Original Article



Prevalence of High-Risk HPV Genotypes (16, 18, 31, and 45) and Cervical Cytological Abnormalities among Women in Eastern India: A Cross-Sectional Pilot Study from a Screening Cohort

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Abstract

Background: Persistent infection with high-risk HPV (HR-HPV) types is a major risk factor for cervical cancer. Regional data on HPV prevalence is essential for effective screening and vaccination strategies.

Materials and Methods: A cross-sectional study was conducted among women attending cervical cancer screening camps in Bhubaneswar. Paired cervical and urine samples were collected. DNA was extracted and analyzed for HR-HPV types 16, 18, 31, and 45 using molecular techniques. Cervical smears were evaluated according to the Bethesda System 2014 for reporting cervical cytology.

Results: Of 125 women included, HR-HPV DNA was detected in 3 (2.4%) cases [HPV16/31, HPV18/45, and HPV16]. All HPV-positive cases had a parity of two. Concordance between cervical and urine samples was 100%. Cytology showed inflammatory changes in most cases (72.8%), with only two cases (1.6%) reported as atypical squamous cells of undetermined significance (ASCUS), one of which was also HPV16 positive.

Conclusion: The prevalence of HR-HPV in this cohort was low. The high concordance between urine and cervical samples suggests urine as a viable non-invasive option for HPV detection. These findings support the integration of urine-based screening and call for broader HPV genotyping and larger-scale studies to better inform regional public health strategies.

Keywords: Cervical Cancer; Cytology; Genotype; Human papillomaviruses; Polymerase chain reaction; Urine-based screening; Viral load

Introduction

Cervical cancer is the fourth most prevalent disease to be reported and the fourth primary factor of cancer death for women worldwide. It is one of the second most prevalent gynecologic diseases in India [1]. According to the Cancer Statistics 2020, women between the ages of 20 and 39 years recorded the second leading death of cervical cancer. 4152 women died from cervical cancer overall, with 50% of them being 50 years old or younger as reported in 2019. Additionally, the growing

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frequency of advanced illness and cervical cancer is being attributed to diagnoses among young women [2]. The underlying factor of nearly every cervical cancer occurrence is a persistent infection with human papillomavirus (HPV). There are 12 HPV types such as 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. But cervical cancer etiology is significantly linked to persistent infection with high-risk human papillomavirus (HR-HPV) strains, including HPV-16, HPV-18, HPV-31, and HPV-45. These HR-HPV strains interfere with the control of the cell cycle by disrupting tumour suppressor proteins such as retinoblastoma protein (Rb) and p53, which promote oncogenesis and cellular transformation [3]. High-risk human papillomaviruses (HR-HPVs) have oncoproteins E6 and E7 that are essential to the molecular pathophysiology of cervical cancer. These viral proteins deactivate tumor suppressors, which impair vital cellular functions [4].

Despite significant regional differences in HPV incidence and related risk factors, cervical cancer comprises over 16.5% of the overall female malignancies in India [5]. In low-income regions improvements in HPV screening and vaccination, there are still significant obstacles to effective prevention and early diagnosis, such as a lack of awareness, cultural hurdles, and limited access to routine cervical smear screenings [6]. The Papanicolaou (Pap) examination is essential for cervical cancer screening and early diagnosis when it comes to cytological examination of cervical smears. An essential diagnostic marker of HPV-associated infections and the possible development of cervical dysplasia is the detection of cytological abnormalities, such as low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), and atypical squamous cells of unknown significance. Cervical neoplasia risk is significantly influenced by these aberrant cytological patterns, which mimic the fundamental histological alterations imposed through high-risk HPV strains [7]. However, there is limited regional data linking specific HR-HPV types to these cytological abnormalities in Bhubaneswar. This study aims to assess the prevalence of HPV types 16, 18, 31, and 45 among women in Bhubaneswar and their association with abnormal cervical smear patterns. The findings will provide crucial epidemiological insights into the regional distribution of high-risk HPV genotypes and associated cervical abnormalities, which is crucial for designing effective screening, prevention, and vaccination strategies to reduce the burden of cervical cancer in India.

Materials and Methods

This cross-sectional observational study was conducted over a ten-month period from July 2023 to April 2024, enrolling a total of 125 women who attended the gynecology outpatient department for routine cervical screening or with gynecological complaints. Participants were recruited from three key urban healthcare and outreach centers in Bhubaneswar, Odisha: Capital Hospital, the Indian Medical Association (IMA) House Screening Point, and the Anganwadi Training Centre. The inclusion of diverse community-based sites ensured a broader representation of the local female population. Women aged 21 years and above who were willing to provide informed consent and undergo cervical examination and sample collection were included. Pregnant women, those with a known history of cervical carcinoma, or who had undergone total hysterectomy were excluded from the study. A detailed clinical history was recorded from each participant, including age, parity, and presenting complaints. A per speculum examination was performed to inspect the cervix for any abnormalities. Women presenting with visible cervical lesions underwent an initial visual inspection after the application of 5% acetic acid (VIA) to identify potential abnormalities.

Cervical and urinary samples were collected from all participants as part of the screening protocol. Cervical scrapings were obtained using a cervical brush and transferred into auto-transfer medium bottles for preservation. Concurrently, freshly voided urine samples were collected in sterile containers. Collected cervical samples were also immediately smeared onto clean glass slides, fixed in 95% ethanol, and stained using the Papanicolaou staining technique. The slides were examined under a light microscope by an experienced cytopathologist and were categorized according to the Bethesda System 2014 for reporting cervical cytology [8]. Detailed observations were recorded, including cellular morphology, presence of inflammation or atypia, and identification of any intraepithelial lesions or malignancy. The cytological findings were used to provide diagnostic insights and to correlate with the molecular data obtained from HPV testing, thereby contributing to a comprehensive cervical cancer screening and surveillance framework.

All biological specimens were transported under strict cold-chain conditions to maintain sample integrity. The cervical swab and urine samples were sent to the Regional Medical Research Centre (RMRC), Bhubaneswar, for virological analysis, specifically focusing on HPV DNA detection and typing. Total DNA extraction was performed using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA), following the manufacturer's protocol. HPV typing was performed using the TruenatTM HPV-HR Test Kit, a chip-based real-time duplex polymerase chain reaction (PCR) test for detecting HPV-HR types 16, 31 and 18, 45, provided by Molbio Diagnostics, Goa, India. This method enables the detection of high-risk HPV types, including HPV-16, HPV-31, HPV-18, and HPV-45. The procedure involved sample preparation, initial inactivation and nucleic acid purification from cervical squamous cells in cervical swab samples and cells in urine samples. Positive controls (known HPV-positive samples) and negative controls were included in each run to ensure quality assurance. Quantitative micro-PCR was performed to detect and differentiate high-risk HPV types (only in the cervical sample).

All collected data were compiled and analyzed using Jamovi statistical software Version 2.6.26 [9, 10]. Descriptive statistics were used to summarize demographic and clinical variables. Continuous variables such as age and parity were expressed

as mean \pm standard deviation (SD), while categorical variables such as cytological findings, clinical examination results, microscopic observations and prevalence of high-risk HPV genotypes were reported as frequencies and percentages.

Results

A total of 125 women participated in the study. The mean age of the participants was 39.2 years (standard deviation = 11.1 years), with a median age of 37 years, and the age range spanned from 24 to 72 years. Most women had one or two children, with 40.0% (n = 50) having two children and 36.8% (n = 46) having one child. A smaller proportion were nulliparous (4.0%, n = 5), while six women (4.8%) had more than three children. Majority of them belonged to lower middle class (n=86; 68.8%) and lower class (n=39; 31.2%), as per revised Kuppuswamy socioeconomic status class classification [11].

On per speculum examination, 49.6% (n = 62) of the women had a clinically healthy cervix. Among the remaining participants, 22.4% (n = 28) had signs of endocervicitis, 11.2% (n = 14) showed cervical erosion, and 5.6% (n = 7 each) had either chronic cervicitis or cervix flushed with the vault. Atrophic changes were observed in 3.2% of the women (n = 4), and 2.4% (n = 3) were reported to have an unhealthy cervix. None of the women showed any abnormality upon VIA examination.

Cytological examination of cervical samples revealed that 98.4% (n = 123) of cases were negative for intraepithelial lesion or malignancy (NILM), while 1.6% (n = 2) were reported as atypical squamous cells of undetermined significance (ASCUS). Microscopic analysis of the samples additionally revealed inflammation in 72.8% of cases (n = 91), inflammation with atrophy in 6.4% (n = 8), and atrophy alone in 4.0% (n = 5). No additional microscopic abnormalities were noted in 16.8% of the samples (n = 21). Shift in flora suggestive of bacterial vaginosis was encountered in 18 women (14.4%) followed by candidial infection, found in 11 women (8.8%) [Figure 1].

In this study, HPV DNA was detected in only 3 (2.4%) of the cervical swab and urine samples collected from participants. Table 1 presents data on these three women, each with a parity of two. Clinically, one woman presented with cervical erosion, another was considered healthy, and the third was labeled as unhealthy. Cytological evaluation revealed that two women had results categorized as NILM (Negative for Intraepithelial Lesion or Malignancy), while one showed ASCUS (Atypical Squamous Cells of Undetermined Significance). High-risk human papillomavirus (HPV) was detected in all cases, with cycle threshold (Ct) value of 31 to 33, which indicates that HPV DNA was detected at a relatively low concentration, suggesting a low viral load. The specific HPV types identified included HPV16/31, HPV18/45, and HPV16, highlighting the presence of oncogenic strains even in cases with absent or minimal cytological abnormalities. This underscores the importance of integrating HPV testing with cytological and visual screening methods for comprehensive cervical cancer risk assessment. Follow-up was advised for these three women. Also, there was 100% concordance rate between HPV (negative/positive) results in paired cervical and urine samples, in the entire screening cohort.

Age	Parity	Findings upon per speculum examina- tion	Impression upon cervi- cal cytology	Additional findings upon cervi- cal cytology	HPV status in cervical sample	HPV status in urine sample	Genotype and viral load in cervical sample
36	2	Cervical erosion	NILM	Inflammation	+	+	Detected (HPV16/31), Ct Value= 33
37	2	Healthy	NILM	Inflammation	+	+	Detected (HPV18/45), Ct Value= 33
37	2	Unhealthy	ASCUS	Inflammation	+	+	Detected (HPV16), Ct Value= 30

Table 1: Clinical, Cytological, and Virological Profile of Women with Positive HPV Status in the study cohort.

Discussion

Around 40 genotypes of HPV belonging to the Alpha genus are capable of infecting the genital tract, though only some have been linked to cervical cancer [12]. These genotypes are categorized based on their cancer-causing potential into high-risk and low-risk groups, with Group 1, classified as high-risk and carcinogenic to humans, includes twelve HPV types (16, 18,

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Table 2: Proposed Measures for Strengthening HPV-Related Cervical Cancer Prevention Strategies in India.

ACTIONABLE STRATEGIES

Incorporation of routine HPV DNA testing into public cervical cancer screening programs alongside cytology Expansion of awareness and education campaigns

Strengthening infrastructure for sample collection, storage, and molecular testing

Promotion and validation of self-collection methodologies

Scaling-up of HPV vaccination programs

Conduction of larger, population-based studies

REMARKS

Especially in women above 30 years of age, to enhance early detection of high-risk infections

To improve understanding of HPV, cervical cancer risk, and the importance of regular screening among women, especially in urban and peri-urban underserved communities

Especially in district hospitals and community health centers to facilitate broader reach

Self-collection methodologies utilising urine or vaginal swabs for HPV testing to increase screening uptake, especially in culturally sensitive and low-resource settings Particularly targeting adolescent girls, and ensuring coverage of genotypes 16 and 18

To generate robust epidemiological data and better define regional genotype prevalence, coinfection patterns, and associated risk factors

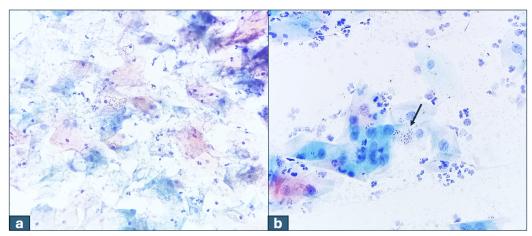


Figure 1: Microphotograhs of cervical cytology: (a) Shift in flora suggestive of bacterial vaginosis [Papanicolaou stain, 400x]; (b) Fungal elements morphologically consistent with Candida species [Papanicolaou stain, 400x]

31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) [12]. HPV 16 and 18 are the most oncogenic types, responsible for approximately 70% of cervical cancers worldwide [12, 13, 14, 15]. In invasive cervical cancer, HPV 16 is the predominant type, detected in 60-80% of cases across different studies. Bhatla et al. [13] reported HPV 16 in 49.4% cases overall and 73.6% of cervical cancer cases in a multi-centric study, while Sowjanya et al. [14] found HPV 16 in 66.7% of cervical cancer cases in southern India. A study by Deodhar et al. [15] reported HPV 16 in 72.4% of cervical cancer cases in western India. HPV 18 is detected in 10-20% of cases of cervical cancer. Bhatla et al. [13] reported HPV 18 in 14.2% of cervical cancer cases. Several studies have noted that HPV 18 is more commonly associated with adenocarcinoma of the cervix compared to squamous cell carcinoma [16]. HPV 31 and 45 are also significant oncogenic types, with HPV 45 being phylogenetically related to HPV 18 and HPV 31 related to HPV 16 [12, 17].

In India, the incidence of HPV fluctuates significantly among populations and geographical areas, reflecting differences in study populations, sampling methods, HPV detection techniques, and regional factors. According to a study done in Delhi and Noida, the prevalence of HPV in healthy people was 3.5% [18]. According to Dutta et al., a population-based study conducted in Eastern India revealed that the prevalence of HPV was 9.9%, with HPV-18 being the most common form at 1.4% and HPV-16 at 0.6% [19]. Another study from Uttar Pradesh has recorded that 9.9% of asymptomatic people tested positive for HPV, with the most common genotypes being HPV-16 (63.7%) and HPV-31 (6.7%) [20]. A multi-centric study conducted by the Indian Council of Medical Research (ICMR) across different geographical regions reported an overall HPV prevalence of 10.3% among women attending gynecology outpatient departments without any cervical abnormalities [13].

Approximately 80% of HR-HPV infections are intermittent and are not reflected in lesions since the virus generally is shedded away in 1–2 years. The majority of the remaining 20% cause low-grade cervical intraepithelial neoplasia (CIN I), which is indicative of an infection that is productive but non-progressive and frequently regresses by itself. Persistent

HR-HPV infection, frequently in conjunction with co-infections and unfavourable host factors, considerably raises the risk of high grade cervical intraepithelial neoplasia (CIN II/III) and invasive cervical cancer, even though the majority of infections are for a short time and asymptomatic [12, 21]. This cross-sectional study aimed to assess the prevalence of HR-HPV genotypes (16, 18, 31, and 45) and associated cervical cytological abnormalities among women attending a health facility in Bhubaneswar. The findings provide important baseline data on cervical health in this population and have implications for screening strategies in similar low-resource settings. Socioeconomic factors can significantly influence women's awareness of cervical cancer, access to screening services, and healthcare-seeking behavior, thereby resulting in lower participation in screening programs or HPV testing [22, 23]. A large-scale study from rural West India found a 10.3% prevalence of high-risk HPV infection among middle-aged women, with increased risk linked to low socioeconomic status, early sexual activity, and vulnerable social groups such as widows and separated women [23]. Although the majority of women in the present study cohort belonged to the lower-middle and lower socioeconomic classes, definitive conclusions regarding the association between socioeconomic status and HPV prevalence cannot be drawn due to the limited sample size and low HR-HPV positivity rates, restricting a stratified statistical analysis.

On clinical examination, nearly half of the women had a healthy cervix, while the remainder showed changes such as endocervicitis (22.4%), cervical erosion (11.2%), and cervix flushed with the vaginal vault. These abnormalities are commonly encountered in outpatient settings and may or may not correlate with cytological or virological findings, as in our study [24]. Integration of visual inspection, cytology, and HPV testing has been shown to enhance detection rates and triage efficiency [25]. Parity analysis indicated that most women had one or two children, a trend consistent with contemporary Indian reproductive patterns. Some studies suggest that higher parity is associated with a greater risk of persistent HPV infection and cervical neoplasia, potentially due to hormonal or cervical trauma-related factors [26]. However, such associations are best evaluated when stratified by HPV status. In the present cohort, women with three or more children did not show any epithelial cell abnormalities upon cytology and were negative for HPV. However, this observation did not reach statistical significance, possibly due to the small sample size and low representation of high-parity women in the study population.

In the current study cohort, there was the low prevalence of cytological abnormalities. Only 1.6% (n=2) of women had atypical squamous cells of undetermined significance (ASCUS), while 98.4% were negative for intraepithelial lesion or malignancy (NILM). These findings are consistent with several population-based studies from India, where NILM rates have been reported to range from 85% to 98% in general screening populations [14, 27]. This high rate of normal cytology could reflect a relatively low burden of HPV-related disease in the screened cohort or good immune-mediated clearance of transient infections. Inflammation was the most frequent associated microscopic finding, present in over 70% of the samples. Inflammatory changes in cervical cytology are common and often reflect underlying bacterial, fungal, or viral infections, or nonspecific cervicitis [28]. Although inflammation alone is not considered a premalignant lesion, persistent inflammatory changes may obscure epithelial abnormalities or indicate an environment conducive to HPV persistence and progression [29].

In the current study, HPV DNA has been identified in just 2.4% (n=3) of participants' cervical swabs and urine samples, which is consistent with data from Noida and Delhi. The found incidence might be attributed to regional variances in HPV exposure, demographics, and lifestyle behaviours. This relatively low detection rate highlights the necessity of integrating region-specific preventative measures like early screening and vaccination with more research into the molecular epidemiology of HPV genotypes in this population. In all the three positive samples, HPV DNA was detected at a relatively low concentration, suggesting a low viral load [30]. In real-time PCR, a Ct value (as a surrogate for viral load) around 33 is near the detection threshold, implying that while infection is present, it is not highly active or abundant at the time of testing [30]. Also, the absence of high-grade lesions (e.g., LSIL or HSIL) in this cohort may reflect a genuinely low underlying prevalence of oncogenic HPV types, or the limitations of cytology alone in detecting early lesions [30]. Incorporating HPV DNA testing is known to improve early detection, especially for lesions missed by cytology [31]. Notably, 100% concordance between cervical and urine samples for HPV detection. Indian studies have reported a moderate to substantial concordance between cervical and urine samples for HPV detection, and may serve as a useful, more accessible alternative, especially for initial screening [32, 33]. In summary, the present study provides important information on the frequency of HPV genotypes and molecular variations among Bhubaneswar women, pointing to important trends that can guide focused preventative efforts like immunisation and early screening initiatives. Given the regional variability in HPV prevalence and type distribution, public health strategies must be localized and evidence-driven and we propose few actionable steps, enumerated under Table 2.

Conclusion

This pilot study highlights a low prevalence (2.4%) of high-risk human papillomavirus (HR-HPV) types—specifically HPV16, 18, 31, and 45—among women attending cervical screening clinics in Bhubaneswar, Odisha. Despite the low detection rate, the identification of oncogenic HPV strains, including in cytologically normal samples, underscores the limitations of relying solely on cytology and visual inspection for early detection. The findings emphasize the need for an

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integrated, multi-modal cervical cancer screening approach that includes HPV DNA testing, particularly in low-resource settings. Importantly, complete concordance was observed between cervical and urine samples for HPV detection, indicating that urine-based HPV testing could be a reliable, non-invasive alternative for cervical cancer screening, especially in culturally sensitive settings. Expanding the HPV genotyping panel and incorporating larger community-based cohorts in future studies will be crucial to better understand the burden of HR-HPV and its role in cervical oncogenesis in this region. These initiatives are essential for creating focused preventative plans, such as immunisation and early screening, to lower the incidence of cervical cancer in India.

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