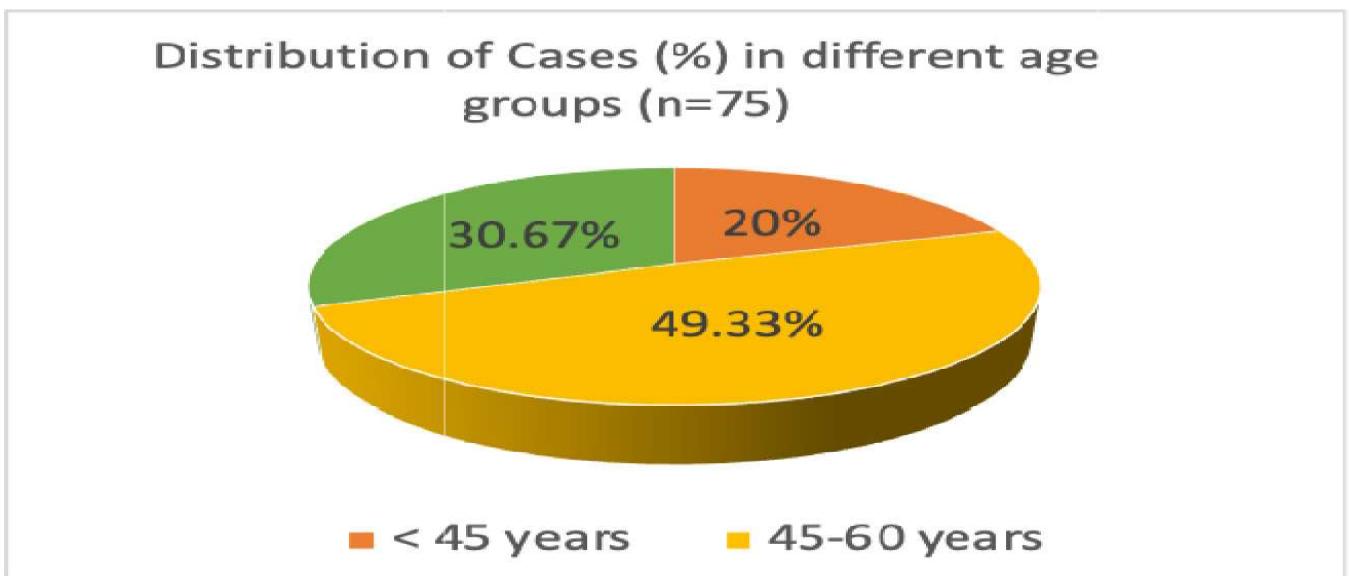


Fig. 2: Distribution of cases (%) in different age group (n=75)

Data Availability: The various pretexts Performa used and/or analyzed during the present study are available with the corresponding author.

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.

Abbreviations:

HPV	: Human papilloma virus
NAT-1	: N-acetyltransferase-1 gene
PCR-RFLP	: Polymerase chain reaction-restriction fragment length polymorphism
OR	: Odds ratio
CI	: Confidence interval
SNP	: Single nucleotide polymorphism

References

1. Agoff SN, Lin P, Morihara J, Mao C, Kiviat NB, Koutsky LA. p16(INK4a) expression correlates with degree of cervical neoplasia: a comparison with Ki-67 expression and detection of high-risk HPV types. *Mod Pathol*. 2003 Jul; **16**(7) : 665-73. *Mod Path*. 2003; **16**(7) : 665-673.
2. Bhatt Deepti, Verma Amit Kumar; Bharti, Prahalad Singh; "BCL-2 (-938C>A), BAX (-248G>A), and HER2 Ile655Val Polymorphisms and Breast Cancer Risk in Indian Population." *J Oncol* ; 2021: 8865624, 2021.
3. Bhushann P, Meka S, Jarjapu, SK Vishwakarma et al, "Influence of BCL2-938 C>A promoter polymorphism and BCL2 gene expression on the progression of breast cancer," *Tumor Biology*. 2016; **37** (5) : 6905–6912.
4. Costa S, Medeiros R, Vasconcelos A, Pinto D, Lopes C. A slow acetylator genotype associated with an increased risk of advanced cervical cancer. *J Cancer Res Clin Oncol*. 2002 Dec; **128**(12):678-82. Epub 2002 Nov 12.
5. Danial NN, Korsmeyer SJ. "Cell death," *Cell*. 2004; **116** (2) : 205–219.
6. Globocan. International Agency for Research on Cancer (IARC), WHO. 2012.
7. Hein DW, Doll MA et.al. Molecular genetics and epidemiology of the NAT1 and NAT2 acetylation polymorphisms. *Cancer Epidemiol Biomarkers Prev*. 2000 Jan; **9**(1) : 29-42.
8. Hein DW, Rustan TD, Doll MA et. Al. Acetyltransferases and susceptibility to chemicals. *Toxicol Lett*. 1992 Dec; 64-65 Spec No:123-30.
9. Hemanth Kumar AK, Ramesh K, Kannam T, Sudha V, Harsbabu Hemlatha, Lavanya J, Swaminathan Seumya, Rama chandran Geeta. N-acetyltransferase gene polymorphisms & plasma isoniazid concentrations in patients with tuberculosis. *Indian J Med Res*. 2017; **145**(1) : 118-123. doi: 10.4103/ijmr.IJMR_2013_15.
10. Hirvonen A. Polymorphic NATs and cancer predisposition. *IARC Sci Publ*. 1999; **148** : 251-70.
11. Jeffcoate SN. Tumour of corpus uteri; Jeffcoate's principles of Gynaecology 5th edition. 1987; pp. 395-416.
12. Kerr JFR, Wyllie AH, Currie AR. "Apoptosis: a basic biological phenomenon with wide ranging implications in tissue kinetics," *British Journal of Cancer*. 1972; **26** (4) : 239–257.
13. Lorincz AT, Reid R, Jenson AB, Greenberg MD, Lancaster W, Kurman RJ et al. Human Papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. *Obster Gynecol*. 1992; **79** : 328-37.
14. Majno G, Joris I. "Apoptosis, oncosis, and necrosis. An overview of cell death," *American Journal of Pathology*. 1995; **146** (1) : 3–15.
15. O'Brien MA, Kirby R. "Apoptosis: a review of proapoptotic and anti-apoptotic pathways and dysregulation in disease," *Journal of Veterinary Emergency and Critical Care*. 2008; **18** (6) : 572–585.
16. Schneider P, Tschopp J. "Apoptosis induced by death receptors," in *Pharmacochimistry Library*, U. Gulini, M. Gianella, W. Quaglia, and G. Marucci, Eds. 2000; **31** : 281–286, Elsevier, Amsterdam, Netherlands.
17. Wong RS. "Apoptosis in cancer: from pathogenesis to treatment," *Journal of Experimental & Clinical Cancer Research*. 2011 : **30**(1) : 87.