

PREVALENCE OF HIGH RISK HPV TYPES IN CERVICAL CANCER PATIENTS OF EASTERN UTTAR PRADESH, INDIA

**Mitali Das¹ Shyam Babu Prasad¹ Suresh Singh Yadav¹ Satyajit Pradhan²
Gopeshwar Narayan¹ Sunita Singh^{3*}**

¹Cancer Genetics Laboratory, Department of Molecular and Human Genetics, Banaras Hindu University,
Varanasi, Uttar Pradesh, India 221005

²Department of Radiotherapy & Radiation Medicine, Banaras Hindu University, Varanasi, Uttar Pradesh,
India 221005

³Department of Zoology, Mahila Mahavidyalaya, Banaras Hindu University, Varanasi, Uttar Pradesh,
India 221005

*Correspondence author; e-mail: sunitas@bhu.ac.in

Abstract:

Cervical cancer has taken a devastating form in India threatening women lives alarmingly. It is the second most common cancer among women in this country. The major risk factor is HPV infection. The only available preventive measure, HPV vaccination, is still not widely applied throughout the country. Before applying preventive measures en masse in India, region wise repository of information particularly about prevalent HPV types should be generated. This is an informative study describing HPV prevalence in cervical cancer patients of eastern Uttar Pradesh, not yet been explored. We observed that 97.5% of cervical cancer cases are infected with HPVs. Among these, the most prevalent type is HPV16 followed by HPV18 affecting 77.8% and 23.5% cases respectively. Interestingly, a small fraction of patients have also been detected to be HPV negative. Overall, this study suggests HPV16 and HPV18 to be most potential prophylactic targets in this region.

Keywords: Cervical cancer, Indian women, HPV genotyping, HPV16, HPV18.

Introduction

Cancer of the uterine cervix, commonly called cervical cancer, is a gynecological cancer considered to be the fourth leading cause of women death worldwide (1). The cancer initiates on the squamocolumnar junction between the columnar epithelium of the endocervix and the squamous epithelium of the ectocervix, a region that face continuous metaplastic changes throughout women's lifecycle (2). This cancer is unique since the malignant transformation is caused by the infection of Human Papillomavirus (HPV) (3). An HPV infection remains inert at its initial phase and takes 10-20 years to develop cancer (2). Persistent HPV infection is essential but not sufficient for development of cervical cancer. Several cofactors and molecular events are involved in the progression of the disease. Worldwide 528,000 new incidents and 266,000 deaths were reported in 2012. In India, 123,000 new cases and 67,000 deaths were reported in the same year. Thus, India accounts for 23.3% of all new cases diagnosed and about 25% of deaths occurring due to cervical

cancer worldwide. This is almost the one fourth of world cervical cancer burden shared by India. Though, cervical cancer is placed as fourth most common cancer amongst women worldwide, in India, it is still the second most common cancer amongst women accounting for 22.9% of all cancers affecting females following breast cancer (27%). Cervical cancer diagnosis rate in India is highest amongst the age group of 55-59 years (78.6 per 100,000 women), whereas the rate is 41.9 per 100,000 women at the age group of 40-44 years. The incidence rate in United States of America is 14.8 per 100,000 women at the age of 40-44 years and 12.7 per 100,000 women at the age of 55-59 years. Compared with the developed nations, cervical cancer is far more alarming in India. Several socio-economic factors such as lower age of marriage, lack of personal hygiene, multiple sex partners and overall unawareness to gynecological health may be the common reasons of the high incidence and mortality rate in India. Higher incidence rate at older age also indicates the lack of screening (4). In spite of being curable, cervical cancer has taken a devastating form in India.

Cervical cancer has an infectious etiology as the main causative agent is Human Papillomavirus (HPV) (3). HPV is a DNA virus containing a 6800-8000 bp genome encoding eight genes performing early (E) and late (L) functioning in the life cycle. Proteins encoded by early genes E6 and E7 have oncogenic properties required for malignant transformation of cervical squamous epithelial cells (5).

There are at least 150 different genotypes of HPV screened and sequenced till date (6). According to their transforming capacities, they are subdivided in two categories the high risk and low risk HPV types. HPVs causing malignant transformation are labeled as high risk types and those causing benign transformation included in the low risk group. Fifteen high risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82) are directly associated with human cervical and other carcinomas. The most prevalent low risk HPV types 6 and 11 are associated with benign genital lesions and warts (6, 7). High risk HPV types 16/18 are most prevalent throughout the world, accounting for almost 70% of all cervical cancer cases. (7, 8). In India, HPV16 infection is most prevalent in cervical tumors followed by HPV18. HPV16 infection has been found in more than 90% cervical cancer patients from different parts of India and HPV18 in 3-20% cases along with other HPV infections (8-11).

Materials and methods:

Study cohort:

A total of 156 cervical cancer patients were screened for HPV types. Cervical primary tumor biopsy samples were collected from these patients diagnosed with cervical cancer and referred to Department of Radiotherapy & Radiation Medicine. All biopsies were obtained after appropriate informed written consent of the subjects and approval of the institutional review board/ethical committee of the Institute of Medical Sciences, Banaras Hindu University. Biopsy tissues, collected in cryovials were immediately kept and transported on ice and stored at -70°C for further use. The patients belonged to eastern Uttar Pradesh region and mostly from lower socio-economic background. Patients were clinically tested for tumor histology and tumor stage was determined by The International Federation of Gynecology and Obstetrics (FIGO) system.

HPV DNA amplification by PCR:

DNA from biopsy samples was extracted with standard Phenol-Chloroform-Isoamylalcohol method. High quality DNA samples were first subjected to PCR amplification for a house keeping gene, GAPDH, as an internal control to check the integrity and adequacy of the DNA. Overall HPV detection was done by amplification of MY09/11 primers (12) for L1 consensus region. Type specific primers for HPV16 and HPV18 (13) were used to detect these two high risk HPVs. Each $15\mu\text{L}$ PCR reaction mixture contains $7.5\mu\text{L}$ of $2\times$ PCR Master Mix (Thermo Scientific, USA), 3.5 mM MgCl_2 , 0.6 pmol of forward (F) and reverse (R) primers and 50ng of DNA template. The annealing temperatures for each primer set was determined by optimization through different temperature gradients using DNA sample isolated from HPV16 and HPV18 positive cervical cancer cell line CaSki (10). A template negative control was kept for each set of PCR reaction to confirm the exclusion of any kind of microbial contamination in reagents. The list of primers used is given in Table 1. All the PCR reactions were performed in thermal cycler (Applied Biosystems, USA). The PCR products were visualized and imaged by gel documentation system (Alfa Imager, USA) after 2% agarose gel electrophoresis. The samples positive for L1 consensus but amplify neither HPV16 nor HPV 18 are considered to be infected with other HPV types that we did not include in our study. The samples positive for internal control but negative for L1, HPV16 and HPV18 were considered to be HPV negative.

Results:

HPV-typing (genotyping for HPVs) showed 55.6% samples positive for exclusively HPV16, 22.2% samples were positive for both HPV16 and HPV18,

and exclusive HPV18 infection was found in 1.3% samples. Thus, HPV16 accounts for 77.8% and HPV18 accounts for 23.5% of total infection of the study population. The samples positive for L1 consensus sequence but negative for both HPV16 and HPV18 are assumed to be infected with other high risk HPV types. 15.7% samples were found to be infected with other HPV types. 5.2% samples did not amplify any of the three HPV primer sets indicating that these tumors were independent of HPV infection (Figure 1). Thus in this study population, 94.8% cases are HPV positive and HPV16 and HPV18 together account for 79.1% cases.

Discussion:

Since cervical cancer is caused by an infectious agent, it is curable. HPV vaccine is an obvious means to eradicate cervical cancer. In India, HPV vaccination is still not widely used and also its administration is controversial. Hence in the present context, cervical cancer screening is indispensable in India (14). HPV DNA detection may also be an early detection tool for cervical cancer (15). Besides this, in such a socio-culturally distinct country like India, a region based repository of information about prevalent HPV types associated with cervical cancer will be helpful in taking any further measure(s) for cervical cancer control.

HPV prevalence in cervical cancer patients had been reported from different parts of India. In a study cohort of 41 cervical cancer patients of Andhra Pradesh, 87.8% specimen was associated with high risk HPVs. Among them, 66.7% patients were HPV16 positive and 19.4% samples were HPV18 positive (16). In North Indian population, 74.3% of high grade squamous intraepithelial lesions are HPV16 positive and 7.4% of the same grade was HPV 18 positive (17). In Karnataka, 96.7% cervical cancers were found to be HPV positive. HPV 16 accounts for 89.7% cases (18). In Tamil Nadu, HPV16 and HPV18 infect 57% and 18% of cervical cancer respectively (19). In rural western India, 86.5% cervical cancer cases were found to be infected by HPV16 and HPV18 (20). In a study population of North Bengal, it had been shown that 97.5% cervical cancer cases were positive for HPVs. HPV16 accounts for 82.5% cases and HPV18 for 17.95% cases (21). In a multi-centre study, Basu *et al.* (22) had shown that throughout India, HPV16 and HPV18 infection prevail in 76.3% cervical cancer patients and they found no significant regional changes on this account. According to ICO Information on HPV and Cancer 2014, 68.9% cervical cancer cases in India are infected with HPV16 and 13.8% with HPV18.

On the other hand, a worldwide analysis showed that 94.1% of cervical cancer cases were HPV positive, 50.9% among them were HPV16 positive and 31.6% were HPV18 positive (23). According to ICO Information on HPV and Cancer 2014 (24), 70% cervical cancer cases throughout the world are HPV positive, 55.4% cases are HPV16 positive and 14.6% are HPV18 positive.

Our study is a case-only type study and has shown that in eastern Uttar Pradesh 94.8% of cervical cancer cases are HPV positive and 77.8% cases are HPV16 positive. The percentages are lying within the range of varieties of numbers reported from different parts of India and concordant with HPV16 prevalence worldwide. 23.5% positivity for HPV18 in this region is marginally higher than the reported ones. However, the results indicate that available vaccines against high risk HPV types 16 and 18 can be applied to reduce more than 79% of cervical cancer burden particularly in this region of India.

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Table 1: List of primers used for the study.

Oligonucleotide Name	Sequence (5' - 3')	Amplicon Size (bp)
MY09/11	F- GCM CAG GGW CAT AAY AAT GG	450
	R-CGT CCM AAR GGA WAC TGA TC	
HPV16	F-AAG GCC AAC TAA ATG TCA C	217
	R-CTG CTT TTA TAC TAA CCG G	
HPV18	F-ACC TTA ATG AAA AAC CAC GA	100
	R-CGT CGT TTA GAG TCG TTC CTG	
GAPDH	F-ACT CCT TTT GCA GAC CAC AG	249
	R-TTG GCA GGT TTT TCT AGA CG	

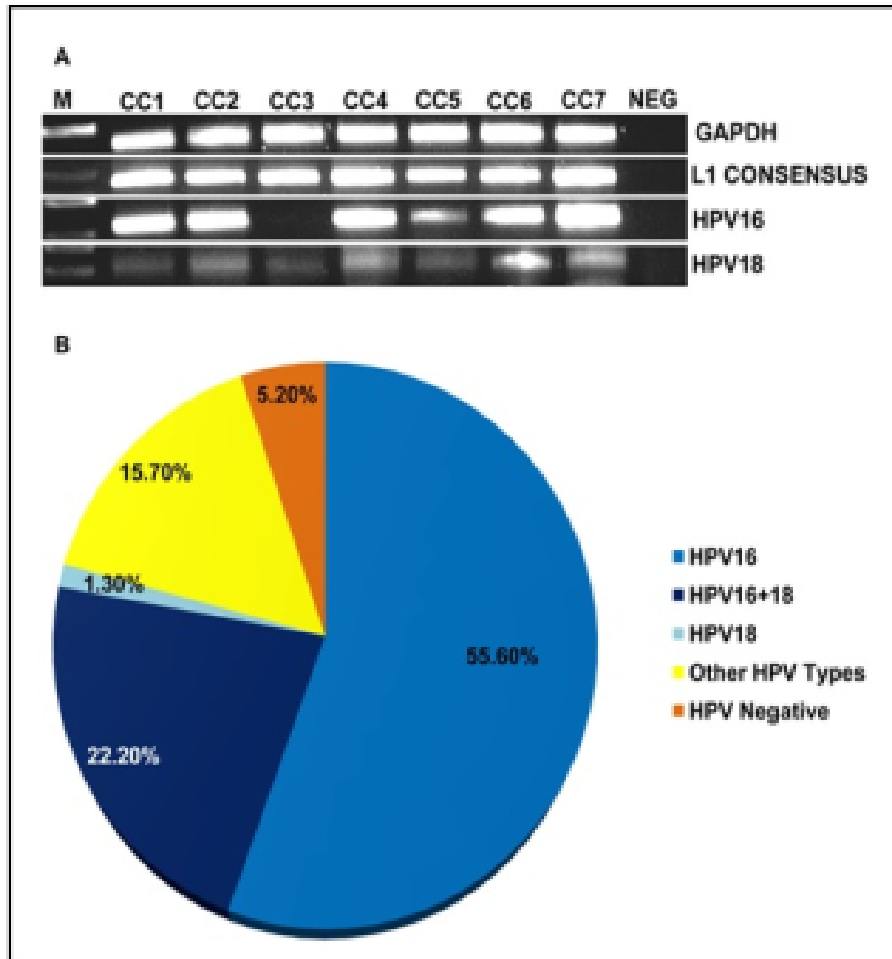
Figure 1

Figure 1: HPV genotyping. A) Electrophoregram showing HPV gene expression in cervical cancer samples; CC = cervical cancer sample; M = DNA ladder; NEG = negative control. B) Pie chart showing the frequency of HPV types in the study population.

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