Original Article

Diagnostic Performance of Pap Smear and HPV DNA Co-testing in Cervical Cancer Screening: Experience from a Tertiary Care Hospital in North India

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ABSTRACT

Background: Cervical cancer remains one of the leading causes of cancer-related morbidity and mortality among women in developing countries, including India. Due to the long preinvasive phase of the disease, early detection through screening offers an effective opportunity for prevention. The Papanicolaou (Pap) smear has long been the cornerstone of cervical cancer screening; however, the addition of human papillomavirus (HPV) DNA testing has significantly improved detection of high-grade lesions.

Objective: To evaluate the utilization, cytological findings, and diagnostic performance of Pap smear combined with HPV DNA testing (co-testing) among women attending a tertiary care hospital in North India, and to assess demographic and clinical factors associated with abnormal screening results.

Materials and Methods: This prospective observational study was conducted between March 2021 and August 2022 in a tertiary care hospital in North India. A total of 50 women fulfilling inclusion criteria underwent cervical cancer screening using both Pap smear and HPV DNA testing. Women with abnormal results were further evaluated using colposcopy-guided cervical biopsy. Demographic and clinical variables were compared between screening-positive and screening-negative groups using Chi-square/Fisher's exact test and Mann–Whitney U test. Statistical analysis was performed using SPSS version 23.

Results: Among the 50 women screened, 7 (14%) were identified as positive on co-testing. The mean age of participants was 42.56 ± 8.78 years. Screening positivity was significantly associated with higher age (median 48 vs 41 years, $p = 0.047$) and postmenopausal status (40% vs 7.5%, $p = 0.008$). Histopathological confirmation revealed one case of squamous cell carcinoma and one case of cervical intraepithelial neoplasia grade 2 (CIN-2). HPV DNA positivity was strongly correlated with abnormal cytology ($p < 0.001$).

Conclusion: Co-testing with Pap smear and HPV DNA improves detection of cervical premalignant and malignant lesions. Increasing age and postmenopausal status were significant predictors of screening positivity. Implementation of combined screening strategies may enhance early detection of cervical cancer in tertiary care settings.

Keywords: *Cervical cancer, Pap smear, HPV DNA testing, co-testing, cervical screening.*

INTRODUCTION

Cervical cancer remains a major global health concern and continues to be one of the leading causes of cancer-related morbidity and mortality among women, particularly in low- and middle-income countries. According to the GLOBOCAN 2020 estimates, cervical cancer is the fourth most common cancer among women worldwide, with approximately 604,000 new cases and 342,000 deaths annually (1). India contributes substantially to this burden, with nearly 123,907 new cases and 77,348 deaths reported each year, accounting for a significant proportion of cancer incidence and mortality among Indian women (2).

A distinctive feature of cervical cancer is its prolonged pre-invasive phase, during which premalignant changes develop gradually before progressing to invasive carcinoma. This extended natural history provides an important window of opportunity for early detection and intervention. Effective screening strategies can identify cervical intraepithelial neoplasia (CIN) and treat these lesions before they progress to invasive disease, thereby significantly reducing cervical cancer incidence and mortality (3).

For several decades, the Papanicolaou (Pap) smear test has served as the cornerstone of cervical cancer screening. This cytological method enables microscopic evaluation of exfoliated cervical cells to detect premalignant lesions and early invasive carcinoma. Organized cytology-based screening programs implemented in developed countries have resulted in a marked reduction in cervical cancer incidence and mortality (4). However, despite its advantages, Pap smear screening has certain limitations. The sensitivity of the Pap test for detecting high-grade squamous intraepithelial lesions (HSIL) is reported to range between 70–80%, and false-negative results may occur due to sampling or interpretation errors (5).

Persistent infection with high-risk human papillomavirus (HPV) has been firmly established as the primary etiological factor responsible for the development of cervical cancer. Among more than 200 identified HPV genotypes, approximately 14 are considered oncogenic, with HPV types 16 and 18 accounting for nearly 70% of cervical cancer cases worldwide (6). Consequently, detection of high-risk HPV DNA has emerged as an important tool in cervical cancer screening.

HPV DNA testing detects viral nucleic acids in cervicovaginal samples and has demonstrated higher sensitivity compared with cytology for detecting high-grade cervical lesions (CIN2+) (7). Furthermore, a negative HPV test result provides longer reassurance against the development of significant cervical disease, thereby allowing safe extension of screening intervals (8). However, HPV testing may detect transient infections that may resolve spontaneously and not progress to malignancy, resulting in relatively lower specificity compared with cytology.

To overcome the limitations associated with individual screening methods, a combined approach known as co-testing, which includes both Pap smear cytology and HPV DNA testing, has been recommended in several international screening guidelines. This strategy combines the high sensitivity of HPV testing with the higher specificity of cytology, thereby improving overall diagnostic accuracy (9).

Large randomized controlled trials have demonstrated that HPV-based screening detects more high-grade precancerous lesions at the initial screening compared with cytology alone, and when coupled with appropriate treatment, leads to a reduction in the incidence of invasive cervical cancer (10). Consequently, several international organizations recommend HPV-based screening or co-testing as an effective strategy for cervical cancer prevention.

Despite these advances, cervical cancer screening coverage remains inadequate in many parts of India due to limited awareness, socioeconomic barriers, and restricted access to organized screening programs (11). Opportunistic screening remains the predominant approach in most healthcare settings, and data regarding the effectiveness of co-testing strategies in tertiary care centers are still limited.

Therefore, the present study was undertaken to evaluate the utilization and diagnostic performance of Pap smear combined with HPV DNA testing (co-testing) among women attending a tertiary care hospital in North India. The study also aimed to assess cytological findings, correlate screening results with histopathological confirmation, and identify demographic and clinical factors associated with abnormal screening outcomes.

MATERIALS AND METHODS

Study Design

This prospective observational study was conducted at gynecology OPD of General Hospital, SGPGIMS, Lucknow between March 2021 and March 2022.

Study Population

The study included women attending the gynecology outpatient department who fulfilled the eligibility criteria.

Inclusion Criteria

Sexually active women aged 25–65 years presenting with:
- White discharge per vaginum
- Postcoital bleeding
- Unhealthy cervix on speculum examination
- Willingness to participate and comply with follow-up

Exclusion Criteria
- Women who had undergone total hysterectomy
- Women previously treated for cervical carcinoma or premalignant lesions
- Pregnant women
- Patients with visible malignant growth on the cervix
- Women unwilling to participate

A total of 50 eligible women were included in the study after obtaining written informed consent.

Data Collection

A detailed clinical history was recorded including:
- Age
- Age at marriage
- Age at first childbirth
- Parity
- Contraceptive history
- Sexual history
- Socioeconomic background

General physical examination and systemic examination were performed. A per-speculum examination using Cusco's speculum was carried out to assess cervical appearance.

Sample Collection

Cervical samples were collected as follows:
- Pap smear: Collected using Ayre's spatula and cytobrush. Two smears were prepared, immediately fixed in alcohol, and sent for cytological evaluation according to the Bethesda reporting system.
- HPV DNA testing: A cervical sample was collected using a cervical broom and preserved in fixative medium for testing using the Hybrid Capture II technique.

Further Evaluation

Women with:
- Abnormal Pap smear findings
- Positive HPV DNA results

were referred for colposcopy-guided cervical biopsy for histopathological confirmation.

Statistical Analysis

Data were analyzed using SPSS version 23.
Statistical tests used included:
- Chi-square test
- Fisher's exact test
- Mann–Whitney U test

A p-value < 0.05 was considered statistically significant.

RESULTS

Overall, 7 women (14%) out of 50 women were identified as screening positive by co-testing (Pap smear and HPV DNA). Among these, 2 cases (28.6%) were histologically confirmed as malignant or premalignant lesions (one squamous cell carcinoma and one CIN-2), underscoring the clinical utility of cotesting in early detection.

Table 1: Baseline Characteristics of Study Participants and Their Association With Screening Positivity

Variable	Category		Screening_positive		chi sq	p-value
			No	Yes		
Parity	P 0 or 1	No	4	2	2.12	0.146
		%	67.0%	33.0%		
	P >= 2	No	39	5		
		%	88.6%	11.4%		

LMP	Pre Menopausal	No	37	3	7.02	**0.008**
		%	92.5%	7.5%		
	Post Menopausal	No	6	4		
		%	60.0%	40.0%		
ADDICTION	Yes	No	2	0	0.34	0.56
		%	100.0%	0.0%		
	No	No	41	7		
		%	85.4%	14.6%		
Mode of delivery	Vaginal	No	29	5	0.00	0.970
		%	85.3%	14.7%		
	Cesarean	No	12	2		
		%	85.7%	14.3%		

Parity did not demonstrate a statistically significant association with screening outcomes (p = 0.146). Although nulliparous or primiparous women showed a higher proportion of positivity (33%) compared to multiparous women (11.4%), the small sample size limited statistical power. Likewise, mode of delivery (vaginal vs. cesarean, p = 0.97) and addiction history (tobacco use, p = 0.56) were not significantly correlated with screening results. Menopausal status was strongly associated with abnormal findings: 40% of postmenopausal women screened positive compared to only 7.5% of premenopausal women (p = 0.008).

Table 2: Comparison of Age, Age at Marriage and Age at First Childbirth According to Screening Outcome

Variable	Screening Result				Mann Whitney Test	
	Negative		Positive			
	Mean	SD	Mean	SD	Z-value	p-value
AGE	41.44	7.735	49.43	12.109	-1.988	**0.047**
AGE AT 1ST CHILDBIRTH	26.65	16.774	22.71	3.729	-0.421	0.674
AGE AT MARRIAGE	21.07	4.818	20.14	3.436	-0.449	0.653

Age emerged as a significant determinant of screening positivity. Women with positive results had a higher median age (48 years vs. 41 years, p = 0.047) compared to those with negative results. Mean age for 1st childbirth was 26.65 years in screening negative women and 22.71 years in screening positive women, but p-value is 0.674 means no association was found between age of 1st childbirth with screening result. Mean age at marriage was 21 years in screening negative women and 20 years in screening positive women, p-value is 0.653 showing no association between age at marriage and screening results.
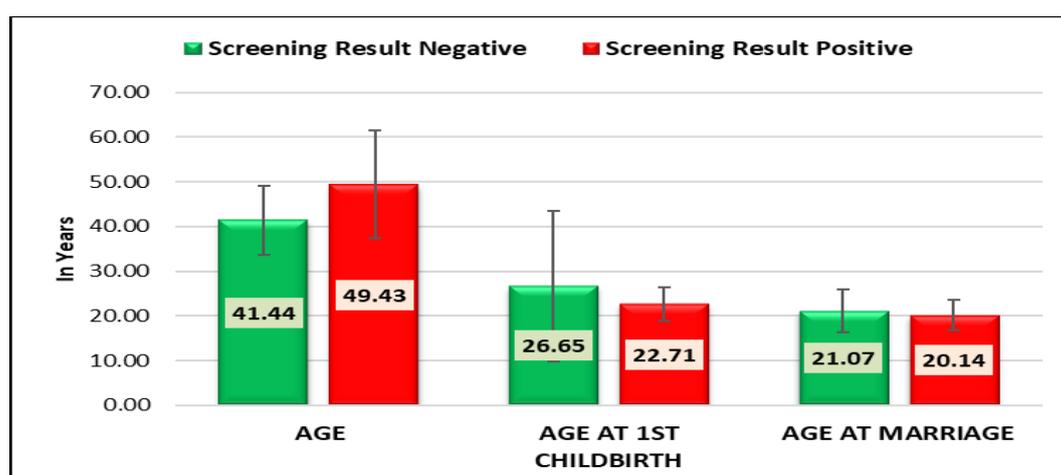

**Table 3: Association Between Presenting Complaints and Screening Positivity**

Variable	Category		Screening positive		chi sq	p-value
			No	Yes		
COMPLAINT	Pain Abdomen	No	4	0	8.28	0.142
		%	100.0%	0.0%		

					chi sq	p-value
AUB	No	17	2			
	%	89.5%	10.5%			
Cervicitis	No	11	2			
	%	84.6%	15.4%			
PMB	No	1	2			
	%	33.3%	66.7%			
PCB	No	3	0			
	%	100.0%	0.0%			
Screening	No	7	1			
	%	87.5%	12.5%			

The most frequent presenting complaint was abnormal uterine bleeding (AUB) (n = 19), followed by cervicitis (n = 13), postmenopausal bleeding (n = 3), and postcoital bleeding (n = 3). Although no overall significant association was observed between presenting complaint and screening positivity (p = 0.142), a noteworthy trend was identified: two-thirds (66.7%) of women with postmenopausal bleeding were screening positive, suggesting that PMB may serve as a clinical red flag for underlying cervical pathology.

Table 4: Association Between HPV DNA Results and Pap Smear Cytology

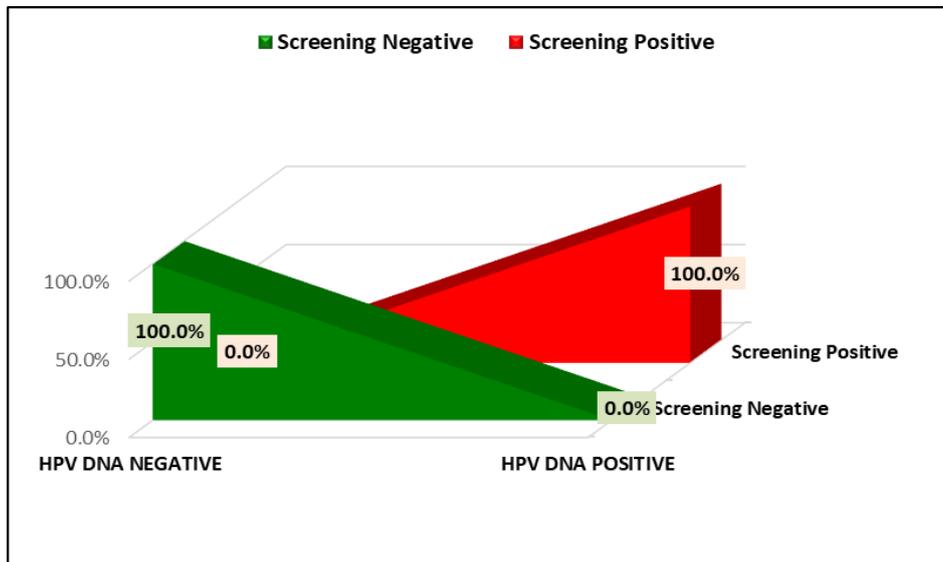| PAP SMEAR		HPV DNA_positive		chi sq	p-value
		No	Yes		
HSIL	No	0	2		
	%	0.0%	100.0%	12.80	<0.001
NILM	No	43	5		
	%	89.6%	10.4%		
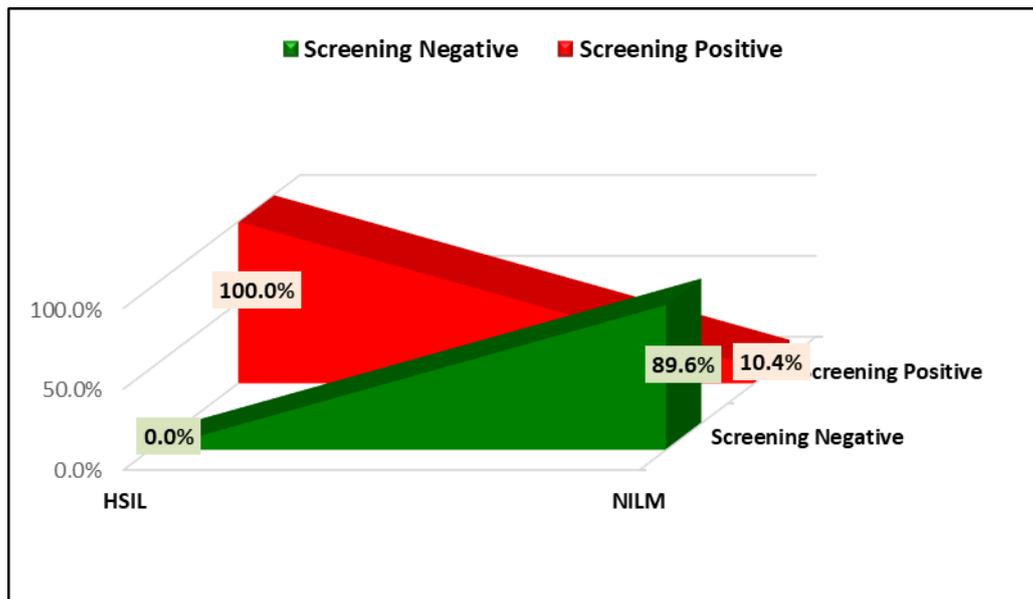
HPV DNA positivity was detected in 7 women (14%). Among these, 2 cases were reported as HSIL on Pap smear, both of which were confirmed on biopsy (CIN-2 and squamous cell carcinoma). The remaining 5 HPV-positive women had NILM cytology; biopsy revealed chronic cervicitis in one case and no dysplasia in the others. Importantly, all abnormal Pap smears were HPV DNA positive, whereas no HPV-negative patient demonstrated cytological abnormality (p < 0.001). This strong correlation underscores the complementary role of HPV testing in enhancing sensitivity, while Pap smear retains higher specificity in confirming clinically relevant lesions.

Table 5: Association Between HPV DNA Status and Overall Screening Outcome

HPV DNA		Screening_positive		chi sq	p-value
		No	Yes		
NEGATIVE	No	43	0	50.00	<0.001

	%	100.0%	0.0%		
POSITIVE	No	0	7		
	%	0.0%	100.0%		

When cotesting was considered (Pap smear + HPV DNA), all screening-positive women were HPV DNA positive, reinforcing the high sensitivity of HPV testing.

However, the presence of false positives (HPV-positive but biopsy-negative cases) highlights the importance of cytological correlation and histopathological confirmation to avoid overtreatment.

These findings highlight the increased vulnerability of older and postmenopausal women to cervical pathology.

Key Interpretations
- Age and menopausal status are significant predictors of screening positivity, aligning with global evidence that cervical cancer risk increases with age.
- HPV DNA testing demonstrated superior sensitivity, detecting all abnormal Pap smears, but with reduced specificity due to false positives.
- Cotesting enhances diagnostic accuracy, with Pap smear providing specificity and HPV DNA offering sensitivity, thereby reducing the risk of missed high-grade lesions.
- Clinical symptomatology such as postmenopausal bleeding may serve as a surrogate marker for higher screening positivity, warranting vigilant evaluation.

DISCUSSION
The present study demonstrates that co-testing with Pap smear and HPV DNA testing improves the detection of cervical premalignant and malignant lesions, with age and menopausal status emerging as significant predictors of screening positivity. These findings are consistent with previous studies conducted both in India and internationally.

Several studies have reported that HPV DNA testing has higher sensitivity compared with cytology alone for the detection of high-grade cervical lesions. A large pooled analysis of randomized trials conducted in Europe demonstrated that HPV-based screening significantly increased detection of CIN2+ lesions compared with cytology (10). Similarly, Castle et al. reported that HPV testing showed superior sensitivity for detecting precancerous cervical lesions, while cytology remained more specific (7).

In the present study, HPV DNA positivity was detected in 14% of women screened. All abnormal Pap smear results were associated with HPV positivity, which highlights the strong correlation between HPV infection and cervical epithelial abnormalities. Similar observations have been reported in several international studies evaluating the performance of HPV-based screening (9).

Age and menopausal status were significantly associated with screening positivity in the present study. Women with abnormal screening results were significantly older compared with those with negative results. This finding aligns with

previous research demonstrating that the risk of cervical neoplasia increases with age due to persistent HPV infection and cumulative exposure to risk factors (10).

Another important observation in this study was the higher proportion of screening positivity among women presenting with postmenopausal bleeding, which is widely recognized as an important clinical warning sign for underlying gynecological malignancy. Previous studies have also emphasized the need for careful evaluation of postmenopausal bleeding due to its association with cervical and endometrial pathology (3).

Overall, the findings of the present study support the growing body of evidence indicating that combined screening using Pap smear and HPV DNA testing enhances diagnostic accuracy and improves early detection of cervical precancerous lesions. The complementary strengths of the two methods—high sensitivity of HPV testing and higher specificity of cytology—make co-testing an effective strategy for cervical cancer screening.

CONCLUSION
The present study demonstrates that co-testing using Pap smear and HPV DNA testing enhances the detection of cervical premalignant and malignant lesions. Age and postmenopausal status were identified as significant predictors of screening positivity.

HPV DNA testing exhibited high sensitivity, detecting all cytological abnormalities, while Pap smear provided greater specificity in identifying clinically relevant lesions. The combination of these modalities improves diagnostic accuracy and reduces the likelihood of missed high-grade lesions.

Given the substantial burden of cervical cancer in India, integration of co-testing into routine screening programs may significantly improve early detection and prevention strategies, particularly in tertiary care settings.

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