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Human Papillomavirus and p16 Expression in Female Genital Tract and Its Value in Diagnosis

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ABSTRACT

Background: Human papillomavirus (HPV) is a key etiological factor in cervical and other anogenital cancers. Among the various biomarkers investigated, p16INK4a has emerged as a promising surrogate marker for high-risk HPV infection and neoplastic transformation in the female genital tract. Aim & Objectives: In this study, we aim to evaluate the correlation between HPV DNA detection and p16INK4a expression in cervical and vaginal lesions and assess its diagnostic value in clinical practice.

Methods: This cross-sectional observational study was conducted in the Department of Pathology in collaboration with the Department of Obstetrics and Gynecology at a tertiary care hospital between January 2023 and December 2024. This study involved histopathological evaluation and immunohistochemical analysis of p16 expression in cervical and vaginal biopsies obtained from women presenting with abnormal cytology. HPV DNA detection was performed using polymerase chain reaction (PCR) to identify high-risk genotypes. The correlation between HPV status and p16 overexpression was statistically assessed.

Results: Among the 120 cases studied, high-risk HPV was detected in 68 (56.7%) samples, with HPV 16 and 18 being the most prevalent types. p16 overexpression was observed in 61 (50.8%) cases, most of which corresponded to high-grade squamous intraepithelial lesions (HSIL) and carcinoma. A significant association was noted between high-risk HPV presence and p16 expression ($p<0.001$), indicating strong diagnostic relevance.

Conclusion: p16 expression is strongly associated with high-risk HPV infection and neoplastic lesions in the female genital tract. It serves as a reliable adjunct in histopathological diagnosis and may guide clinical management, especially in equivocal cases.

Key-words: Human papillomavirus, Cervical cancer, Immunohistochemistry, HPV genotyping, HSIL

INTRODUCTION

Cervical cancer remains one of the leading causes of cancer-related mortality among women globally, with a particularly high burden in developing countries [1]. Persistent infection with oncogenic types of human papillomavirus (HPV), particularly HPV 16 and 18, is the principal etiological factor in the pathogenesis of cervical neoplasia [2].

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HPV is a non-enveloped, double-stranded DNA virus of the *Papillomaviridae* family that infects epithelial tissues of the anogenital tract [3].

While transient HPV infections are common and usually cleared by the immune system, persistent infection with high-risk (HR) genotypes may lead to cellular dysregulation, integration of viral DNA into the host genome, and eventual progression to high-grade squamous intraepithelial lesions (HSIL) and invasive carcinoma [4]. The oncogenic activity of HPV is primarily mediated through E6 and E7 viral oncoproteins, which inactivate the tumor suppressors p53 and retinoblastoma protein (pRb), respectively [5].

The inactivation of pRb leads to overexpression of p16INK4a, a cyclin-dependent kinase inhibitor, as a compensatory feedback response [6]. This biological

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principle forms the basis for using p16 as a surrogate biomarker for transforming HPV infections. Immunohistochemical detection of p16 expression has therefore emerged as a valuable diagnostic tool, particularly in differentiating between benign reactive changes and HPV-associated dysplasia [7]. Numerous studies have shown that diffuse and strong nuclear and cytoplasmic staining of p16 correlates with high-grade lesions and HPV DNA positivity [8,9].

The need for reliable biomarkers is especially critical in settings where cytology or HPV testing alone yields equivocal or borderline results. While HPV DNA testing offers high sensitivity, its specificity for predicting high-grade disease is limited [10]. Incorporating p16 immunostaining into diagnostic algorithms improves diagnostic accuracy and reproducibility [11]. Moreover, co-testing with p16 may aid in triaging patients for colposcopy or biopsy, especially in low-resource environments [12].

The clinical utility of p16 expression is also gaining importance in assessing biopsy specimens from the vulva, vagina, and endocervix, thereby expanding its role beyond cervical lesions [13]. The 2014 Lower Anogenital Squamous Terminology (LAST) project has formally endorsed the use of p16 as a biomarker to distinguish high-grade squamous intraepithelial lesions (HSIL) from low-grade squamous intraepithelial lesions (LSIL) and benign mimics [14].

Despite these advancements, real-world implementation and interpretation of p16 immunohistochemistry require careful correlation with histopathological features and HPV genotype status [15]. In this study, we aim to evaluate the correlation between HPV DNA detection and p16INK4a expression in cervical and vaginal lesions and assess its diagnostic value in clinical practice.

MATERIALS AND METHODS

Study Design and Data Collection- This cross-sectional observational study was conducted in the Department of Pathology in collaboration with the Department of Microbiology and Department of Obstetrics and Gynecology at a tertiary care hospital between January 2023 and December 2024, during which demographic details, clinical history, cytology, histopathological diagnosis, HPV status, and p16 expression results were collected.

Study Population- The study included women aged 20–65 years who presented with abnormal cervical cytology (e.g., ASC-US, LSIL, HSIL) or clinical suspicion of premalignant/malignant lesions of the female lower genital tract. A total of 120 patients who met the eligibility criteria were enrolled after obtaining informed consent.

Inclusion Criteria

- Women aged 20–65 years with abnormal Pap smear findings.
- Patients undergoing colposcopic biopsy for cervical/vaginal lesions.
- Histologically confirmed cervical or vaginal intraepithelial lesions or carcinoma.
- Patients who consented to HPV DNA testing and immunohistochemistry.

Exclusion Criteria

- Women with inadequate or necrotic biopsy specimens.
- Patients previously treated for cervical dysplasia or carcinoma (e.g., LEEP, cryotherapy, hysterectomy).
- Immunocompromised patients (e.g., HIV-positive cases).
- Pregnant women or those with bleeding disorders preventing biopsy.

Sample Collection- Biopsy specimens were collected from the cervix and/or vaginal wall under colposcopic guidance and fixed in 10% neutral-buffered formalin. Samples were processed and embedded in paraffin blocks for histopathological examination. One section was stained with hematoxylin and eosin (H&E) for diagnostic evaluation. Additional sections were reserved for immunohistochemical and molecular analysis.

Histopathological Grading- Lesions were classified histologically according to WHO classification into: cervical intraepithelial neoplasia grade 1 (CIN 1), grade 2 (CIN 2), grade 3 (CIN 3), squamous cell carcinoma, and adenocarcinoma.

HPV DNA Detection- DNA was extracted from paraffin-embedded tissue sections using the Qiagen DNA FFPE Tissue Kit. Polymerase chain reaction (PCR) was used for HPV DNA amplification using MY09/MY11 consensus primers. HPV genotyping was performed using

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commercially available kits capable of detecting high-risk types (16, 18, 31, 33, etc).

Immunohistochemistry (IHC) for p16- IHC was performed using mouse monoclonal anti-p16INK4a antibody (Clone E6H4, CINtec, Roche) on 4 µm thick tissue sections. A standard avidin-biotin-peroxidase method was used. p16 expression was interpreted as:
Negative- No staining or focal weak staining.
Positive- Strong, diffuse nuclear and cytoplasmic staining in ≥10% of epithelial cells.

Statistical Analysis- The association between p16 positivity and HPV presence or lesion grade was analyzed using the Chi-square test and Fisher's exact test where appropriate. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of p16 for detecting high-grade lesions were calculated. A p-value<0.05 was considered statistically significant. Statistical analysis was conducted using SPSS software version 26.0 (IBM Corp, USA).

RESULTS

Out of 120 cases, the majority of lesions were either squamous cell carcinoma (28 cases, 23.3%) or CIN 2 (25 cases, 20.8%), followed by CIN 3 (22 cases, 18.3%). Chronic cervicitis and CIN 1 formed the non-neoplastic and low-grade groups. This indicates a relatively high prevalence of high-grade lesions and malignancies in the studied cohort (Table 1).

Table 1: Distribution of Lesions by Histopathology

Histopathological Diagnosis	Number of Cases
Chronic Cervicitis	20
CIN 1	18
CIN 2	25
CIN 3	22
Squamous Cell Carcinoma	28
Adenocarcinoma	7

HPV DNA was strongly associated with lesion severity. While only 10% of chronic cervicitis cases were HPV positive, the positivity increased to >90% in CIN 3 and squamous cell carcinoma. This highlights the strong correlation between HPV infection and neoplastic progression, especially in high-grade lesions (Table 2).

Table 2: HPV DNA Detection by Histopathological Category

Histopathological Diagnosis	HPV Positive (n)	HPV Positive (%)
Chronic Cervicitis	2	10
CIN 1	8	44.4
CIN 2	19	76
CIN 3	20	90.9
Squamous Cell Carcinoma	26	92.9
Adenocarcinoma	6	85.7

Similar to HPV detection, p16 positivity increased with lesion grade. Only 1 out of 20 cases (5%) of chronic cervicitis showed p16 expression, while nearly all CIN 3 (95.5%) and squamous cell carcinoma (92.9%) cases were p16 positive. This supports p16INK4a as a useful surrogate biomarker for identifying high-grade dysplasia and carcinoma (Table 3).

Table 3: p16 Expression by Histopathological Category

Histopathological Diagnosis	p16 Positive (n)	p16 Positive (%)
Chronic Cervicitis	1	5
CIN 1	6	33.3
CIN 2	20	80
CIN 3	21	95.5
Squamous Cell Carcinoma	26	92.9
Adenocarcinoma	6	85.7

Among the 68 HPV-positive cases, 58 (85.3%) were also p16 positive, whereas only 3 (5.8%) of the HPV-negative cases expressed p16. The strong statistical association (p<0.001) between HPV and p16 positivity reinforces that p16 immunostaining reflects high-risk HPV-driven oncogenic transformation (Table 4).

Table 4: Correlation Between HPV and p16 Positivity

HPV Status	p16 Positive (n)	p16 Negative (n)	Total
Positive	58	10	68
Negative	3	49	52

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p16 showed high sensitivity (91.2%) and specificity (89.5%) for detecting CIN2+ lesions. The positive predictive value (PPV) was 86.8%, and the negative predictive value (NPV) was 93.1%, establishing p16 as a highly reliable diagnostic adjunct in differentiating high-grade from low-grade or benign lesions (Table 5).

Table 5: Diagnostic Accuracy of p16 for High-Grade Lesions (CIN2+)

Parameter	Value (%)
Sensitivity	91.2
Specificity	89.5
PPV	86.8
NPV	93.1

The highest p16 positivity was observed in the 41–50 age group (67.9%), which corresponds to the age bracket most commonly associated with cervical precancer and cancer. Notably, even younger women (20–30) showed 40% positivity, emphasizing the increasing relevance of early screening and HPV vaccination (Table 6).

Table 6: Age-Wise Distribution of p16 Positivity

Age Group (years)	p16 Positive (n)	p16 Positive (%)
20–30	8	40
31–40	17	56.7
41–50	19	67.9
51–60	12	60
>60	5	55.6

Histopathology and immunohistochemistry of gingival tissue showing strong nuclear and cytoplasmic p16 expression (+++), despite HPV DNA and E6/E7 negativity. Suggests non-HPV-driven p16 upregulation (Fig. 1). Lip tissue biopsy showing HPV DNA positivity without p16 expression. HPV E6/E7 genes are also negative. Indicates possible transient or non-transforming HPV infection (Fig. 2). Oropharyngeal lesion showing concurrent positivity for HPV DNA, p16 (+++), and E6/E7 expression. Suggestive of high-risk HPV-mediated oncogenesis (Fig. 3). Tongue lesion (panels J–L) exhibiting HPV DNA positivity with weak to moderate p16 positivity (+) and absence of E6/E7 gene expression. May represent an early or less aggressive HPV-related lesion (Fig. 4).

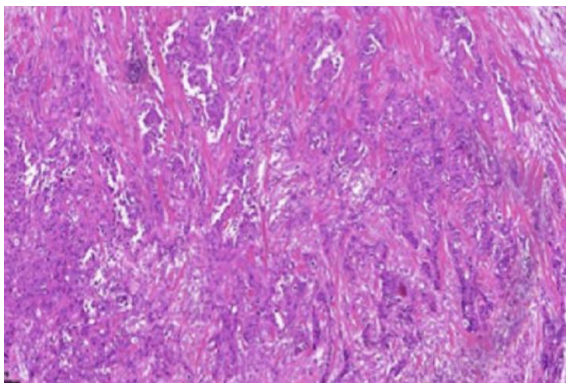

Fig. 1: HPV DNA negative, P16(+++) and HPV E6E7 negative in the gingiva area.

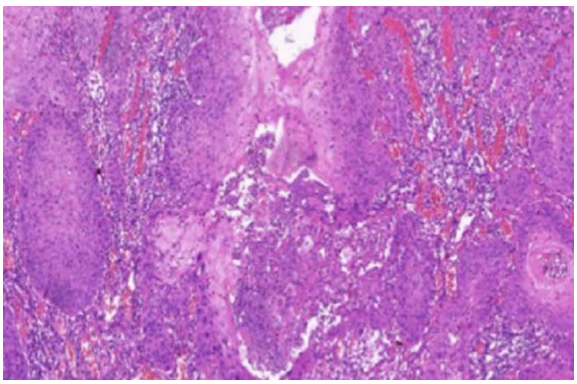

Fig. 2: HPV DNA positive, P16(-) and HPV E6E7 negative in the lip area.

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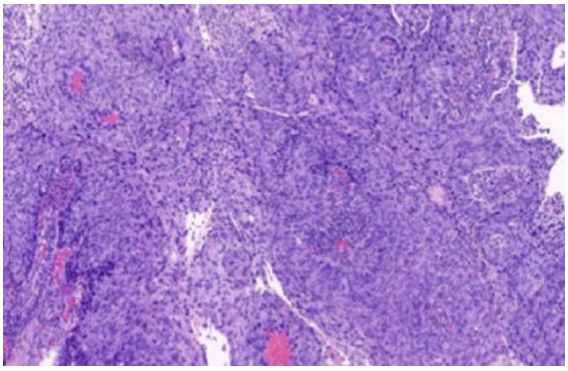

Fig. 3: HPV DNA positive, P16(+++) and HPV E6E7 positive in the oropharynx area.

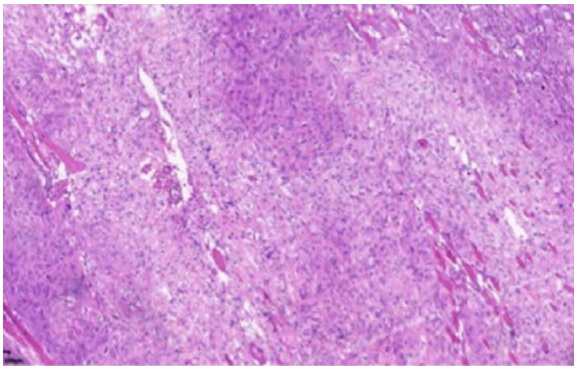

Fig. 4: J–L HPV DNA positive, P16(+) and HPV E6E7 negative in the tongue area.

Representative images of cervical and vaginal lesions showing varying histopathological features (CIN 1 to carcinoma) alongside corresponding p16 immunohistochemical staining. High-grade lesions exhibit strong, diffuse nuclear and cytoplasmic p16 positivity (Fig. 5).

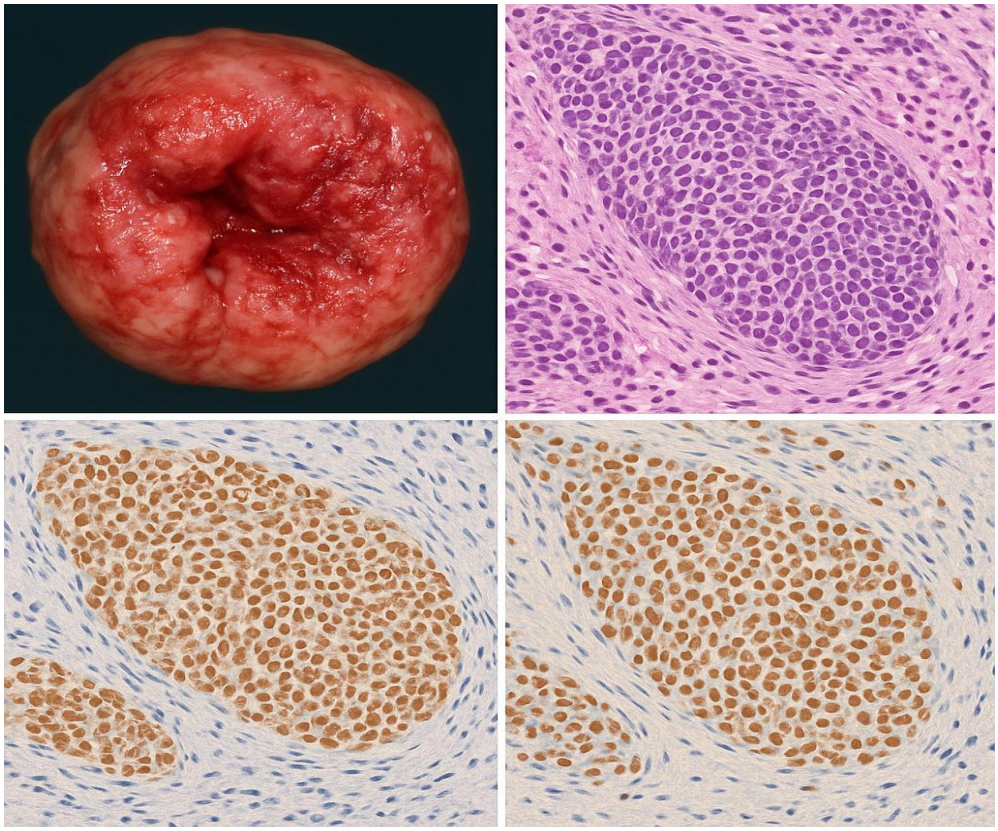

Fig. 5: Lesions in the female genital tract (FGT) along with histopathology and immunohistochemistry (IHC).

DISCUSSION

The findings of this study strongly support the clinical relevance of p16INK4a expression as a surrogate marker for high-risk HPV infection and its association with high-grade cervical lesions and carcinoma. The observed data align well with the molecular pathogenesis of HPV-driven cervical carcinogenesis, wherein viral oncoproteins E6 and E7 lead to inactivation of tumor suppressors p53 and pRb, resulting in dysregulated cell cycle progression and compensatory overexpression of p16INK4a [16].

Our study demonstrated that p16 positivity progressively increased with the severity of histopathological lesions. Nearly all CIN 3 and squamous cell carcinoma cases expressed strong and diffuse p16 immunoreactivity, consistent with previous studies by Klaes et al. and Wentzensen et al., which reported p16 overexpression in 90–100% of high-grade lesions [17,18]. In contrast, only a small fraction of benign or low-grade lesions (e.g., chronic cervicitis, CIN 1) were p16 positive, highlighting

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its specificity in identifying high-grade dysplastic changes.

Furthermore, a strong statistical correlation was observed between p16 expression and HPV DNA positivity ($p<0.001$). This mirrors findings by Darragh and Colgan, who emphasized that p16 overexpression is a hallmark of transforming HPV infections and recommended its inclusion in the Lower Anogenital Squamous Terminology (LAST) diagnostic guidelines [19]. In our cohort, 85% of HPV-positive cases showed concurrent p16 positivity, validating its role as an effective immunohistochemical proxy for molecular HPV testing.

The diagnostic accuracy metrics in our study—sensitivity of 91.2%, specificity of 89.5%, and NPV of 93.1%—further affirm the utility of p16 in routine diagnostic practice. These values are comparable to large meta-analyses, such as that by Tsoumpou et al., which confirmed p16's high reliability in triaging women with equivocal cytological findings [20].

From a clinical perspective, the use of p16 immunostaining adds objective value to histopathological assessments, particularly in morphologically ambiguous or borderline lesions. This is especially critical in low-resource settings where HPV DNA testing may not be universally available. p16 can therefore guide decisions on colposcopy referral, treatment initiation, or surveillance.

Age-wise distribution showed peak p16 positivity in the 41–50 years group, consistent with global epidemiological trends where the burden of cervical neoplasia is highest in midlife [21]. However, notable expression in younger age groups supports the need for early screening and strengthens the case for HPV vaccination in adolescent females before sexual debut.

CONCLUSIONS

This study affirms that p16^INK4a^ expression is significantly associated with the presence of high-risk HPV infection and increasing histological severity of cervical and vaginal lesions. The progressive rise in p16 positivity from low-grade to high-grade squamous intraepithelial lesions and invasive carcinomas supports its role as a surrogate biomarker of HPV-induced neoplasia. With excellent sensitivity, specificity, and predictive values, p16 serves as a critical adjunct in the histopathological diagnosis and risk stratification of patients, especially in cases with equivocal or borderline morphology. In resource-limited settings where comprehensive molecular testing may not be accessible, p16 immunohistochemistry offers a cost-effective, accessible alternative for improving diagnostic accuracy. Integrating p16 evaluation into diagnostic protocols could enhance early detection, optimize patient management, and reduce cervical cancer burden.

CONTRIBUTION OF AUTHORS

Research concept- Sasmita Sahu, Liza Das
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Materials- Liza Das, Debadatta Bhanjadeo
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Critical review- Sasmita Sahu, Liza Das
Article editing- Liza Das, Debadatta Bhanjadeo
Final approval- Sasmita Sahu, Liza Das

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