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ORIGINAL RESEARCH

Comparative Analysis of HPV DNA Testing and Pap Smear Screening: Evaluating Their Efficacy in Cervical Cancer Prevention and Early Detection Strategies

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ABSTRACT

Introduction: Cervical cancer remains a significant public health concern, with high morbidity and mortality rates worldwide. Early detection and prevention are crucial in reducing the burden of this disease. Traditional Pap smear screening has been the cornerstone of cervical cancer prevention for decades. However, advancements in medical technology have introduced HPV DNA testing as a potential primary screening tool. **Objective:** The study aims to evaluate the efficacy of Human Papillomavirus (HPV) DNA detection and high-risk genotyping as a primary screening tool for cervical cancer prevention in women attending a tertiary care hospital in North India. The focus is on determining the prevalence of hrHPV infections, assessing the potential for early detection of cervical neoplasia. **Methods:** A cross-sectional observational study was conducted from January 2023 to June 2024, involving 395 women aged 25-65 years presenting to the gynaecology department of a tertiary care hospital in North India. Endo-cervical brushings were collected and subjected to HPV DNA testing using polymerase chain reaction (PCR). Genotyping of hrHPV was performed for HPV-positive samples. Data for cytological examination was also collected and analysed. **Results:** Out of the 395 women screened, 50 (12.65%) tested positive for HPV DNA. hrHPV 16 was detected in 21 (42%) of the total positive HPV DNA cases, followed by hrHPV 39/68 in 16 (32%), hrHPV 31, 33, 35, 52, 58, 51, 56 or 66 in 9 (18%), and HPV 18 in 4 (8%) cases. No co-infection was reported in our study. Squamous Cell Carcinoma (SCC) and Atypical squamous cells of undetermined significance (ASC-US) was confirmed cytologically in 6% of hrHPV cases. **Conclusion:** HPV DNA detection and genotyping for hrHPV is highly effective as primary screening tools for cervical cancer. The prevalence of hrHPV in North Indian women underscores the importance of incorporating HPV-based screening into national cervical cancer prevention strategies. Further research is recommended to assess long-term outcomes and cost-effectiveness.

Keywords: Human Papillomavirus, hrHPV, cervical cancer, genotyping, Pap smear, Molecular diagnostics

INTRODUCTION

Cervical cancer remains a significant public health concern, ranking as the third most commonly diagnosed cancer and the fourth leading cause of cancer-related mortality among women globally. It is the leading cause of cancer-related deaths in 36 countries and ranks as the second most common cause of cancer-related deaths in 49 countries[(1,2)]. Persistent infection with high-risk types of Human Papillomavirus (HPV), particularly HPV 16 and 18, is a necessary precursor for cervical cancer development, with nearly 99.7% of invasive cases globally being attributed to HPV infection [(3,4)]. Despite the declining global incidence of cervical cancer due to increased awareness, improved screening practices, and HPV vaccination, disparities remain, especially in low- and middle-income countries where cervical cancer still accounts for a significant proportion of cancer-related deaths[(4,5)].

India, for instance, bears a disproportionate burden, accounting for about one-fifth of all new cervical cancer cases and nearly one-fourth of global cervical cancer-related deaths in 2022[(6).] Among Indian women, cervical cancer is the second most common cancer, contributing to 9% of new cancer cases and 1.3% of cancer-related deaths, with a five-year prevalence rate of 50.02% [(6,7).] These statistics highlight the urgent need for enhanced screening and preventive strategies.

Traditional cervical cancer screening methods, such as the Papanicolaou (Pap) smear, have significantly reduced cervical cancer incidence in high-resource settings. However, its limitations in sensitivity (ranging between 50–75%) and its reliance on cytological interpretation reduce its effectiveness [(8,9).]

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In contrast, HPV DNA testing, a molecular approach that detects high-risk HPV genotypes, has emerged as a more sensitive screening tool, with detection rates exceeding 95% in some studies[10,11]. Unlike the Pap smear, HPV DNA testing identifies infections before cytological abnormalities develop, allowing for earlier intervention and potentially reducing progression to invasive cancer [12,13].

Globally, the World Health Organization (WHO) now recommends HPV DNA testing as the primary screening method, either alone or as part of a "screen-and-treat" approach that eliminates the need for histological confirmation before initiating treatment for positive cases[14]. This strategy is particularly advantageous in resource-limited settings, where follow-up compliance and healthcare infrastructure are challenges. Research indicates that HPV DNA testing is more effective in detecting cervical intraepithelial neoplasia grade 2 or higher (CIN2+) lesions, further supporting its integration into national screening programs[15]

This study aims to evaluate and compare the efficacy of HPV DNA testing and Pap smear screening in cervical cancer prevention. By analysing their sensitivity, specificity, and practical application in diverse populations, the research seeks to provide evidence-based recommendations to optimize cervical cancer screening strategies, especially in resource-constrained regions where the burden of disease remains disproportionately high.

MATERIALS AND METHODS

This study is based on an observational hospital based cross-sectional design, spanning over a period of 18 months, from January 2023 to June 2024.

The study population included women aged 25 to 65 who presented to the gynaecology outpatient department for routine cervical cancer screening or with symptoms suggestive of cervical pathology.

For women who satisfied the inclusion criteria and gave consent to participate in the study, sample for Pap smear and HPV testing were taken during gynaecological examination. Women with prior history of malignancy or undergoing treatment were excluded from the study. Detailed demographic and clinical information were collected from each participant, including age, marital status, age of marriage, age of first childbirth, parity, sterility,previous screening history and vaccination status.

Cervical samples were collected using a cytobrush and placed in a transport medium suitable for HPV DNA testing. Samples were stored at 4°C till further processing.

DNA was extracted from the cervical samples using a commercially available DNA extraction kit (TRUPCR® Tissue DNA Extraction kit) following the manufacturer's protocol.

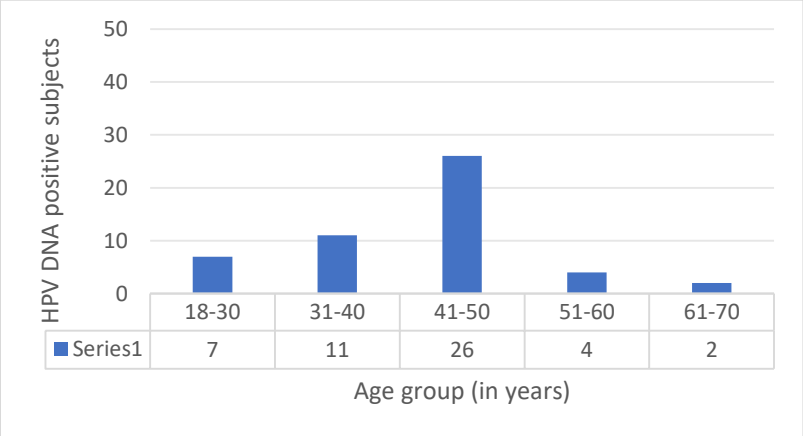
For the Real Time PCR reaction and analysis, the TRUPCR® HPV HR with 16/18 genotyping kit was used. This kit employs fluorescent reporter dye probes to detect 14 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), while also providing specific genotyping for HPV types 16 and 18. The kit operates with three independent reactions in parallel tubes: the first detects HPV HR genotypes 16, 31, 33, 35, 52, 58, 51, 56, and 66 (FAM channel); the second detects genotypes 18, 45, and 59 (FAM channel) and provides HPV 18 genotyping (HEX) along with an endogenous internal control (IC) (TEXAS RED) to ensure reliable results; the third reaction identifies HPV HR genotypes 39 or 68 (FAM) and performs HPV 16 genotyping (TEXAS RED).

A concurrent Pap smear was taken for cytological examination. Samples were stained using the Papanicolaou method and examined under a microscope for cytological abnormalities. The results were classified according to the Bethesda system[32]

RESULTS AND OBSERVATION

Out of the 395 women screened, 50 (12.65%) tested positive for HPV DNA.

Fig. 1 Age group of HPV DNA positive subjects



Of the 50 women who tested positive for HPV DNA, the majority were in the 41-50 age group, accounting for 26 cases (52%). This was followed by 11 women (22%) in the 31-40 age group, and 7 women (14%) in the 18-30 age group.

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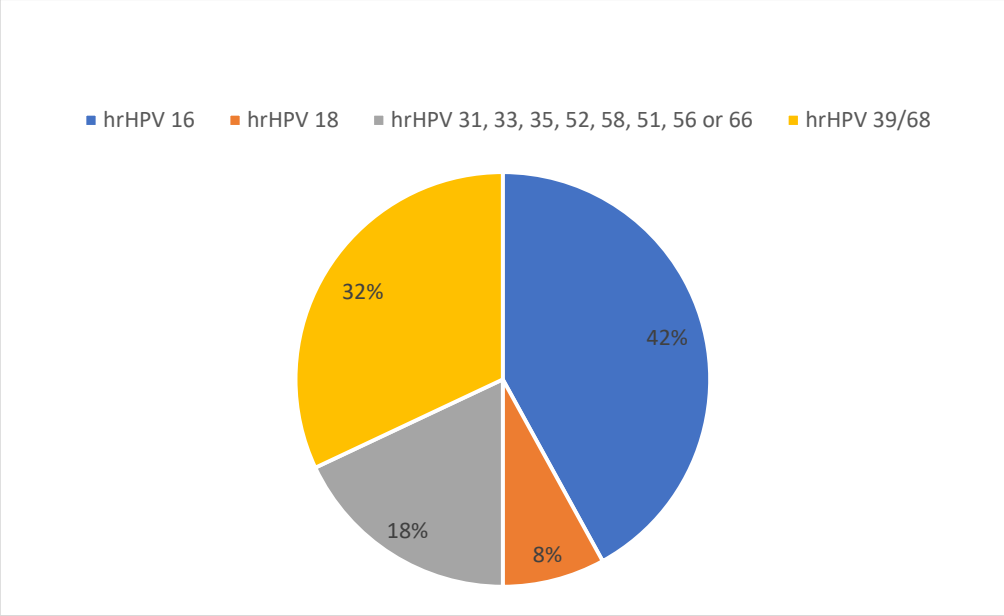
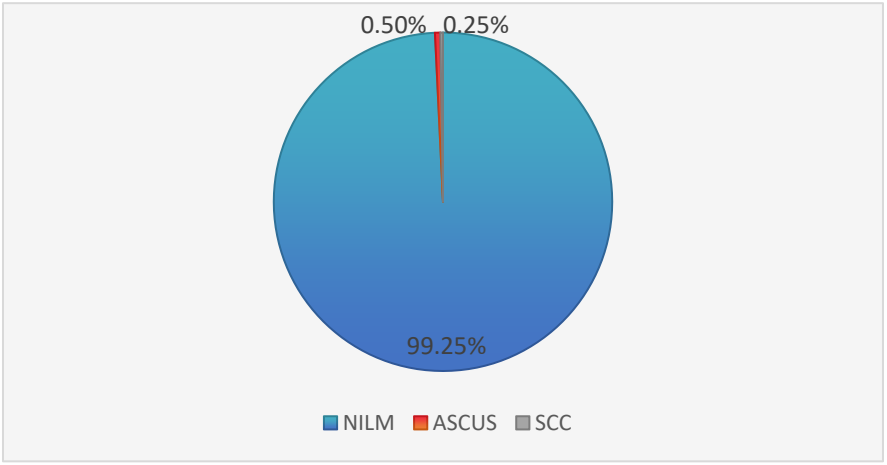
Fig. 2 Distribution of positive HPV DNA subjects by high risk genotypes



hrHPV 16 was the most frequently detected, (21). followed by hrHPV 39/68, (16). Other hrHPV types (31, 33, 35, 52, 58, 51, 56, or 66) identified in 9 cases. least frequently detected HPV 18, (4). No cases of co-infection were reported.

Fig. 3 Pie chart shows the distribution of Pap smear results among the 395 women screened. It categorizes the cases as follows:

- **Squamous Cell Carcinoma (SCC):** 0.25%
- **Atypical Squamous Cells of Undetermined Significance (ASC-US):** 0.50%
- **Negative for intraepithelial malignancy (NILM):** 99.25%



This visualization highlights the relatively small proportion of cases with significant cytological findings.
Out of 395 women screened, 104 women presented for routine screening. Out of these 104 women, HPV DNA was detected in 9 (8.65%) In 291 women presenting with complain, HPV DNA was detected in 41 (14.08%) endo-cervical specimen screened.

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Out of 395 women screened, only 2 were vaccinated against Human Papillomavirus.

DISCUSSION

The findings of this study underscore the critical role of HPV DNA testing as a highly sensitive screening tool in cervical cancer prevention, outperforming traditional Pap smear cytology in both early detection and improved patient outcome.

Age-Related HPV Prevalence

The age distribution of HPV-positive cases in this study revealed a higher prevalence among women aged 41–50 years (52%), followed by those aged 31–40 years (22%) and 18–30 years (14%). This differs from the meta-analysis by Sanjosé et al.[16], which noted peak HPV prevalence in women under 25 years of age, followed by a decline and a second peak in women over 45. The discrepancy may arise from differences in sexual behaviour, screening practices, or HPV vaccination coverage in different regions.Because of majority of our patients presented with symptoms that manifested later in life, variation in age group was observed. Notably, a similar trend was observed in a multicentre study in India, where HPV prevalence peaked in the 40–49 age group[17].

Prevalence and Genotyping of HPV

The prevalence of HPV DNA in this study (12.65%) aligns with recent literature reporting a prevalence range of 5% to 36.4% globally[18-23]. High-risk HPV (hrHPV) 16 was identified as the most predominant genotype, accounting for 42% of total HPV-positive cases. This result is consistent with several studies that highlight HPV 16 as the leading high-risk genotype associated with cervical neoplasia[24-27]. However, variations in hrHPV genotype prevalence were observed. For instance, hrHPV 18 positivity in this study was 8%, higher than reported in the ATHENA trial (0.5%)[28]and findings by Parvez et al. (0.4%)[29]but lower than Vijayaraghavan et al., who reported 22% positivity[30]. These disparities reflect the variable epidemiology of hrHPV genotypes across populations, influenced by geographic, demographic, and behavioural factors.

Sensitivity and Specificity of HPV DNA Testing

This study demonstrated the superior sensitivity (100%) of HPV DNA testing compared to Pap smear cytology, which showed a lower sensitivity (88%). This finding corroborates previous studies indicating that HPV DNA testing can detect up to 95% of cervical precancerous lesions, whereas Pap smear sensitivity ranges from 70–80%[31-34]. However, the specificity of HPV DNA testing may be slightly reduced, as evidenced in this study, where 94% of HPV DNA-positive cases were cytologically negative. This reinforces the need for follow-up protocols, such as colposcopy, to monitor women with high-risk HPV types despite normal cytology [35-37].

Challenges in Implementing HPV DNA Testing

Despite its advantages, the implementation of HPV DNA testing as a primary screening tool faces several challenges. Resource constraints, including the lack of trained personnel and infrastructure in low- and middle-income countries, remain significant barriers. Additionally, the lower specificity of HPV DNA testing necessitates careful consideration to avoid overtreatment in cases of transient HPV infections, particularly in younger women.

Combined Screening Approach

To address these challenges, a dual strategy integrating HPV DNA testing with Pap smear cytology has been proposed. HPV DNA testing could serve as the primary screening tool, with Pap smear triage for positive cases to improve specificity. Such an approach has been endorsed by WHO and other health authorities to balance the sensitivity of HPV testing with the specificity of cytological evaluation, thereby reducing false-positive rates and unnecessary treatments[38].

CONCLUSION

While the sensitivity of HPV DNA testing makes it an invaluable tool for early detection, its lower specificity and implementation challenges in resource-constrained settings necessitate complementary strategies. A combined approach utilizing HPV DNA testing followed by cytological triage could enhance diagnostic accuracy and optimize resource utilization.

Negative HPV-DNA testing provides significant reassurance, allowing for longer screening intervals and reducing unnecessary follow-ups, as women who test negative for high-risk HPV are at very low risk for developing cervical cancer in the near future. On the other hand, positive HPV-DNA testing enables early identification of women at higher risk, allowing for timely monitoring and interventions to prevent the progression of precancerous lesions into cervical cancer. While it is important to note that not all HPV-DNA-positive cases progress to cancer—many infections are transient and resolve naturally—almost all cases of cervical cancer are caused by persistent infections with high-risk HPV (hrHPV) types. This highlights the critical role of HPV DNA testing in effective cervical cancer prevention strategies.

Future research should focus on evaluating the long-term clinical outcomes, cost-effectiveness, and feasibility of widespread HPV-based screening in diverse populations. Expanding these efforts will be pivotal in achieving the global goal of eliminating cervical cancer as a public health problem, particularly in high-burden regions like India.

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