Role of HPV Genotyping and p16 Expression in the Detection of Precancerous Cervical Lesions

Noor-E-Ferdous[1], Nigar Sultana[2*], Khodeza Khatun[3], Farzana Sharmin[4], Latifa Akter [5], Zaheen Naveed Haque[6]

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*Corresponding author

ABSTRACT

Background: Persistent infection with high-risk human papillomavirus (HPV) is the primary cause of cervical cancer. Although HPV DNA testing is widely used for screening, its specificity for high-grade cervical lesions is limited. p16 immunohistochemistry has emerged as a surrogate marker of transforming HPV infection. This study aimed to evaluate the role of HPV genotyping and p16 expression in detecting precancerous cervical lesions, particularly high-grade squamous intraepithelial lesions (HSIL). **Methods & Materials**: This cross-sectional study included 72 screen-positive women aged 30–60 years attending the colposcopy clinic of Bangladesh Medical University (BMU), Shahbagh, Dhaka in 2022-2023. Colposcopy-directed cervical biopsy and/or LEEP specimens were examined histopathologically as the gold standard. p16 immunohistochemistry was performed and interpreted as diffuse (positive) or focal/negative. HPV genotyping was conducted to detect high-risk HPV types. Diagnostic performance indicators including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated. **Results**: Histopathology confirmed HSIL (CIN II/III) in 61.1% of cases. Diffuse p16 expression was significantly associated with HSIL ($p < 0.001$; OR 142.7, 95% CI 16.6–1229.1). p16 demonstrated high sensitivity (84.1%) and specificity (96.4%), with PPV of 97.4%, NPV of 79.4%, and overall accuracy of 88.9%. In contrast, HPV genotyping was not significantly associated with HSIL ($p = 0.675$) and showed lower diagnostic performance. **Conclusion**: Diffuse p16 expression shows superior diagnostic performance compared with HPV genotyping alone for detecting high-grade precancerous cervical lesions. Incorporating p16 immunohistochemistry into routine diagnostic evaluation may enhance risk stratification and reduce overtreatment.

Keywords: *Cervical intraepithelial neoplasia (CIN); p16 immunohistochemistry; Human papillomavirus (HPV); High-grade squamous intraepithelial lesion (HSIL); Cervical cancer screening.*

1. *Associate Professor, Department of Gynecological Oncology, Bangladesh Medical University (BMU), Dhaka, Bangladesh (ORCID: 0000-0003-1793-0792)*
2. *Associate Professor, Department of Obstetrics and Gynaecology, Bangladesh Medical University, Dhaka, Bangladesh (ORCID: 0000-0003-1793-0792)*
3. *Associate Professor, Department of Obstetrics and Gynaecology, Bangladesh Medical University, Dhaka, Bangladesh*
4. *Associate Professor, Department of Gynecological Oncology, Bangladesh Medical University (BMU), Dhaka, Bangladesh*
5. *Associate Professor, Department of Obstetrics and Gynaecology, Bangladesh Medical University, Dhaka, Bangladesh*
6. *Medical Student, Holy FamDily Red Crescent Medical College, Dhaka, Bangladesh*

INTRODUCTION

Cervical cancer remains one of the leading causes of cancer-related morbidity and mortality among women worldwide, particularly in low- and middle-income countries (LMICs). Persistent infection with high-risk human papillomavirus (HPV), especially genotypes 16 and 18, is well established as the primary etiological factor in the development of cervical intraepithelial neoplasia (CIN) and invasive cervical carcinoma [1]. Although most HPV infections are transient and resolve spontaneously, persistent high-risk HPV infection can lead to the development of precancerous cervical lesions, which may progress to invasive cancer if left untreated [2]. Early detection of precancerous lesions, particularly high-grade squamous intraepithelial lesions (HSIL; CIN II/III), is crucial for preventing progression to invasive disease. Conventional cytology (Pap smear) has significantly reduced cervical cancer incidence in many countries; however, its sensitivity is limited and subject to interobserver variability. As a result, adjunctive biomarkers have been increasingly investigated to improve diagnostic accuracy and risk stratification [3]. HPV DNA testing has emerged as a highly sensitive screening tool for detecting high-risk HPV infection. While HPV testing is valuable for identifying women at risk, its specificity for predicting high-grade lesions is relatively low, as many infections are transient and not associated with clinically significant disease. Therefore, additional biomarkers that reflect transforming HPV infection are needed to distinguish transient infections from lesions with malignant potential [4]. p16INK4a (p16) is a cyclin-dependent kinase inhibitor that is overexpressed in cells affected by oncogenic HPV due to inactivation of the retinoblastoma (Rb) pathway by the viral E7 oncoprotein. Diffuse, strong p16 expression is considered a surrogate marker of transforming HPV infection and is strongly associated with high-grade cervical lesions. Immunohistochemical detection of p16 has therefore been incorporated into routine diagnostic practice to improve the accuracy and reproducibility of CIN grading [5-7]. Despite the established roles of HPV genotyping and p16 expression and their relative diagnostic performance in detecting precancerous cervical lesions remain areas of ongoing investigation, particularly in resource-constrained settings. This study aimed to evaluate the role of HPV genotyping and p16 immunohistochemical expression in the detection of precancerous cervical lesions and to assess their diagnostic accuracy in identifying high-grade disease.

METHODS & MATERIALS

Study Design and Setting

This cross-sectional observational study was conducted at the Colposcopy Clinic of Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbagh, Dhaka, from October 2022 to March 2023.

Study Population and Sampling

The study population comprised screen-positive women (VIA, Pap smear, and/or HPV DNA positive) aged 30–60 years who were referred to the colposcopy clinic and

had colposcopically suspected high-grade squamous intraepithelial lesions (HSIL). Purposive sampling was used to recruit eligible participants. Women who were pregnant or lactating, had a history of hysterectomy, known cervical cancer, prior treatment for CIN, or refused consent were excluded. A total of 72 eligible women were enrolled in this study.

Study Procedure

After obtaining informed written consent, demographic, obstetric, and clinical information were collected using a semi-structured questionnaire. Colposcopy was performed using the Swede scoring system [8]. Colposcopy-directed punch biopsy or Loop Electrosurgical Excision Procedure (LEEP) specimens were obtained from suspected HSIL cases. Histopathological examination served as the reference standard. Cervical intraepithelial neoplasia was graded as CIN I (LSIL) and CIN II/III (HSIL) [9].

p16 Immunohistochemistry

Formalin-fixed paraffin-embedded tissue blocks were sectioned at 4 µm and mounted on poly-L-lysine–coated slides. Immunohistochemical staining for p16INK4a was performed using monoclonal mouse anti-human p16 antibody (E6H4 clone, DAKO, Denmark) with EnVision secondary detection system. Diffuse, strong nuclear with or without cytoplasmic staining extending from the basal layer upward was interpreted as positive (block-positive). Focal, patchy, or absent staining was considered negative according to LAST criteria [10].

HPV Genotyping

HPV genotyping was performed to detect high-risk HPV types, particularly HPV 16 and 18 [11].

Outcome Measures and Statistical Analysis

The primary outcome was histologically confirmed HSIL (CIN II/III). Diagnostic performance of p16 expression and HPV genotyping was assessed by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy.

Data were analyzed using SPSS version 26. Descriptive statistics were expressed as mean ± standard deviation or frequency and percentage. Associations were evaluated using chi-square tests, and $p < 0.05$ was considered statistically significant.

Ethical Considerations

Ethical approval was obtained from the IRB of BSMMU. Written informed consent was obtained from all participants. Confidentiality was maintained throughout the study.

RESULTS

A total of 72 women were included in the study. The majority of participants were aged 30–39 years (45.8%), followed by 40–49 years (36.1%), while 18.1% were aged 50 years or older. The mean age was 40.96 ± 7.69 years (range: 30–60 years). *Table I* shows that 16.7% had CIN I, 27.8% CIN II, and 33.3% CIN III, with HSIL (CIN II/III) comprising 61.1% of cases. Benign lesions accounted for 22.2%. Diffuse p16 expression was observed in 52.8% of cases, while HPV DNA was detected in 16.7%, predominantly HPV 16 (15.3%), with HPV 18 identified in 1.4%.

Table I
Distribution of histopathological diagnoses, p16 immunohistochemistry expression, and HPV genotyping results among study participants (n = 72).

Variable	Frequency (n=72)	Percentage (%)
Histopathology		
CIN I	12	16.7
CIN II	20	27.8
CIN III	24	33.3
Chronic cervicitis	6	8.3
Chronic cervicitis with squamous metaplasia	10	13.9
P16 IHC		
Diffuse/full thickness	38	52.8
Negative	22	30.6
Focal/patchy	12	16.7
HPV genotyping		
Not detected	60	83.3
HPV 18	1	1.4
HPV 16	11	15.3

In *Table II*, a strong association was observed between diffuse p16 expression and HSIL ($p < 0.001$). Among p16-positive cases, 84.1% had HSIL, compared with 15.9% among p16-negative cases. Diffuse p16 positivity significantly increased the odds of high-grade lesions (OR 142.7; 95% CI: 16.6–1229.1). In contrast, HPV genotyping was not significantly associated with HSIL ($p = 0.675$; OR 1.26; 95% CI: 0.43–3.67).

Table II
Association of HPV DNA and p16 expression with high-grade cervical lesions (n=72).

	Histopathology		p value OR (95%CI)
	HSIL (n=44)	LSIL/Benign (n=28)	
P16 IHC			
Positive (n=38)	37 (84.1)	1 (3.6)	<0.001
Negative (n=34)	7 (15.9)	27 (96.4)	142.7 (16.6 –1229.1)
HPV genotyping			
HPV 16/18/others (n=20)	13 (29.5)	7 (25.0)	0.675
Not detected (n=52)	31 (70.5)	21 (75.0)	1.26 (0.43-3.67)

As shown in *Table III*, diffuse p16 expression demonstrated high sensitivity (84.09%; 95% CI: 69.93–93.36) and specificity (96.43%; 95% CI: 81.65–99.91) for detecting CIN II/III. The positive predictive value (PPV) was 97.37%, negative predictive value (NPV) was 79.41%, and overall diagnostic accuracy was 88.89%. HPV DNA testing showed lower diagnostic performance, with sensitivity of 29.55% (95% CI: 16.76–45.20), specificity of 75.00% (95% CI: 55.13–89.31), and overall accuracy of 47.22%. Overall, diffuse p16 expression demonstrated superior diagnostic performance compared with HPV genotyping for detecting high-grade cervical lesions.

Table III
Diagnostic performance of p16 Expression and HPV DNA for detecting high-grade cervical lesions (CIN II/III) (*n*=72).

Diagnostic Parameter	p16 Diffuse % (95% CI)	HPV DNA % (95% CI)
Sensitivity	84.09 (69.93–93.36)	29.55 (16.76–45.20)
Specificity	96.43 (81.65–99.91)	75.00 (55.13–89.31)
PPV	97.37 (84.32–99.61)	65.00 (45.80–80.32)
NPV	79.41 (66.08–88.42)	40.38 (33.71–47.44)
Overall accuracy	88.89 (79.28–95.08)	47.22 (35.33–59.35)

DISCUSSION

This study evaluated the diagnostic role of HPV genotyping and p16 immunohistochemical expression in detecting precancerous cervical lesions, particularly high-grade squamous intraepithelial lesions (HSIL). Histopathology confirmed HSIL (CIN II/III) in 61.1% of cases, highlighting the high burden of clinically significant lesions among colposcopy-suspected women.

A key finding of this study is the strong association between diffuse p16 expression and HSIL. Diffuse p16 positivity was significantly associated with high-grade lesions ($p < 0.001$), with an odds ratio of 142.7. The diagnostic performance of p16 was notable, demonstrating high sensitivity (84.1%) and excellent specificity (96.4%), along with a high positive predictive value (97.4%). These findings support the established biological role of p16 as a surrogate marker of transforming high-risk HPV infection. Overexpression of p16 occurs due to inactivation of the retinoblastoma (pRb) pathway by the HPV E7 oncoprotein, leading to deregulated cell cycle progression. Therefore, diffuse "block-positive" staining reflects oncogenic HPV activity and correlates strongly with high-grade dysplasia [12].

Our findings are consistent with previous studies demonstrating that p16 immunostaining improves diagnostic accuracy and reduces interobserver variability in grading CIN lesions [13-15]. Several authors have reported comparable sensitivity and specificity values for p16 in detecting CIN II/III, reinforcing its value as an adjunctive diagnostic tool, particularly in morphologically equivocal cases [15-17]. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions (LAST) recommendations also endorse p16 immunostaining to distinguish HSIL from LSIL when histological interpretation is uncertain [18].

In contrast, HPV genotyping alone did not show a statistically significant association with HSIL in our study ($p = 0.675$). Although HPV 16 was the most frequently detected genotype among positive cases, overall HPV detection rates were relatively low. This may reflect transient infections, viral clearance, sampling variability, or limitations of genotype detection. While HPV DNA testing is highly sensitive for identifying women at risk, its specificity for high-grade lesions is limited because many infections do not result in clinically significant disease. This finding underscores the importance of combining virological and biomarker-based approaches rather than relying solely on HPV detection.

This study has several limitations. The sample size was relatively small and derived from a single tertiary care center, which may limit generalizability. Purposive sampling may introduce selection bias. Additionally, long-term follow-up was not performed to assess progression or regression outcomes.

Despite these limitations, our findings demonstrate that diffuse p16 expression has superior diagnostic accuracy compared with HPV genotyping alone for detecting high-grade cervical lesions. Incorporating p16 immunohistochemistry into diagnostic algorithms may improve risk stratification and optimize management of women with precancerous cervical lesions.

CONCLUSION

Diffuse p16 expression demonstrates high diagnostic accuracy for detecting high-grade precancerous cervical lesions and performs better than HPV genotyping alone. Incorporating p16 immunohistochemistry into routine evaluation may improve diagnostic precision and help reduce unnecessary treatment in women with suspected cervical intraepithelial neoplasia.

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