ORIGINAL ARTICLE

Distribution and Prevalence of High-risk Human Papillomavirus Infection in Women of Western Uttar Pradesh, India: A Hospital-based Study

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Abstract

Aim: Cervical cancer caused by human papillomavirus (HPV) is heterogenic in nature with a regional variation in its distribution. It is crucial to detect high-risk HPV, and thus, the present study aims to find the distribution and prevalence of HPV genotypes by DNA testing and its correlation with cervical cytology. The results of this study would be helpful in the development of newer and efficacious HPV vaccine to make it regionally more specific.

Materials and methods: A cross-sectional study was conducted in a tertiary-care hospital. A total of 217 women presented at the outpatient Department of Obstetrics and Gynaecology with different clinical conditions. Women with history of malignancy and pregnancy were excluded from the study. Detailed history was taken on a preformed pro forma, and cervical samples were detected for abnormal cytology by Pap smear and genotyping by HPV DNA testing by polymerase chain reaction.

Results: The overall prevalence of HPV was 5.5% (12/217), and HPV types 59, 56, 51, 33 and 18 were found prevalent in this study. The higher number of HPV DNA positivity found was in low-grade squamous intraepithelial lesion constituting (66.6%), followed by inflammatory smear (20.6%) and normal cytology with (1.1%).

Conclusion: It has been observed that there is a high prevalence of HPV genotypes 59, 56, 51, 33, and 18. Our study highlights the importance of considering other high-risk genotypes which are not covered by the vaccines currently available in India; therefore, it is necessary to redesign the vaccine by including these genotypes to reduce the incidence of carcinoma cervix.

Keywords: Cervical cancer, High-grade squamous intraepithelial lesion, Human papillomavirus DNA, Human papillomavirus vaccines, Low-grade squamous intraepithelial lesion, Pap smear.

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Introduction

Human papillomavirus is a highly prevalent sexually transmitted infection with varying degree of genotypic distribution worldwide.[1,2] Although the majority of lesions are benign and cleared away by host immune system,[3] due to long persisting period of HPV infection, it may escape from immune response and lead to the development of preinvasive lesions.[4] In order to prevent the progression from premalignant lesion to cancer, early detection could be the important step. Prophylaxis used for the prevention of HPV infection provides protection against few predominant genotypes included in its formation, due to its highly heterogeneous nature regional data on genotypic distribution are essential for the development of newer vaccines with expanded coverage of high-risk genotypes.

Based on the above mentioned background, this study was aimed to find the distribution and to estimate the prevalence of high-risk genotype of HPV in the region of Western Uttar Pradesh and to correlate the HPV DNA testing with Pap smear. The results of the current study might help to decrease the incidence of carcinoma cervix and will be helpful in the development of newer efficacious vaccines.

Materials and Methods

The present study was conducted in Department of Microbiology in collaboration with Department of Gynaecology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh. Women of reproductive age-group were included in the study from May 2019 to April 2021. Pregnant women with the history of malignancy were excluded from the study. Detailed history was taken in a preformed pro forma questionnaire followed by clinical examination. The study was approved by the Institutional Ethics Committee. All subjects under study were informed the objective of the study, and written informed consent was taken prior sample collection.

Pap smear and HPV DNA sampling was done by clinicians with the help of Ayre spatula by rotating at 360° in and outside the

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surface of cervix. The collected sample was quickly smeared on a labeled grease-free slide immediately fixed by 95% alcohol and sent to laboratory for cytological examination by the Bathesda system of classification (2014).

Scraped cell material for HPV DNA testing was then transferred to preserving solution (Preserv Cyt® Solution Marlborough, Massachusetts, USA) and stored at 4°C till it reaches the molecular laboratory for detection of genotypic HPV by real-time polymerase chain reaction. DNA extraction was done by phenol–chloroform extraction method. The process includes OasigTM 2× qPCR master mix kit (Genesig, Primerdesign Ltd. UK), 0.5 µM concentrations of each primer/probe mix, FAM labeled; 0.5 µM concentration of internal extraction control primer/probe, VIC labeled; and 25 ng DNA. Polymerase chain reaction cyclic condition is as follows—denaturing step of 95°C for 5 minutes, followed by 50 cycles of 95°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute.

Statistical Analysis

Data were analyzed in Statistical Package for Social Sciences version 22 and Excel. Mean and standard deviation were calculated. Data were tabulated and proportions were calculated and compared using Chi-square and Student's *t* test wherever applicable. *p* value below 0.05 was considered statistically significant.

Results

In present study, the mean age of the participants was 32.9 years and SD ± 8.9, and most of them belonged to age-group 21–50 years (Table 1). All women included in the study were married and had a parity of up to one to six; 215 (99.1%) women were gravid while only two (0.9%) were nulliparous while women with parity of four or more were 36 (16.5%). Majority of them belonged to the strata of low socioeconomic status. Major complaints registered by the subjects were abdominal pain, vaginal discharge, frequency in micturition, and irregular menstruation together constituting 151 (69.6%) of women while the asymptomatic cases were 66 (30.4%) (Table 2).

On Pap smear, it was observed that 82.9% women were negative for intraepithelial lesion or malignancy while inflammation was present in 13.4%, atypical squamous cell of undetermined significance (ASCUS) in 0.92%, and low-grade squamous intraepithelial lesion (LSIL) in 2.8%. On correlation, highest number of HPV-positive women were found in LSIL (66.6%) followed by inflammatory Pap smear cytology (20.7%), and 1.1% HPV positivity found in women with normal cytology. None of them were found positive for HPV in ASCUS and high-grade squamous intraepithelial lesion (Fig. 1).

Table 1: Sociodemographic characteristics of the study group participants

Age at marriage	*Number (217)*	*%*
21–30	133	61.3
31–40	80	36.9
41–50	4	1.8
Age at first pregnancy		
21–30	81	37.3
31–40	126	58.1
41–50	8	3.7
Parity		
0	2	0.9
1–3	179	82.5
>3	36	16.6
Hygiene		
Good	52	23.9
Bad	165	76.1
Socioeconomic status		
Below poverty line	176	81.1
Average	27	12.4
Good	14	6.4

Patients below 30 years of age were 44.2% who harbored HPV positivity of 4.1% which was found to be lower than what has been found in women above 30 years 55.8% where it has been found 6.6% (Fig. 2).

Overall prevalence of HPV infection was 12 (5.5%) with the frequency of high-risk genotypes 59, 56, 51, 33, and 18. Among all the HPV-positive women, majority had infection of high-risk HPV type 59 (33.3%) while in the remaining cases, HPV types 18, 33, 51, and 56 were prevalent with 16.7% each. The distribution of HPV genotypes in various cytological categories were 6 (50%) patients with inflammatory smears (type 18, type 33, type 56, and type 59), two (16.6%) patients of HPV genotype 51 found with normal Pap smear, four (33.3%) patients with LSIL found positive for type 56 and type 59 (Table 3).

Discussion

To the best of our knowledge, the current study is the first to report the prevalence of HPV genotype distribution among the women of reproductive age-group in the Western part of Uttar Pradesh

Table 2: HPV prevalence in cervical screening population

Age (in years)	*N = 217*	*%*	*Abnormal cytology (N = 37)*	*%*	*HPV DNA (N = 12)*	*%*
21–30	96	44.2	9	24.3	4	33.3
31–40	99	45.7	16	43.2	6	50
41–50	22	10.1	12	32.5	2	16.7
Complaints and symptoms						
Pain in abdomen, micturition, vaginal discharge, irregular menstruation	151	69.6	35	94.6	10	83.3
Asymptomatic	66	30.4	2	5.4	2	16.7

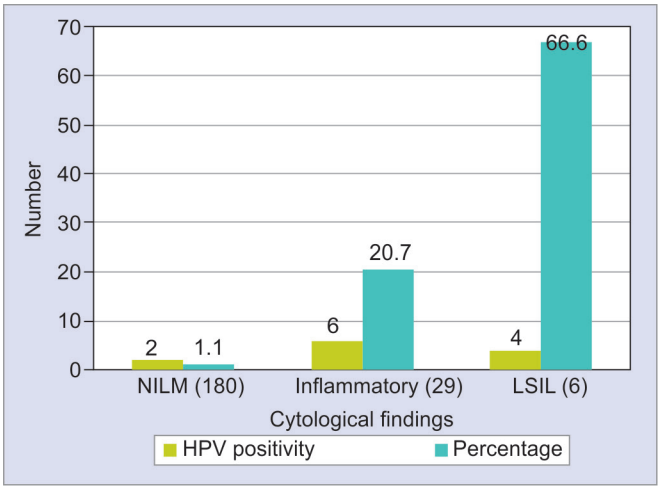

Fig. 1: Correlation between cervical cytology and HPV positivity (NILM, negative for intraepithelial lesion or malignancy; LSIL, low-grade squamous intraepithelial lesion)

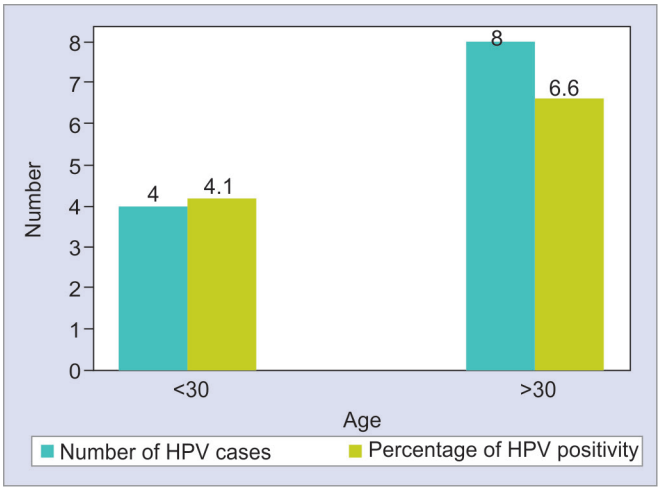

Fig. 2: Correlation of age and HPV-positive cases

(Ghaziabad). Overall, the prevalence of HPV infection found in this study was 5.5% which is lower in comparison to other studies by Datta et al.[5] who reported 7% while Sahasrabuddhe et al. reported HPV infection to be as high as 41.7%.[6]

The most prevalent high-risk HPV type recorded in our study was type 59 which contributed to 33.3%, followed by HPV type 18, 33, 51, and 56 contributing to 16.6% each. A similar result was obtained by another prospective study, where HPV type 59 was highly prevalent along with other HPV types 16, 45, 67, 31, and 51.[7]

In our study, HPV DNA testing showed higher HPV positivity in LSIL with 66.6% which was in consistent with the study done by Matah et al. who have also reported similarly,[8] whereas prevalence of HPV infection in inflammatory smear and in normal cervical cytology was 20.7 and 1.1%, respectively. These findings are comparatively lower to what Senapati et al. reported around 54.3% in inflammatory smear and 19.1% in normal cervical cytology.[9] It was evident from the study that the prevalence of HPV infection increases with increasing grade of cervical abnormality. On correlation, it has been observed that the LSIL had higher percentage followed by inflammatory smear and least number

Table 3: Distribution and correlation of high-risk HPV genotypes with cervical cytology

Cytology (number of patients)	*Number of HPV-positive cases (12)*	*Percentage of high-risk HPV*	*p value*	*HPV type*
NILM ($n = 180$)	2	16.7	0.0001*	Type 51, type 51
Inflammatory ($n = 29$)	6	50.0	0.0006*	Type 18, type 18, type 33, type 33, type 56, type 59
ASCUS ($n = 2$)	0	0	0.7	0
LSIL ($n = 6$)	4	33.3	0.0001*	Type 59, type 59, type 59, type 56

*Statistically significant; NILM, negative for intraepithelial malignancy; ASCUS, atypical squamous cell of undetermined significance; LSIL, low-grade squamous intraepithelial lesion

of positivity seen in women with normal cytology report. These findings are in agreement with Jovanovic et al.[10] as they have also mentioned high HPV positivity rate with increasing abnormality in cervical cytology.

It has been observed that women above 30 years of age were found positive for LSIL in comparison to ASCUS and inflammatory smear which found positivity below 30 years of age, and these findings are in concordance with Misra et al. where increasing age played significant role in progression of abnormal cytology.[11] According to Xu et al.,[12] women in their twenties and thirties are more likely to acquire HPV infection due to the presence of immature transformation zone. On the other hand, women 30 years of age or older who carry mature stable transformation zone are less prone to acquire new HPV infection, but they can give positive HPV DNA test due to its long-standing persistent infection of past that has not been cleared immunologically.

In our study, asymptomatic women contributed 30.41% among them while 3.03% HPV positivity has been found with normal cytology which is very low in comparison to Sontakke et al. who reported as high as 44.2%.[13] However, the percentage of HPV positivity is low in asymptomatic women but small proportion of women with negative Pap smear could with persisting HPV infection increase the chances of acquiring epithelial cell abnormalities and may eventually develop into cancer. Therefore, HPV DNA testing is the most acceptable tool in screening of cervical cancer along with Pap smear cytology.[14]

After analysis of several studies, it has been found that the most predominant genotype are 16 and 18 responsible for causing approximate 75% of cervical carcinoma in India;[7,15–18] in other words, we can say that they are highly mentioned and most commonly detected high-risk genotypes.[19] Interestingly in our study, HPV type 16 was not found, whereas the prevalence of HPV 18 was comparatively low 16.6%. Therefore, author wants to bring special attention that other genotypes should be considered for more effective and inclusive prophylaxis to eradicate the HPV infection.

However, in India, the vaccines approved and recommended for HPV are a quadrivalent vaccine (Gardasil™ marketed by Merck) and a bivalent vaccine (Cervarix™ marketed by Glaxo Smith Kline) and recently introduced gender-neutral nano-valent vaccine (Merck & Co. Inc.).[20,21] These vaccines confer protection to related strains, and by taking this study into cogitation, it has been concluded that other high-risk strains may have the tendency to actively transform the epithelial cells into malignant cells. This is clinically significant to evaluate the distribution of high-risk HPV. The evaluation plays major role in the development of more effective HPV vaccines.[16]

One of the limitations of our study is that only women attending outpatient department at tertiary-care center were included; however, to know more precise data on distribution and prevalence rate in this geographical region, we need to expand this study to community level by including both rural and urban sector. Due to lack of resources and funding, we couldn't extend this study to correlate with histopathology.

Conclusion

Human papillomavirus type 59 was found as the most predominant genotype in Ghaziabad. Our study highlights the importance of including such high-risk genotypes in development of vaccine to make it regionally more specific. Therefore, it is imperative to continuous analysis of geographical distribution of HPV genotypes in other regions of India to develop efficacious newer HPV vaccines.

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