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Original Research Article

HPV DNA TESTING AS A PRIMARY TOOL FOR CERVICAL SCREENING IN THE COMMUNITY AND ITS CORRELATION WITH CERVICAL CYTOLOGY

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ABSTRACT

Background: Cervical cancer remains one of the leading causes of cancer-related morbidity and mortality among women worldwide. Early detection of cervical epithelial abnormalities through screening methods such as the Papanicolaou (Pap) smear and Human Papillomavirus (HPV) DNA testing plays a crucial role in identifying precancerous lesions and preventing disease progression. **Objectives:** To evaluate the prevalence of cervical cytological abnormalities, determine HPV DNA positivity, and analyze the correlation between HPV DNA detection and Pap smear findings among women undergoing cervical cancer screening.

Materials and Methods: This cross-sectional study included **286 women** who underwent cervical screening. Data were analyzed with respect to demographic characteristics, parity, menstrual history, presenting complaints, per speculum examination findings, Pap smear results, and HPV DNA detection. Cytological findings were classified according to standard Pap smear reporting, and HPV DNA testing was performed to determine viral positivity. Statistical analysis was conducted to assess the association between cytological abnormalities and HPV DNA positivity.

Results: Most participants were 41–45 years old, and multiparity (≥3 deliveries) was common. The most frequent symptom was white discharge per vagina, and 22% had an unhealthy cervix on examination. Pap smear showed normal cytology in 52.4%, while 47.6% had abnormalities, mainly inflammatory changes with few cases of ASCUS, LSIL, and HSIL. HPV DNA positivity strongly correlated with abnormal cytology, with all ASCUS, LSIL, and HSIL cases testing HPV positive. The association between abnormal Pap smear findings and HPV DNA positivity was highly statistically significant (χ^2 = 221.45, $p < 0.0001$).

Conclusion: The study demonstrates a strong correlation between cervical cytological abnormalities and HPV DNA positivity. Combined screening with Pap smear and HPV DNA testing enhances the detection of premalignant cervical lesions and can improve early diagnosis and prevention strategies for cervical cancer.

Keywords: Cervical cancer screening, Pap smear, Human Papillomavirus Deoxyribonucleic Acid(HPV DNA), Atypical Squamous Cells of Undetermined Significance(ASCUS), Low-Grade Squamous Intraepithelial Lesion(LSIL), High-Grade Squamous Intraepithelial Lesion(HSIL).

INTRODUCTION

Cervical cancer is a significant contributor to cancer-related deaths among women and stands as a leading cause of morbidity and mortality worldwide. It is the second most prevalent cancer in women from less developed regions, accounting for approximately 570,000 new cases in 2018, which represents 84% of the global incidence. Screening for cervical cancer has been widely recommended due to its potential to reduce associated mortality and morbidity, as noted in prior research studies. Various screening methods have been developed, including the Papanicolaou (Pap) smear, liquid-based cytology (LBC), and human papillomavirus (HPV) testing.[1,2]

The effectiveness of the traditional Pap smear shows considerable variation, potentially impacting the detection rates of high-grade cervical intraepithelial neoplasia (CIN). To address these limitations, LBC was introduced, offering advantages such as faster reporting, reduced likelihood of missed cases, and the possibility of HPV testing from residual fluid. LBC was first approved by the US Food and Drug Administration (USFDA) in 1996 with the Thin Prep® system. Since its introduction, LBC has gained widespread acceptance, particularly in the United States, where it is now employed in over 90% of cases. However, a small percentage of false-negative results remain a challenge.[3,4]

In recent years, HPV DNA testing has emerged as a molecular-based method with greater sensitivity for assessing cervical cancer risk. While promising as a primary screening tool, especially in developing countries, its implementation is still experimental due to high costs.[12] High-risk HPV testing is highly sensitive, although its specificity depends on the frequency of screening and subsequent evaluation protocols.[5]

This study aims to assess and compare the diagnostic efficacy of LBC and HPV testing in detecting both pre-invasive and invasive cervical lesions. The findings could provide valuable insights into optimizing the use of HPV DNA testing as a complementary screening method for cervical cancer.

MATERIALS AND METHODS

Study Design: Prospective study.

Study Area: The present study was conducted in villages under rural health training centre, pasumamula, KAMSRC.

Study Population: Women aged between 35 - 55 years of age who attending to the outpatient department. In villages under rural health training centre, pasumamula, KAMSRC

Study Period: The study was carried out for a period of 24 months ie from March 2023 to March 2025.

Sample Size:

Sample size = 286.

Inclusion Criteria: Women aged between 35 to 55 years.

Exclusion Criteria

1. Women who are pregnant.
2. Women with history of malignancy.
3. Women who didn't give consent for the study.

Method of data collection:

Institutional ethical committee clearance was obtained prior to the start of study. A written and informed consent was taken from women who are participating in the present study. Women were educated about the need of HPV screening and the detailed information regarding the tests conducted were explained before taking consent.

Using a predesigned, semi-structured questionnaire socio-demographic information was collected. Cervical cytology and HPV DNA sampling was carried out with the help of Brush by rotating at 360° in and outside the surface of cervix. The acquired sample was immediately spread on a grease-free slide with a label and fixed with 95% ethanol before being transported to a lab for cytological analysis using the Bathesda system of categorization (2014). Scraped cell material was then transferred to a preserving solution and kept at 4°C until it was transported to a molecular lab for Conventional polymerase chain reaction genotypic HPV detection The phenol-chloroform extraction procedure was used to get DNA.

Data Analysis: Data collected was entered into MS-Excel 2013 spreadsheet. The collected data was analyzed using IBM statistical package for social sciences (IBM SPSS) version 24 software (trail version)

Statistical tests: Continuous variables were reported as mean ± standard deviation (SD) while categorical variables were expressed as absolute values and percentages.

RESULTS

Table 1: Distribution of Participants by demographic

Age (years)	Frequency	Percentage
35–40	50	17.5%
41–45	115	40.2%
46–50	81	28.3%
51–55	40	14.0%
Total	**286**	**100%**
Parity		
Nulliparous	9	3.1%
P2	80	28.0%

P3	103	36.0%
P4	56	19.6%
>P5	38	13.3%
Menstrual History		
Regular	228	79.7%
Irregular	32	11.2%
Postmenopausal	26	9.1%

The majority of participants belonged to the 41–45 years age group (40.2%), followed by 46–50 years (28.3%), indicating that most women screened were in their early to mid-forties.

Most participants were multiparous, with parity 3 (36%) being the most common, suggesting that a large proportion of women had multiple pregnancies. The majority of women (79.7%) had regular menstrual cycles, indicating that most participants were in the reproductive age group.

Table 2: White Discharge per Vagina (WDPV) and Cervical Appearance

WDPV	Frequency	Percentage
Yes	120	42%
No	166	58%
Total	**286**	**100%**
Cervical Appearance		
Healthy	223	78%
Unhealthy	63	22%
Total	**286**	**100%**

White discharge per vagina was reported by 42% of participants, suggesting a common gynecological complaint among the screened population. On per speculum examination, 22% of women had an unhealthy cervix, indicating the need for further diagnostic evaluation.

Table 3: Distribution Based on Presenting Complaints

Presenting Complaint	Frequency	Percentage
Abnormal uterine bleeding	34	11.9%
Postmenopausal bleeding	10	3.5%
Postcoital bleeding	6	2.1%
White discharge per vagina	236	82.5%

White discharge per vagina was the most common presenting complaint (82.5%), followed by abnormal uterine bleeding (11.9%).

Table 4: Findings on Per Speculum Examination

Findings	Frequency	Percentage
Old tear	26	9.1%
Ulcer	61	21.3%
Polyp	6	2.1%
Unhealthy vagina	31	10.8%
Discharge present	120	42%
Bulky uterus	16	5.6%
Normal adnexa	286	100%

The most common finding was vaginal discharge (42%), followed by cervical ulceration (21.3%).

Table 5: Distribution Based on Pap Smear Findings

Pap Smear Finding	Frequency	Percentage
ASCUS	20	7.0%
HSIL	1	0.3%
LSIL	3	1.0%
Inflammatory	54	18.9%
Inflammatory – BV	36	12.6%
Inflammatory – Candida	10	3.5%
Inflammatory – Squamous metaplasia	5	1.7%
Inflammatory – Trichomonas	6	2.1%
Negative	151	52.8%
Total	**286**	**100%**

Most participants (52.8%) had negative cytology, while 8.3% showed premalignant lesions (ASCUS, LSIL, HSIL).

Table 6: Correlation Between HPV DNA and Pap Smear Findings

Pap Smear Finding	HPV Positive	HPV Negative	Total
ASCUS	20	0	20
HSIL	1	0	1
Inflammatory	3	51	54
BV	0	36	36
Candida	0	10	10
Squamous metaplasia	1	4	5
Trichomonas	0	6	6
LSIL	3	0	3
Negative cytology	0	151	151
Total	**28**	**258**	**286**

Chi-square test = 221.45, p < 0.0001 (statistically significant)

HPV DNA was detected in 9.8% of participants, indicating the presence of HPV infection in a small but significant proportion of the study population. A strong association was observed between abnormal cytology (ASCUS, LSIL, HSIL) and HPV DNA positivity, indicating that HPV infection is strongly linked with premalignant cervical lesions.

DISCUSSION

The present study found that the highest proportion of participants were in the 41–45 years age group (40.2%), followed by 46–50 years (28.3%), 35–40 years (17.5%), and 51–55 years (14%). This distribution is consistent with the findings of Nwesar et al,[6] who reported a mean age of 42.6 years among women undergoing cervical screening. Similarly, Gupta et al,[7] noted that the majority of women with cervical abnormalities belonged to the 40–50 years age group, highlighting that women in their forties are at a higher risk for developing cervical intraepithelial neoplasia (CIN) and invasive carcinoma. Bhaksi et al,[8] emphasized that the peak prevalence of high-risk HPV (HR-HPV) infections and cervical dysplasia is observed in women aged 40–50 years, particularly in populations with limited access to routine screening. These findings suggest that women in their forties are at a critical age where cervical cancer screening should be actively promoted.

Parity, or the number of pregnancies carried to viability, is an important risk factor for cervical cancer. The present study found that multiparity was common, with 36% (103 participants) having three deliveries (P3), 28% (80 participants) having two (P2), and 32.9% (94 participants) having four or more pregnancies (P4 and >P5). Only 3.1% (9 participants) were nulliparous. These findings align with Nwesar et al,[6] who reported that high parity (≥3 pregnancies) was significantly associated with HPV positivity, with 60% of HPV-positive women in their study having at least three full-term deliveries. Gupta et al,[7] also reported that women with multiple pregnancies were at higher risk of developing chronic cervicitis, HPV infections, and cervical dysplasia. Bhaksi et al,[8] further supported this association by noting that each additional pregnancy increases the risk of HPV persistence, as hormonal changes and cervical trauma during delivery may contribute to HPV infection progressing to dysplasia. This emphasizes the need for routine screening in multiparous women, particularly those with three or more deliveries.

The present study observed that 79.7% (228 participants) had regular menstrual cycles, 11.2% (32 participants) had irregular cycles, and 9.1% (26 participants) were postmenopausal. This is similar to findings from Gupta et al,[7] where postmenopausal women accounted for 10% of the study population and had a higher prevalence of HPV infections and cervical abnormalities. Bhaksi et al,[8] highlighted that postmenopausal women are more prone to developing cervical atrophy, which may lead to diagnostic difficulties in Pap smear screening. Additionally, hormonal fluctuations in premenopausal women may contribute to an increased risk of persistent HPV infection. Therefore, postmenopausal women should not be excluded from routine cervical cancer screening programs.

The present study found that 42% (120 participants) had white discharge per vagina (WDPV), while 58% (166 participants) did not report this symptom. This aligns with Nwesar et al,[6] who found that 48% of HPV-positive women presented with vaginal discharge. Gupta et al,[7] also reported that 78% of HPV-positive women had persistent vaginal discharge, which was significantly associated with chronic cervicitis and CIN. White discharge is often linked to bacterial vaginosis, Candida infections, and Trichomonas vaginalis, as evidenced by Pap smear findings. Bhaksi et al,[8] noted that persistent WDPV should be evaluated through cytology and HPV DNA testing, as it may indicate underlying precancerous lesions.

Among the presenting complaints, the present study found that WDPV was the most common symptom (82.5%), followed by abnormal uterine bleeding (AUB) (11.9%), postmenopausal bleeding (3.5%), and post-coital bleeding (2.1%). Gupta et al,[7] similarly found that AUB and WDPV were the most common complaints among HPV-positive women, with AUB affecting 14% of cases. Nwesar et al,[6] reported intermenstrual bleeding in 10% of HPV-positive cases and emphasized that post-coital bleeding is a significant red flag for cervical dysplasia and malignancy

The present study found that 78% of participants had a healthy cervix, while 22% had an unhealthy cervix. In comparison, Gupta et al,[7] found that 30% of HPV-positive women had an unhealthy cervix,

suggesting that a clinical speculum examination is crucial for early detection. Bhaksi et al,[8] emphasized that visual inspection methods, such as VIA and VILI, are effective in detecting cervical pathology and should be used in resource-limited settings.

Most participants (52.8%, 151 individuals) had normal cytology. Inflammatory changes were common: 18.9% had non-specific inflammation, 12.6% had bacterial vaginosis, 3.5% had Candida, 1.7% had squamous metaplasia, and 2.1% had Trichomonas vaginalis. Epithelial abnormalities included 7.0% with ASCUS, 1.0% with LSIL, and 0.3% with HSIL.

Nwesar et al,[6] reported a higher prevalence of cytological abnormalities, with ASCUS in 37%, LSIL in 18%, and HSIL in 11% of cases. Bhaksi et al,[8] noted that the sensitivity of Pap smear for detecting CIN2+ lesions varies widely from 39% to 88%, underscoring the need for HPV DNA testing as an adjunct screening tool.

In the present study, HPV DNA testing was positive in 9.8% (28 participants), while 90.2% (258 participants) tested negative. Gupta et al,[7] reported an HPV DNA positivity rate of 21.8% among women undergoing cervical screening Nwesar et al,[6] found that 21% of their study participants tested positive for HPV DNA. Bhaksi et al,[8] highlighted that HPV prevalence varies globally, with developing countries reporting higher rates due to lack of widespread vaccination and organized screening programs.

A notable aspect of the present study is the HPV DNA positivity rate of 25.9%, which is higher than the 21% reported by Nwesar et al,[6] but within the range of similar studies. The slightly higher prevalence in the present study could be attributed to differences in sample population characteristics, sexual activity, and screening techniques. The HPV DNA test is crucial for detecting high-risk HPV types that are associated with cervical neoplasia. Gupta et al,[7] reported that sensitivity of HPV DNA testing was 90%, and specificity was 84.61%, making it a valuable tool in early detection of pre-invasive lesions. Bhaksi et al,[8] emphasized that HPV DNA testing is more sensitive (90–97%) than cytology (64–89%) in detecting CIN2+ lesions. This is in agreement with the present study, where HPV DNA testing significantly correlated with cytological abnormalities, reinforcing its role in cervical cancer screening.

The findings of the present study highlight the importance of incorporating HPV DNA testing alongside Pap smear cytology for effective cervical cancer screening. Several studies have demonstrated that HPV testing has higher sensitivity for detecting high-grade cervical intraepithelial lesions (CIN2+) compared with cytology alone. Therefore, combined screening (co-testing) improves early detection of precancerous lesions and reduces the risk of progression to invasive cervical cancer.[9,10,11]

CONCLUSION

The present study demonstrates that HPV DNA testing significantly enhances the sensitivity of cervical cancer screening when combined with Pap smear cytology. Women in the 40–50 years age group showed a higher prevalence of cervical abnormalities, indicating the importance of prioritizing routine screening in this population. High parity (≥3 deliveries) was also associated with increased HPV positivity and abnormal cytological findings, suggesting its role as a potential risk factor for cervical pathology. A strong statistical association between HPV DNA positivity and premalignant lesions (ASCUS, LSIL, and HSIL) highlights the value of HPV testing in identifying women at risk for developing high-grade cervical lesions. Furthermore, the high negative predictive value of HPV testing indicates that women who test negative for HPV may safely undergo screening at longer intervals. Therefore, incorporating HPV DNA testing alongside Pap smear screening (co-testing) can improve early detection of cervical precancerous lesions, particularly in women above 30 years of age. Strengthening public health awareness, promoting HPV vaccination, and implementing regular cervical screening programs are essential strategies for reducing the burden of cervical cancer.

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