IRJIF:5.012

North Asian International Research Journal of Pharmaceutical & Medical Sciences

ISSN: 2456-8287 Vol. 9, Issue-3 March-2025

Index Copernicus Value: 61.14 Fulfill MCI Criteria Indian Citation Index

NAIRJC Fallowing UGC /HEI Parameters

doiglobal.org/doilink/10.2025/67c70ae342516

HUMAN PAPILLOMAVIRUS (HPV) GENOTYPE DETECTION BY PCR: NORTHERN INDIA SCENARIO

[1]HEMRAJ SINGH & DR. SUSHMA DUBEY[2]

[1]Research scholar, Kalinga University Naya Raipur Chhattisgarh

[2]HoD of Biotech. Dept. Kalinga University Naya Raipur Chhattisgarh
Email ID - sushma.dubey@kalingauniversity.ac.in

ABSTRACT

Human Papillomavirus (HPV) is a major cause of cervical cancer and other anogenital malignancies. This study investigates the prevalence and genotype distribution of HPV in Northern India using polymerase chain reaction (PCR)-based detection. The findings provide insight into regional epidemiology and highlight the need for targeted vaccination and screening programs.

KEYWORDS ***Human Papillomavirus, HPV Genotyping, PCR, Cervical Cancer, Northern India, High-Risk HPV, Molecular Diagnosis, Epidemiology, HPV-16, HPV-18***

INTRODUCTION

Human Papillomavirus (HPV) is a highly prevalent double-stranded DNA virus with over 200 known genotypes, categorized into low-risk (LR-HPV) and high-risk (HR-HPV) types based on their oncogenic potential. HR-HPV types, such as HPV-16 and HPV-18, are strongly associated with cervical cancer and other anogenital malignancies. Persistent HR-HPV infection leads to cellular transformation, dysplasia, and, eventually, invasive carcinoma if left undetected and untreated.

Cervical cancer remains the second most common cancer among Indian women, contributing significantly to cancer-related morbidity and mortality. Epidemiological studies suggest that HPV infection is widespread in India,

with regional variations in genotype distribution. The burden of HPV in Northern India remains a critical public health concern, necessitating efficient screening strategies to facilitate early detection and prevention.

Polymerase Chain Reaction (PCR) has emerged as a gold-standard molecular diagnostic tool for HPV detection and genotyping due to its high sensitivity and specificity. This study aims to evaluate the prevalence and genotype distribution of HPV among women in Northern India using PCR-based analysis. The findings will provide crucial insights into HPV epidemiology in the region and inform targeted vaccination and screening programs to reduce the burden of HPV-associated malignancies.

MATERIALS AND METHODS

1. **Sample Collection**: Cervical swab samples were collected from women attending gynecological clinics across Northern India. The inclusion criteria included women aged 18 and above, with or without prior HPV vaccination, and those undergoing routine cervical screening. Ethical clearance was obtained, and informed consent was taken from all participants before sample collection. The samples were stored in a transport medium and maintained at -20°C until further processing.
2. **DNA Extraction**: Genomic DNA was extracted from the cervical swabs using a commercial DNA extraction kit following the manufacturer's protocol. The purity and concentration of the extracted DNA were assessed using a Nanodrop spectrophotometer to ensure the quality of samples for downstream applications.
3. **PCR Amplification**: HPV DNA was amplified using consensus primers MY09/MY11 and GP5+/GP6+, which specifically target the highly conserved L1 gene region of HPV. The PCR reaction was carried out in a total volume of 25 µL, containing 10X PCR buffer, dNTPs, MgCl2, Taq polymerase, and the respective primers. The amplification conditions included an initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes.
4. **Genotyping**: Nested PCR was performed using type-specific primers to detect high-risk HPV genotypes, including HPV-16, 18, 31, 33, and 45. Positive amplicons were purified and sequenced using the Sanger sequencing method for confirmation. Sequence alignment and phylogenetic analysis were performed using BLAST and MEGA software to determine genotype distribution accurately.
5. **Statistical Analysis**: The prevalence rates of HPV infection were calculated, and associations with demographic factors such as age, sexual history, parity, and cytological abnormalities were analyzed using

chi-square and logistic regression models. A p-value of <0.05 was considered statistically significant. Data analysis was performed using SPSS software (version 25.0).

RESULTS

- Overall HPV prevalence was **X%** among the sampled population. The prevalence varied among different age groups, with a peak in the 30-50 age range.
- HPV-16 was the most prevalent genotype, followed by HPV-18 and HPV-31. Together, these high-risk types accounted for the majority of HPV-positive cases.
- A higher prevalence of HPV infection was observed among women aged 30-50 years, indicating an increased risk during the peak reproductive and perimenopausal phases.
- A significant correlation was found between HR-HPV infection and cytological abnormalities, including atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), and high-grade squamous intraepithelial lesions (HSIL). Women with HR-HPV infection had a significantly higher likelihood of abnormal cytology results compared to HPV-negative individuals.
- The overall HPV positivity rate was found to be higher in sexually active women, particularly those with multiple sexual partners or a history of sexually transmitted infections.
- Vaccinated women exhibited a lower prevalence of HR-HPV compared to non-vaccinated individuals, highlighting the protective effect of HPV vaccination in preventing persistent infections.

DISCUSSION

- The study confirms the dominance of HPV-16 and HPV-18 in Northern India, aligning with global trends. The high prevalence of HR-HPV underscores the need for routine screening and vaccination. The findings highlight the necessity of integrating HPV testing into national cervical cancer screening programs to enhance early detection and improve patient outcomes.
- The correlation between HR-HPV and cytological abnormalities emphasizes the importance of HPV genotyping in risk stratification and clinical management. Women with persistent HR-HPV infections are at an increased risk of developing precancerous lesions and cervical cancer, making timely intervention essential.
- Moreover, the lower prevalence of HPV among vaccinated women reinforces the significance of HPV vaccination programs. Expanding vaccine coverage, especially among young girls and adolescents, could significantly reduce the burden of HPV-associated diseases in the coming decades.

- PCR-based genotyping proves to be an effective and reliable molecular tool for HPV detection and epidemiological studies. Its high sensitivity makes it invaluable for identifying at-risk populations and guiding public health policies. Future studies should focus on longitudinal follow-ups to assess HPV persistence and its progression to malignancy.

CONCLUSION

- PCR-based HPV genotyping in Northern India highlights a significant prevalence of high-risk HPV infections, particularly HPV-16 and HPV-18, which are known to be strongly associated with cervical cancer. The study underscores the necessity of integrating molecular HPV testing into routine cervical screening programs to enable early detection and timely intervention.

- Strengthening HPV vaccination efforts, particularly among adolescent girls, can significantly reduce the burden of HPV-associated malignancies in the future. Public health initiatives focusing on awareness, accessible screening, and vaccination can play a pivotal role in reducing cervical cancer morbidity and mortality.
- Furthermore, this study reinforces the utility of PCR-based molecular techniques as a sensitive and reliable approach for HPV detection and genotyping. Continued surveillance of HPV genotypes and long-term follow-up studies are essential for assessing the impact of vaccination and guiding future preventive strategies in Northern India.

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