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ORIGINAL ARTICLE

p16 Immunohistochemistry Improves Treatment Decision-Making in See-and-Treat Management of Cervical Intraepithelial Neoplasia

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ABSTRACT

Background*: The "see-and-treat" approach is commonly used in cervical cancer prevention programs to reduce loss to follow-up by providing immediate treatment after colposcopic diagnosis. However, discrepancies between colposcopic impressions and histopathological findings may affect treatment decision-making. p16 immunohistochemistry has emerged as a biomarker associated with oncogenic human papillomavirus–induced cellular transformation and may improve diagnostic accuracy. This study evaluated the association between p16 expression and clinicopathological characteristics and examined its implications for treatment decision-making in the management of cervical intraepithelial neoplasia (CIN).* ***Methods & Materials****: This cross-sectional study included 72 women aged 30-60 years diagnosed with high-grade lesions on colposcopy using the Swede scoring system attending the colposcopy clinic of Bangladesh Medical University (BMU), Shahbagh, Dhaka in 2022-2023. All participants underwent colposcopy-guided cervical biopsy followed by histopathological examination. Histological diagnoses were categorized as CIN I, CIN II, CIN III, or benign lesions such as chronic cervicitis. p16 immunohistochemistry staining was performed and interpreted as diffuse/block positivity, focal staining, or negative expression. Treatment modalities and treatment adequacy were analyzed.* ***Results****: Histopathology revealed CIN I in 29.2%, CIN II in 23.6%, CIN III in 33.3%, and chronic cervicitis in 13.9% of cases. Overall, 43.1% of colposcopic high-grade lesions were not confirmed as high-grade on histology, indicating potential overtreatment risk in a see-and-treat setting. Diffuse p16 positivity was observed in 52.7% of cases. Overtreatment occurred in 13.9% of patients and was exclusively associated with p16-negative lesions, whereas undertreatment occurred in 22.2% of cases and was observed only among p16-positive lesions ($p < 0.001$).* ***Conclusion****: Substantial discordance exists between colposcopic impressions and histopathological findings. p16 immunohistochemistry may improve treatment decision-making in see-and-treat management by helping identify lesions requiring definitive treatment while avoiding unnecessary procedures.*

Keywords: *Cervical intraepithelial neoplasia; p16 immunohistochemistry; colposcopy; see-and-treat; cervical precancer; treatment decision-making*

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INTRODUCTION

Cervical cancer remains one of the most preventable yet persistent malignancies affecting women worldwide. Despite advances in screening and vaccination, it continues to pose a major public health challenge, particularly in low- and middle-income countries where screening coverage and access to treatment services are limited [1]. Persistent infection with high-risk human papillomavirus (HPV) is recognized as the primary etiological factor in the development of cervical cancer. The disease typically progresses through a well-defined precancerous stage known as cervical intraepithelial neoplasia (CIN), providing an opportunity for early detection and intervention [2]. CIN is classified histologically into three grades based on the extent of epithelial dysplasia. CIN I represents mild dysplasia involving the lower third of the epithelium and often regresses spontaneously. CIN II and CIN III represent moderate and severe dysplasia, respectively, and are associated with a higher risk of progression to invasive carcinoma if left untreated. Early identification and appropriate treatment of high-grade CIN therefore play a crucial role in cervical cancer prevention [3]. Colposcopy is an essential diagnostic procedure used to evaluate women with abnormal cervical screening results. During colposcopic examination, abnormal epithelial changes are identified through magnified visualization after application of acetic acid and iodine solution [4]. Several scoring systems have been developed to improve the accuracy and reproducibility of colposcopic interpretation. Among these, the Swede scoring system is widely used and evaluates features

such as acetowhiteness, vascular patterns, lesion margins, lesion size, and iodine staining to estimate the probability of high-grade disease [5]. In many clinical settings, particularly where follow-up rates are uncertain, a "see-and-treat" strategy is adopted. Under this approach, treatment is performed immediately after colposcopic diagnosis without waiting for histopathological confirmation [6]. The see-and-treat strategy offers several practical advantages. It reduces the number of clinic visits required, minimizes loss to follow-up, and ensures timely treatment for women with suspected high-grade lesions. These advantages are particularly relevant in resource-limited settings where patient retention in screening programs may be challenging [7]. However, the see-and-treat approach has also raised concerns regarding diagnostic accuracy. Colposcopic assessment is inherently subjective and may be influenced by factors such as inflammation, immature metaplasia, or physiological changes within the cervical transformation zone [6]. As a result, discrepancies between colposcopic impressions and histopathological diagnoses are frequently reported. Such diagnostic discordance may lead to overtreatment of lesions that are ultimately found to be low grade or benign, as well as undertreatment of lesions that require more definitive management [8-10]. Given these limitations, there has been increasing interest in identifying adjunct biomarkers that can improve the diagnostic accuracy of cervical lesion assessment. One such biomarker is p16INK4a (p16), a cyclin-dependent kinase inhibitor that regulates the cell cycle. In normal cervical epithelium, p16 expression is minimal. However, infection with oncogenic HPV disrupts the retinoblastoma tumor suppressor pathway through the viral E7 oncoprotein, leading to overexpression of p16 within dysplastic epithelial cells [11]. Diffuse or block-type p16 staining is therefore considered a surrogate marker of HPV-mediated oncogenic transformation. Numerous studies have demonstrated that p16 immunohistochemistry improves diagnostic reproducibility in cervical pathology and helps distinguish high-grade lesions from benign mimics such as reactive changes or immature squamous metaplasia [12,13]. Beyond its diagnostic value, p16 expression may also provide clinically relevant information that could support treatment decision-making. In the context of see-and-treat management strategies, integrating biological markers such as p16 may help clinicians better assess lesion severity and select appropriate treatment modalities [14-15]. Despite growing evidence supporting the diagnostic utility of p16 immunohistochemistry, its potential role in guiding treatment decisions in see-and-treat clinical settings has not been fully explored. Understanding how p16 expression correlates with histopathological severity and treatment outcomes may help refine management strategies for cervical precancerous lesions. Therefore, the present study aimed to evaluate the association between p16 immunohistochemistry expression and clinicopathological characteristics among women with colposcopically diagnosed cervical lesions. In addition, the study examined the implications of p16 expression for treatment decision-making within a see-and-treat management framework.

METHODS & MATERIALS

Study Design and Participants

This cross-sectional observational study was conducted among women aged 30-60 years undergoing colposcopic evaluation for suspected cervical lesions at the Colposcopy Clinic of Bangladesh Medical University (BMU), Shahbagh, Dhaka, from October 2022 to March 2023. Women who were diagnosed with high-grade squamous intraepithelial lesion (HSIL) on colposcopy were eligible for inclusion. A total of 72 patients meeting the inclusion criteria were enrolled in the study. Patients with inadequate biopsy specimens or incomplete clinical data were excluded from the analysis.

Colposcopy Procedure

Colposcopy was performed by trained clinicians using a standard colposcope. A 5% acetic acid solution was applied to the cervix to identify acetowhite changes and abnormal vascular patterns, and suspicious areas were targeted for biopsy. If no clear lesions were identified, Lugol's iodine was applied to highlight areas that failed to take up iodine staining [16]. Colposcopic findings were evaluated using the Swede scoring system, which assesses acetowhiteness, lesion margins, vascular patterns, lesion size, and iodine staining (score range 0–10). A score of 5–6 suggests probable high-grade lesions (CIN2+), while 7–10 indicates high-grade disease or suspected invasive cancer. Women with colposcopic HSIL based on the Swede score underwent colposcopy-guided biopsy [17].

Histopathological Evaluation

All participants underwent colposcopy-guided cervical biopsy. Histopathological diagnoses were categorized as: CIN I, CIN II, CIN III, and benign lesions such as chronic cervicitis with or without squamous metaplasia. CIN lesions were graded according to the proportion of epithelial thickness occupied by dysplastic cells, with CIN I representing mild dysplasia and CIN II–III representing high-grade lesions [18].

p16 Immunohistochemistry

Immunohistochemical staining for p16INK4a was performed on all biopsy specimens to evaluate p16 expression. Paraffin-embedded tissue blocks were sectioned into 4-µm thick sections and mounted on Poly-L-lysine–coated slides. Slides were air-dried and incubated overnight at 37 °C. Deparaffinization was performed using xylene, followed by rehydration in graded alcohol solutions. Antigen retrieval was carried out using Dako FLEX Target Retrieval solution, with slides heated in a water bath at 95–99 °C for approximately 40 minutes. After washing with deionized water, endogenous peroxidase activity was blocked using FLEX peroxidase blocking reagent. Slides were then incubated with the primary antibody against human p16 protein (E6H4 clone, DAKO, Denmark) for 30 minutes. Following washing in Tris-buffered saline, slides were incubated with a peroxidase-labeled polymer secondary antibody. Chromogenic detection was performed using diaminobenzidine (DAB), producing a brown staining signal at the site of antigen expression. Finally, slides were counterstained with Mayer's hematoxylin, dehydrated, and mounted for microscopic examination. Uterine cervical tissue was used as the positive control for p16 immunostaining [19].

Interpretation of p16 Immunohistochemical Staining

p16 staining patterns were interpreted according to established criteria. Diffuse pattern: strong, continuous nuclear and cytoplasmic staining involving the basal and parabasal epithelial layers or full epithelial thickness; Focal pattern: patchy, discontinuous staining involving limited epithelial areas; Negative pattern: absence of staining. Diffuse staining was interpreted as p16 positive, whereas focal or patchy staining and negative staining were categorized as p16 negative [20].

Treatment Modalities

Treatment decisions were made based on routine clinical practice and included: thermocoagulation (thermal ablation),

loop electrosurgical excision procedure (LEEP) and follow-up colposcopy. LEEP involves excision of the cervical transformation zone using a thin wire loop under local anesthesia and allows both treatment and histological evaluation of the excised tissue [21].

Definition of Undertreatment and Overtreatment

Treatment adequacy was assessed by comparing the treatment provided with the histopathological diagnosis. Undertreatment was defined as inadequate management relative to histological severity, including: CIN III treated with thermocoagulation; CIN I or CIN II lesions managed only with follow-up colposcopy without definitive treatment. Overtreatment was defined as unnecessarily aggressive treatment relative to histopathological findings, including: LEEP or thermocoagulation performed for chronic cervicitis; LEEP performed for CIN I or CIN II lesions.

Statistical Analysis

Data were entered into a data collection sheet and analyzed using Statistical Package for Social Sciences (SPSS) version 27.0. Categorical variables were summarized using frequencies and percentages. Associations between p16 expression and clinicopathological characteristics—including age, histopathological diagnosis, treatment modality, overtreatment, and undertreatment—were assessed using Pearson's chi-square test or Fisher's exact test, as appropriate. A p-value <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

Table I shows that the majority were aged 30–39 years (45.8%), followed by 40–49 years (36.1%), while 18.1% were aged ≥50 years. Histopathological diagnoses included: CIN I: 29.2%, CIN II: 23.6%, CIN III: 33.3% and Chronic cervicitis: 13.9%.

All participants were initially diagnosed as having high-grade lesions on colposcopy using the Swede score. However, histopathological evaluation showed that 43.1% of these cases were not confirmed as high-grade lesions, demonstrating substantial diagnostic discordance.

Table – I: p16 Immunohistochemistry Expression with Clinicopathological Characteristics and Treatment Adequacy

Variable	p16 Positive (n=38) n (%)	p16 Negative (n=34) n (%)	Total (N=72) n (%)	p-value
Age Group				0.187†
30–39 years	20 (52.6)	13 (38.2)	33 (45.8)	
40–49 years	14 (36.8)	12 (35.3)	26 (36.1)	
≥50 years	4 (10.5)	9 (26.5)	13 (18.1)	
Histopathology				<0.001†*
CIN I	1 (2.6)	20 (58.8)	21 (29.2)	
CIN II	13 (34.2)	4 (11.8)	17 (23.6)	
CIN III	24 (63.2)	0 (0.0)	24 (33.3)	
Chronic cervicitis	0 (0.0)	10 (29.4)	10 (13.9)	
Treatment Modality				<0.001†*
Thermocoagulation	17 (44.7)	30 (88.2)	47 (65.3)	
LEEP	21 (55.3)	2 (5.9)	23 (31.9)	
Follow-up colposcopy	0 (0.0)	2 (5.9)	2 (2.8)	
Undertreatment				<0.001‡*
Yes	16 (42.1)	0 (0.0)	16 (22.2)	
No	22 (57.9)	34 (100.0)	56 (77.8)	
Overtreatment				<0.001‡*
Yes	0 (0.0)	10 (29.4)	10 (13.9)	
No	38 (100.0)	24 (70.6)	62 (86.1)	

† Pearson Chi-square test, ‡ Fisher's Exact test, Statistically significant at p < 0.05
P16 positive=Diffuse/full thickness, p16 negative= Focal/patchy and Negative

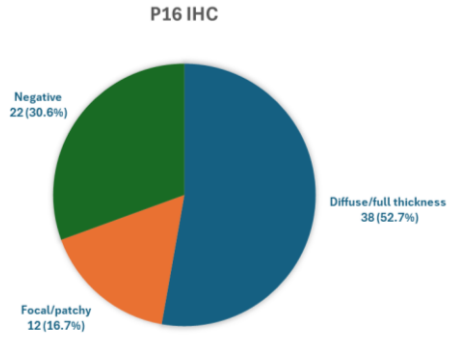

Figure – 1: p16 Expression in Cervical Intraepithelial Neoplasia

Diffuse p16 positivity was observed in 38 patients (52.7%), while 34 patients (47.3%) were p16 negative (*Figure 1*).

Histopathological severity showed a strong association with p16 expression (p < 0.001). Among p16-positive patients: 63.2% had CIN III, 34.2% had CIN II and only 2.6% had CIN I. In contrast, among p16-negative patients: 58.8% had CIN I, 29.4% had chronic cervicitis and none had CIN III.

Treatment approaches included: Thermocoagulation: 65.3%, LEEP: 31.9% and follow-up colposcopy: 2.8%. Undertreatment occurred in 22.2% of cases. All undertreated cases were observed among p16-positive patients. Overtreatment occurred in 13.9% of patients, and all overtreated cases were found among p16-negative lesions. These associations were statistically significant (p < 0.001).

DISCUSSION

This study evaluated the relationship between p16 immunohistochemistry expression, histopathological findings, and treatment decisions among women with colposcopically diagnosed cervical lesions. The results highlight the challenges associated with relying solely on colposcopic assessment and

demonstrate the potential value of p16 immunohistochemistry in improving treatment decision-making within a see-and-treat framework.

One of the most important findings of this study was the substantial diagnostic discordance between colposcopic impressions and histopathological diagnoses. Although all patients were initially diagnosed as having high-grade lesions on colposcopy, histopathological evaluation revealed that 43.1% of these cases were not high-grade lesions. This discrepancy underscores the limitations of colposcopy as a standalone diagnostic tool. Colposcopy is highly sensitive in detecting abnormal epithelial changes but may lack specificity in distinguishing lesion severity. Factors such as inflammation, immature metaplasia, and observer variability may contribute to misclassification [22].

In the context of see-and-treat management strategies, such diagnostic discrepancies may have significant clinical implications. If treatment decisions were based solely on colposcopic findings, a substantial number of women with low-grade lesions or benign conditions could potentially undergo unnecessary procedures [23].

Another key finding was the occurrence of overtreatment in 13.9% of patients, and importantly all overtreatment cases were associated with p16-negative lesions. The absence of diffuse p16 expression is typically associated with lesions that are less likely to represent true high-grade HPV-driven disease. This observation suggests that p16 testing could potentially help identify patients who may not require immediate invasive treatment. Incorporating p16 immunohistochemistry into diagnostic algorithms may therefore reduce unnecessary procedures [24,25].

Conversely, undertreatment occurred in 22.2% of cases, and notably all undertreated cases were observed among p16-positive patients. Diffuse p16 positivity reflects HPV-induced oncogenic transformation and is strongly associated with high-grade CIN lesions. The presence of p16 positivity in undertreated cases suggests that these lesions had higher malignant potential than indicated by the treatment provided [26,27].

Taken together, these findings demonstrate that p16 immunohistochemistry may provide valuable additional information for risk stratification and treatment decision-making [28]. In see-and-treat settings, integrating p16 testing could help clinicians better align treatment strategies with the biological severity of the lesion.

CONCLUSION

Substantial discordance exists between colposcopic impressions and histopathological diagnoses in cervical lesion assessment. p16 immunohistochemistry shows a strong association with lesion severity and treatment adequacy. Incorporating p16 testing into the evaluation of cervical lesions may improve treatment decision-making in see-and-treat management strategies.

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