Section: Miscellaneous

Original Research Article

OUTCOME OF CYTOLOGY AND HPV DNA TESTING OF CERVICAL CANCER SCREENING IN A TERTIARY CARE CENTRE OF NORTH INDIA

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ABSTRACT

Background: Cervical cancer remains a leading cause of morbidity and mortality among women in low- and middle-income countries, primarily due to persistent infection with high-risk human papillomavirus (HPV) and limited access to organized screening. This study evaluated cytological abnormalities, HPV prevalence, and associated risk factors in women attending a tertiary care hospital in North India.

Materials and Methods: A prospective cross-sectional study was conducted over 18 months at Government Doon Medical College and Hospital, Dehradun. The study received approval from the Institutional Ethics Committee prior to commencement. A total of 250 women aged 25□65 years underwent Pap smear examination, and 125 samples were tested for HPV DNA using PCR. Demographic, clinical, and viral co-infection data (HIV, HBV, HCV, syphilis) were collected and statistically analyzed.

Results: Of the 250 Pap smears, 54% were negative for intraepithelial lesion, while 7.6% showed ASC-US, 4.4% ASC-H, and 6.4% HSIL. Among 125 women tested, 30.4% were HPV-positive, with HPV-16 as the predominant genotype. ASC-US smears showed the highest HPV positivity (42.1%). HPV infection was significantly associated with younger age (25□40 years; p=0.04), early sexual debut (p=0.003), and co-infections with HIV, HBV, HCV, and syphilis (all p<0.01).

Conclusion: The study demonstrates a substantial HPV prevalence in this population, with HPV-16 as the dominant oncogenic strain. Cytology alone failed to detect several HPV infections, highlighting the need for integrated HPV DNA testing in screening programs. Comprehensive strategies combining cytology, HPV testing, vaccination, and sexual health services are essential to reduce the cervical cancer burden in India.

Keywords: Cervical cancer, Human papillomavirus (HPV), Pap smear.

INTRODUCTION

Cervical cancer remains a significant and enduring health issue for women, serving as a prominent indicator of global health inequities. Although highly preventable and largely treatable with early detection, it continues to cause thousands of fatalities annually, particularly in regions with deficient health infrastructure.[1] The World Health Organization (WHO) reports that cervical cancer is the fourth most common cancer among women worldwide, with 604,000 new cases and 342,000 deaths reported in 2020. Over 85% of these deaths occurred in low- and middle-income countries (LMICs).[2,3]

In high-income nations, routine screening and timely interventions have markedly improved survival. By contrast, women in LMICs often rely on opportunistic screening or symptomatic diagnosis due to limited access, inadequate infrastructure, and shortage of trained personnel. As a result, many are diagnosed at advanced stages, leading to high morbidity and preventable mortality. Economic, educational, systemic, and cultural barriers further hinder early detection and consistent treatment.[4,5]

This paradox is striking: cervical cancer is preventable and curable in early stages, yet remains a leading cause of cancer-related mortality worldwide. Unequal access to HPV vaccination, screening, and treatment highlights broader disparities within health systems.[6] Low health literacy, stigma surrounding gynecological care, fragmented services, and insufficient public awareness campaigns reduce the effectiveness of existing interventions, especially in LMICs.[4] The burden extends beyond biology, reflecting systemic failures, gender inequities, and socio-economic limitations.

Cervical cancer also imposes a heavy financial strain in resource-poor settings. Families often face catastrophic healthcare costs due to lack of insurance coverage. Each late diagnosis not only diminishes survival chances but also places a physical, emotional, and economic burden on households, thereby perpetuating cycles of poverty and inequality.[7,8]

The disease is primarily caused by persistent infection with high-risk human papillomavirus (HPV) types, particularly HPV-16 and HPV-18. While most HPV infections resolve spontaneously, persistent infections can progress to precancerous lesions over 10□20 years, providing a critical window for detection and treatment.[9] Unfortunately, many health systems fail to utilize this opportunity effectively. Risk factors such as HIV-related immunosuppression, tobacco use, high parity, and long-term hormonal contraceptive use further accelerate progression, underscoring the need for regular screening.[10]

The introduction of the Pap smear in the mid-20th century revolutionized prevention. Countries that implemented structured cytology-based screening observed a 70□80% reduction in mortality.[11] Pap smears remain simple, cost-effective, and non-invasive, but require robust recall systems. Colposcopy improves diagnostic accuracy after abnormal cytology but is resource-intensive, requiring trained personnel and reliable follow-up.[10,14]

HPV DNA testing has emerged as a more sensitive alternative, particularly for detecting high-risk genotypes. When combined with genotyping, HPV testing allows risk-stratified approaches; women positive for HPV-16 or HPV-18 may be prioritized for intensive triage, even without cytological abnormalities. This strategy improves diagnostic precision and optimizes scarce resources.[10-12] National programs adopting HPV testing benefit from longer screening intervals, lower costs, and improved access through self-sampling in remote or culturally sensitive settings.[11]

Given the strengths and limitations of each method, a combination of approaches tailored to local clinical and resource contexts is essential. The present study seeks to analyze the role of different screening modalities, alongside their clinico-demographic and pathological correlations, to determine the most effective strategy in our setting.

MATERIALS AND METHODS

This hospital-based, prospective, cross-sectional study was carried out in the Department of Pathology and Microbiology at Government Doon Medical College and Hospital, Dehradun, over 18 months. A total of 250 women attending gynecology outpatient clinics and meeting the eligibility criteria were enrolled.

Ethics Approval

The study received approval from the Institutional Ethics Committee prior to commencement.

Study Design: This was a hospital-based, prospective, cross-sectional study designed to evaluate the clinical, cytological, and histopathological characteristics of women presenting with gynaecological symptoms. The cross-sectional approach was selected to provide a comprehensive snapshot of relevant findings within the targeted timeframe. Study Population

Women aged 25□65 years, married and sexually active, were included after obtaining informed consent. Those with a history of cervical cancer or hysterectomy were excluded.

Inclusion Criteria

Participants eligible for inclusion were-

(1) Women willing to participate.
(2) Women older than 21 years of age
(3) Women presenting with one or more of the following complaints: chronic vaginal discharge, lower abdominal discomfort or pain, chronic pelvic pain, bleeding following sexual intercourse, irregular menstruation, bleeding after menopause, or frequent urination with discharge.
(4) Abnormal findings on per speculum examination.

Exclusion Criteria: Exclusion criteria included -

(1) Women not willing for procedures.
(2) Women with previously biopsy-confirmed precancerous or cancerous cervical lesions.
(3) Women with frank visible cervical lesions on examination.
(4) Women who had already received treatment for carcinoma of the cervix.

Data Collection

Demographic and clinical variables such as age, parity, body mass index (BMI), age at first sexual intercourse/marriage, and co-infections including HIV, HBsAg, HCV, and syphilis (VDRL) were recorded.

Cytological Examination

Cervical samples were collected using an Ayre□s spatula and cytobrush, immediately fixed in 95% ethanol, and stained using the Papanicolaou method. Smears were reported according to the Bethesda System (2014).

HPV DNA Testing

Out of the total cohort, 125 cervical samples were subjected to PCR-based HPV DNA testing. The

results were categorized as HPV-positive or HPV-negative.

Statistical Analysis

Associations between cytological findings, demographic/clinical variables, and HPV status were analyzed using appropriate statistical tests. A p value <0.05 was considered statistically significant.

RESULTS

Table 1: Specific Cytological Findings

PAP Smear Finding	No. of Participants (n=250)
Negative	135
ASC-US	19
ASC-H	11
Inflammatory Smear	6
Reactive changes	37
Atrophic smear changes	4
Atrophic Vaginitis	5
HSIL	16
AGUS	1
Altered flora	15

As shown in Table 1, among the 250 women screened, the majority (135; 54%) had negative cytology results. Reactive cellular changes were observed in 37 cases (14.8%), while inflammatory smears accounted for 6 cases (2.4%). Atrophic smear changes and atrophic vaginitis were identified in 4 (1.6%) and 5 (2%) participants, respectively. Abnormal cytological findings included ASC-US in 19 cases (7.6%), ASC-H in 11 cases (4.4%), HSIL in 16 cases (6.4%), and AGUS in 1 case (0.4%). Additionally, altered vaginal flora was detected in 15 cases (6%).

Table 2: HPV DNA

HPV DNA Genotype	No. of Participants (n=125)	Percentage (%)
Positive	38	30.4
Negative	87	69.6

As presented in Table 2, out of 125 women tested for HPV DNA, 38 (30.4%) were positive, while 87 (69.6%) tested negative. This indicates that nearly one-third of the study cohort harbored HPV infection, highlighting its significant prevalence in the screened population.

Table 3: Demographic and Clinical Variables vs HPV status

Variable	HPV-Positive (n=38)	HPV-Negative (n=87)	p-Value
25-40 Age (n)	37	64	0.04*
Age of 1st Sexual Intercourse/Marriage	18.04 ± 0.87	18.76 ± 1.91	0.003*
Under weight (<18.5)	5	5	0.05*
HIV	5	0	0.003*
HBsAg	10	2	0.009*
HCV	9	2	0.007*
VDRL	5	0	0.003*

According to Table 3, analysis of demographic and clinical parameters revealed significant associations with HPV positivity. Most HPV-positive women were aged 25□40 years (97.4%), compared to 73.5% in the HPV-negative group (p=0.04). The mean age at first sexual intercourse or marriage was lower in HPV-positive women (18.04 ± 0.87 years) than in HPV-negative women (18.76 ± 1.91 years; p=0.003). Underweight status showed a borderline association (p=0.05). Co-infections were significantly higher in HPV-positive cases: HIV (13.2% vs 0%, p=0.003), HBsAg (26.3% vs 2.3%, p=0.009), HCV (23.7% vs 2.3%, p=0.007), and VDRL reactivity (13.2% vs 0%, p=0.003). These findings suggest that both sexual and viral co-factors play a role in HPV susceptibility.

Table 4: Correlation of PAP Smear Findings with HPV Genotype Positivity

PAP SMEAR FINDINGS	No of cases	HPV GENOTYPE POSITIVITY	Percentage
REACTIVE CELLULAR CHANGES	37	11	29.7%
ASCUS	19	08	42.1%
ASC-H	11	02	18.1%
HSIL	16	04	25%
AGUS	01	00	-

As illustrated in Table 4, correlation between cytological abnormalities and HPV positivity showed variable trends. HPV DNA was detected in 29.7% of cases with reactive cellular changes and in 25% of HSIL cases. Notably, ASC-US smears showed the highest HPV positivity (42.1%), while ASC-H demonstrated 18.1%. The single AGUS case was negative for HPV. These findings emphasize that even low-grade cytological abnormalities such as ASC-US may carry a considerable risk of underlying HPV infection.

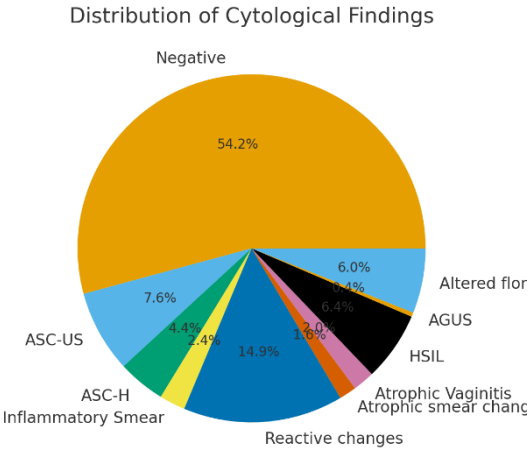

Figure 1: Distribution of Cytological Findings

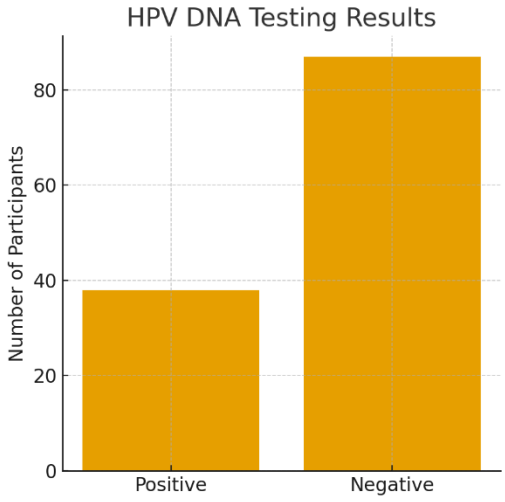

Figure 2: HPA DNA Testing Results

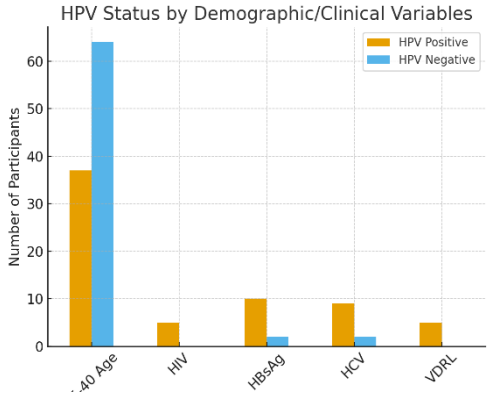

Figure 3: HPV Status By Demographic Variables

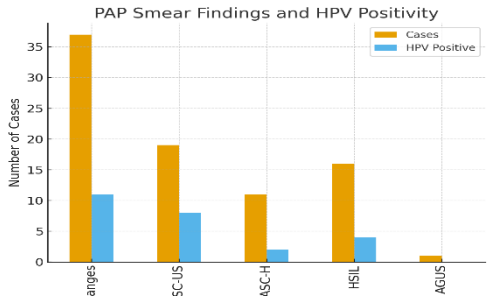

Figure 4: PAP Smear Findings

DISCUSSION

The present study is a hospital-based, prospective cross-sectional study conducted in the Department of Obstetrics and Gynaecology at Government Doon Medical College and associated Doon Hospital, Dehradun, in collaboration with the Departments of Pathology and Microbiology. A total of 250 participants were enrolled over a period of 18 months. The data obtained was analysed statistically and discussed with the bibliographic documents under the headings.

The cytological analysis of the present study revealed that out of 250 pap smears done 54.4% of smears were NILM, while 2.4% showed inflammatory smears. These findings are consistent with Park et al,[1] and Sharma et al,[2] who reported similar proportions of benign and inflammatory smears. These results suggest that the majority of women screened do not have overt cytological abnormalities, but a significant fraction exhibit inflammatory or reactive changes that may warrant close follow-up given the potential association with persistent HPV infection.

The ASC-US rate of 7.6% observed in the present study is somewhat lower than those reported by Singh et al. of 11.2% and Pimple et al. 10.8%. The plausible explanations early age group screening, population.[3-5]

In our study cohort, out of 125 women screened for HPV, 38 women tested positive, while 87 tested negative. HPV positivity was also observed in

women with normal Pap smear results (NILM). This highlights a key limitation of cytology alone in detecting early or latent infections, reinforcing the value of HPV DNA testing for comprehensive screening and also emphasies the utility of molecular testing in conjunction with the cytological screening to further decide the frequency of testing.

The current study evaluated 84 participants with abnormal cytological abnormalities and incidence of HPV genotype positivity in them. A progressive increase in HPV positivity was noted with increasing severity of epithelial abnormalities, consistent with the known etiopathological role of high-risk HPV types in cervical intraepithelial neoplasia.

Among 37 cases with Reactive Cellular Changes (RCC), HPV genotype positivity was noted in 29.7% cases, which may indicate subclinical or latent HPV infection despite benign cytological features. This emphasizes the role of co testing even in seemingly non-neoplastic cases. In the Atypical Squamous Cells of Undetermined Significance (ASCUS) category 42.1% tested positive for HPV. This highlights the clinical dilemma in ASCUS interpretation, where HPV testing plays a vital triage role in determining the need for colposcopic evaluation. In the category of Atypical Squamous Cells□cannot exclude HSIL (ASC-H), 18.2% were HPV-positive. Though the sample size is small, the lower-than-expected positivity rate warrants consideration of differential diagnoses and potential cytological overdiagnosis and the need for colposcopy and biopsy.

In the category of HSIL, 25% were HPV-positive. Although HSIL is generally associated with high-risk HPV infection, the lower positivity rate in our cohort may be due to small sample size or variability in detection

The single case of Atypical Glandular Cells of Undetermined Significance (AGUS) was HPV-negative. Although AGUS is less commonly associated with HPV, especially in glandular pathologies, the absence of HPV further underlines the need for concurrent endometrial evaluation in such cases.

A major strength of our study is the incorporation of HPV genotyping, providing detailed virological insights. The overall HPV positivity rate was 30.4% (38 out of 125 tested women), which is notably higher than the 13.2% rate reported by Pimple et al., likely reflecting differences in cohort selection (symptomatic vs. general population), regional variations in HPV epidemiology, vaccination status, and possibility of higher prevalence of risk behaviors in the sample population.

HPV 16 emerged as the overwhelmingly predominant genotype in our study which is consistent with the global and Indian oncogenic predominance. Its strong association with biopsy-confirmed CIN 1□3 lesions corroborates previous findings that identify HPV 16 as the chief driver of cervical carcinogenesis.

These findings underscore the predominance of HPV 16, but also highlight a diverse genotype profile with co-infections involving high-risk and intermediate-risk strains. Our findings also support stratified screening approaches that prioritize women with HPV 16 positivity for closer surveillance and early intervention, given their higher risk of progression to high-grade lesions.

The analysis of demographic and clinical parameters among HPV-positive and HPV-negative individuals reveals several statistically significant associations, highlighting potential risk factors for HPV infectionA significantly higher proportion of HPV-positive women (97.3%) belonged to the 25□40 years age group compared to 73.6% in the HPV-negative group (p= 0.04), suggesting that sexually active women in this age range may be at higher risk for acquiring HPV. This aligns with global data indicating peak HPV prevalence in younger, sexually active women.

The mean age at first sexual intercourse or marriage was notably lower among HPV-positive women (18.04 ± 0.87 years) than HPV-negative women (18.76 ± 1.91 years), which was statistically significant (p = 0.003). Early sexual debut is a known risk factor for HPV acquisition, likely due to longer exposure duration and vulnerability of the immature cervical epithelium to viral entry.

Our study explored the presence of viral co-infections in the cervical cancer screening cohort and found that 11.2% (28 out of 250 participants) were positive for at least one viral marker. Co-infections were significantly more frequent among HPV-positive women: HIV (13.2% vs. 0%), HBsAg (26.3% vs. 2.3%), HCV (23.7% vs. 2.3%), and VDRL reactivity (13.2% vs. 0%). This substantial co-infection burden is clinically relevant, as chronic viral infections can modulate immune responses and potentially influence HPV persistence and neoplastic progression. This finding suggests that HPV screening programs in India should be operated in integration with comprehensive sexual health services.

CONCLUSION

This study demonstrates a high prevalence of HPV infection (30.4%) among women screened, with HPV 16 as the predominant genotype. Cytology alone missed latent infections, underscoring the value of integrating HPV DNA testing with Pap smears for more accurate detection. ASC-US smears showed the highest HPV positivity, confirming the role of HPV testing as a critical triage tool. Significant associations with younger age, early sexual debut, and viral co-infections highlight multifactorial risks for persistence. Strengthening cervical cancer prevention in India requires combined HPV-based screening, timely vaccination, and integration with sexual health services to reduce disease burden.

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