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Incidence of HPV 16 gene among cervix cancer patients of Kanpur – A cross-sectional study

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Abstract--Cervix cancer is the common cancer type among women in India, the present study was aimed to demonstrate the incidence of HPV 16 in cervix cancer women of Kanpur which will be helpful for implementing screening programs, vaccinations as well as prognosis of the treatments. The study was conducted in 300 cervix cancer confirmed cases of Kanpur. The diagnosis was confirmed with clinical presentation and cytological examinations. The HPV 16 was confirmed with the real time polymerase chain reaction method. The incidence of HPV 16 was correlated with cytological features. Spearman's rank correlation test was used for correlating the findings. The data was expressed in frequency and percentage. Majority of study population were in age group of 41-60years. The incidence of HPV 16 was 34% among cervix cancer patients. The cervix cytological features like inflammatory smear, atrophic smear and intraepithelial lesion and malignancies positively correlated with HPV 16 incidence. The conclusions of the present study may be helpful for planning the screening methods and also for implementing the HPV vaccination programs in Kanpur.

Keywords---HPV 16 gene; cervix cancer; correlation; cervix cytology; PCR.

1 Introduction

Cervical cancer is the fourth most common malignancy worldwide. Every year, 1.3 million Indian women are diagnosed with cervix cancer, which leads to 67,477 fatalities. In India, 432.2 million females aged 15 and up are thought to be at risk for this disease. It is the 2nd most common malignancy in women aged 15 to 44 (Ghanta, Gursale, Burte, & Bhaskar, 2021; Pravallika et al., 2022). Human papillomavirus (HPV) infection is thought to be responsible for 99.7 per cent of all cervix cancer cases. There are many HPV genotypes that invade mucosa and skin epithelia, all of which have certain genetic characteristics, including a helical DNA genome. The most prevalent strain is HPV 16 (Sample, 2020).

Furthermore, cytology has been the accurate approach for predicting Low-grade squamous intraepithelial lesion (LSIL) development. Normal cytology/negative for intraepithelial lesion or malignancy (NILM) or mild cytological anomalies, such as the atypical squamous epithelium abnormalities of unknown aetiology, have a five-year progression rate, whereas more severe cytological anomalies, such as atypical squamous/glandular cells, have accelerated progression. Conversely, LSIL with NILM or somewhat aberrant cytology showed a 10 percent progression rate (Quint, de Koning, Quint, & Pirog, 2013). This variability in progression rate frequently confounds clinicians in the detection of progressing LSIL (Huang et al., 2016; Liao et al., 2014; Mills, Paquette, Castle, & Stoler, 2015). Regardless of cytological evidence, HPV 16 gene positive has been shown to increase the risk of advancement. HPV 16 genotyping has become a sophisticated technique in terms of detection (Burd, 2003). However, there is a prospective study looking into the role of HPV 16 gene positive in the progression of LSIL (McGarry & Kirschner, 1998).

Our study aims to evaluate the incidence of HPV 16 gene among cervix cancer patients of Kanpur. This study also correlates the HPV 16 gene incidence with cervical cytological features of cervix cancer patients as a secondary objective.

2 Materials and Methods

Study setting and data collection

This cross-sectional study was conducted by department of Anatomy in collaboration with department of Obstetrics and Gynaecology at a tertiary hospital in Kanpur during 2016 December to 2020 January, including 300 confirmed cervix cancer patients. Patients with a confirmed diagnosis of cervix cancer aged 20 to 60 years have been included in the study, whereas those with a history of fibroid illness present with cervical growth and significantly distorted cervix were excluded.

With 16.9% as the prevalence of HPV-infections, a 95 percent confidence interval, and a 5% margin of error, the sample size needed was 216 for the Kanpur female population of 2121000. The study comprised 300 patients, taking into account the loss of follow-up (Franceschi et al., 2005).

Sample collection

The gynaecologist or a skilled nurse were involved to harvest cervix cells from squamo-columnar confluence following a standard method, after receiving informed consent from the patients. The samples were treated with 95 percent ethanol and air-dried after a clear Papanicolaou (Pap) film was made on a sterilized microscope slide. The smears have been analysed and inferred following Bethesda 2001 system (Apgar, Zoschnick, & Wright, 2003).

HPV 16 gene evaluation using PCR techniques was done following previous studies (Rajesh, Mohiyuddin, Kutty, & Balakrishna, 2017; Senapati, Nayak, Kar, & Dwibedi, 2017; Del Prete et al., 2019). The QIA amp DNA Blood Mini Kit (50) (Qiagen, India) was used to extract DNA from samples according to the kit specifications. To check for sample quality, the human beta-globin gene was amplified. Multiplex real-time PCR (mRT-PCR) was run on isolated DNA samples using a CFX Real-Time PCR machine (Bio-Rad, USA).

The methodologies used were compliant with relevant committees on human experimentation's (national and institutional) ethical norms, as well as the Helsinki Declaration-1975, which was amended in 2013.

3 Results and Discussion

Cervix carcinoma is the fourth most prevalent cancer in women (Senapati et al., 2017). HPV genotype data are critical for developing HPV tests, vaccinations, as well as other treatment strategies to manage viral infections, and they will undoubtedly add to national epidemiological data on HPV-infection in cervix cancer. The introduction of a unique tailored HPV vaccine in Asian economies such as India would be a huge step forward, not just in terms of reducing cancer rates but also in terms of cost-effective screening programmes (Sreedevi, Javed, & Dinesh, 2015). As a result, we looked at the incidence of main high-risk HPV-16 strain in the female population affected with cervix cancer in Kanpur.

The cervix cancer patients included in this cross-sectional study ranged in age from 20 to 60 years. The majority of those affected were between the ages of 41 and 60. The age range of 21-40 years old had the lower prevalence. An incidence of 102 HPV 16 gene positive cases was noticed in the cervix cancer population of Kanpur. The cytological features observed among the cervix cancer population were tabulated in Table 1.

Table 1

Demographic and cervix cytology details of study participants

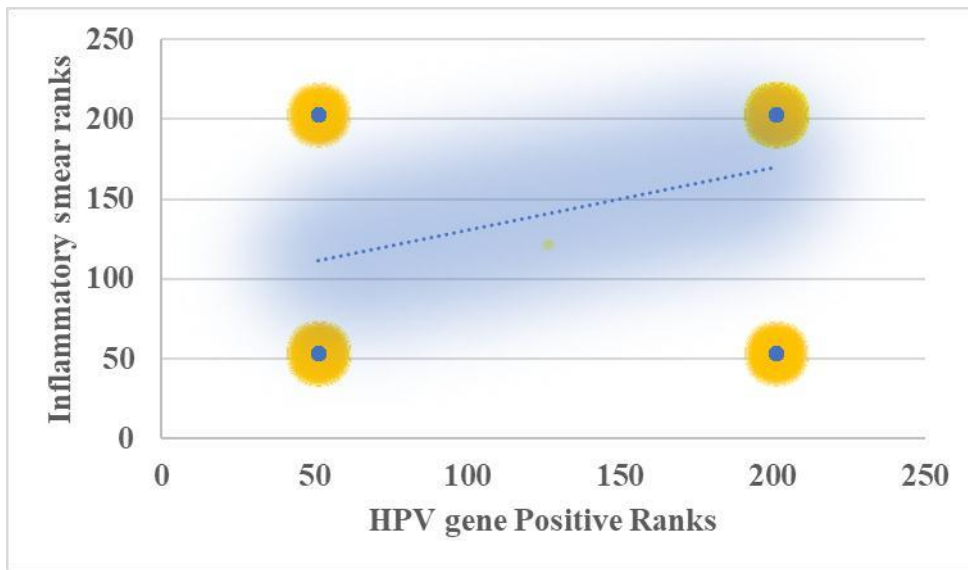
S.No	Patient characteristics (N=300)		Frequency (n)	Percentage (%)
1	Age	21-40 years	89	29.67
		41-60 years	211	70.33
2	HPV 16 gene positive		102	34
3	Atypical squamous cells of undetermined significance (ASCUS)		14	4.66
4	High-grade squamous intraepithelial lesion (HSIL)		14	4.66
5	Low-grade squamous intraepithelial lesion (LSIL)		22	7.33
6	Negative for intraepithelial lesion and malignancies (NILM)		105	35
7	Inflammatory smear		105	35
8	Atrophic smear		38	12.66

Thirty five percent of the patients were negative for intraepithelial lesion and malignancies (NILM). The NILM feature showed positive correlation ($r^2=0.62$) with HPV 16 gene positivity (Table 2 & Figure 1). The cervical reactive abnormalities, such as atrophic and inflammatory, were not statistically significant but were positively correlated with the HPV 16 gene (Table 2 & Figure 1).

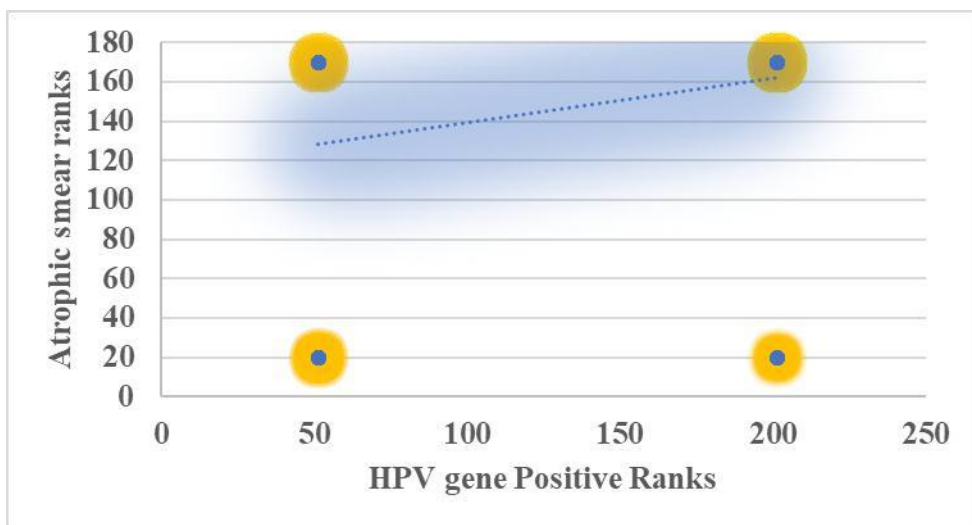
Table 2

Spearman correlation of cervical cytology features among HPV 16 gene-positive cases

Cervix cytology		HPV 16 gene
Atypical squamous cells of undetermined significance (ASCUS)	Correlation Coefficient	0.108
	Sig. (2-tailed)	0.06
	N	300
Low-grade squamous intraepithelial lesion (LSIL)	Correlation Coefficient	0.04
	Sig. (2-tailed)	0.47
	N	300
High-grade squamous intraepithelial lesion (HSIL)	Correlation Coefficient	0.008
	Sig. (2-tailed)	0.89
	N	300
Inflammatory Smear	Correlation Coefficient	0.38
	Sig. (2-tailed)	0.001
	N	300
Atrophic Smear	Correlation Coefficient	0.31
	Sig. (2-tailed)	0.001
	N	300
Negative for intraepithelial lesion and malignancies (NILM)	Correlation Coefficient	0.62
	Sig. (2-tailed)	0.001
	N	300



A



B

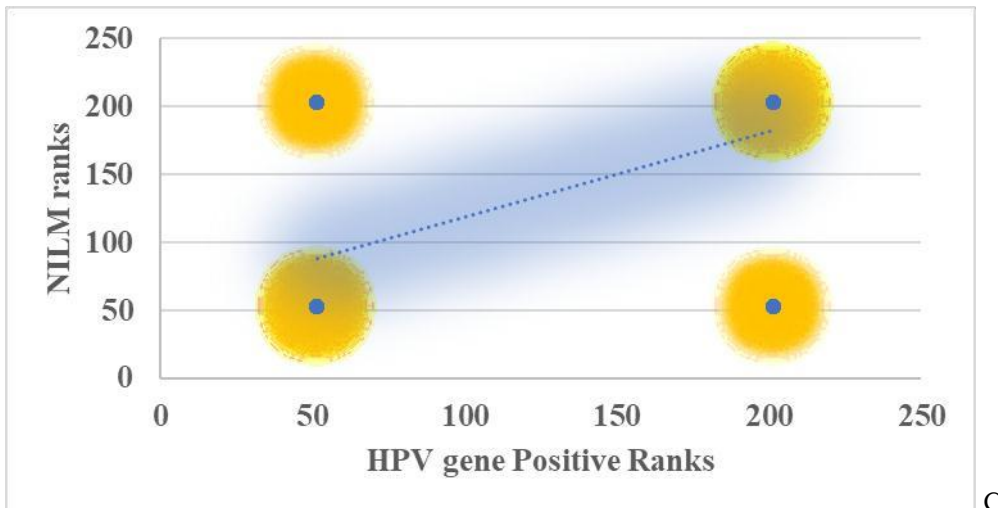


Figure 1: Spearman correlation of HPV 16 gene with cytological features in cervix cancer patients. A-Inflammatory smear, B-Atrophic smear, C-NILM.

In global scenario, studies have shown that Denmark had 1.5 times greater HPV 16/18 incidence than Greenland. Other countries like California, Spain, Japan and Pakistan had a prevalence rate of 3.4, 4.3, 46 and 33 percent respectively. A Finnish study revealed that HPV 16 gene was strongly associated with cervix cancer pathogenesis. Similarly, a study in Mongolia observed 101 positive cases for HPV 16 gene among 374 study population. Our study also showed incidence of 102 cases with HPV 16 gene positivity among 300 included cervix cancer patients. A study conducted during 2012 in eastern Uttar Pradesh revealed 9.9% of oncogenic HPV 16 incidence among 2424 study population (Srivastava, Gupta, & Roy, 2012).

In a study 56 percent (112) of cases exhibited NILM and 32.5 percent of the women had an inflammatory smear (Verma, Verma, Vashist, Attri, & Singhal, 2017). Another study showed a high rate of 73.7 percent inflammatory smear (Kulkarni, Rani, Vimalambike, & Ravishankar, 2013), and in contrast a lesser rate of 14.3 percent was also shown (Lawley, Lee, & Kapela, 1990). Persistent inflammation is linked to cervical intraepithelial neoplasia in 14.3% to 16.7% of women, according to above mentioned studies. In our study, ASCUS, HSIL, LSIL, NILM, inflammatory smear, and atrophic smear was exhibited by 4.66, 4.66, 7.33, 35, 35, and 12.66 percent study population respectively.

Consequences of this HPV 16 infections were revealed by a study in Japan. A study in Japan found that oncogenic HPV incidence is linked to a higher occurrence and development of cytological abnormalities two years later in those with NILM or ASCUS; among these HPV variants, HPV-16 was shown to have a greater predisposition of cytological abnormalities over HPV-52 (Abe et al., 2014).

4 Conclusion

The findings offer the very first evidence on the genotype prevalence among Kanpur women diagnosed with cervix cancer, which will aid in the development of a disease surveillance strategy. This study also provides the regional baseline information for future investigation and post-vaccination monitoring. To lower the cervix cancer incidence in India, an efficient vaccination programme based on regional HPV epidemiology profiles, as well as cervical screening tests, is recommended. Furthermore, HPV surveillance of older women would be beneficial.

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