P16 Immunohistochemistry: A Key to Accurate Diagnosis of High-Risk Cervical Lesions

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Abstract

Background

The expression of p16 protein is a critical biomarker for identifying high-risk HPV-related cervical lesions, including cervical intraepithelial neoplasia (CINII) (CINII) (CINI) and Koilocytosis. This study evaluated the diagnostic utility of p16 immunohistochemistry in cervical biopsies at Muhimbili National Hospital.

Methods

A total of 92 cervical biopsy specimens were analyzed using immunohistochemical staining for p16. The staining results were assessed by two independent pathologists, with p16 positivity defined as strong nuclear and cytoplasmic staining in over 75% of the cells. The association between p16 expression and lesion grades was statistically analyzed using chi-square and Fisher's exact tests.

Results

High p16 expression was observed in cases of high-grade CIN, consistent with global findings. Conversely, low-grade CIN and benign lesions exhibited minimal p16 overexpression. These results underscore the potential of p16 as a reliable biomarker for distinguishing between high-grade and low-grade cervical lesions.

Conclusion

The findings of this study reinforce the diagnostic value of p16 immunohistochemistry in cervical pathology. By accurately identifying high-risk lesions, p16 testing can significantly improve diagnostic precision and reduce the risk of overtreatment in low-resource settings. Further research is warranted to explore the integration of p16 testing into routine cervical cancer screening protocols.

1. Introduction

Cervical cancer remains a major public health concern globally, particularly in low-resource settings such as Tanzania, where it ranks among the leading causes of cancer-related mortality in women [1],[2]. The primary etiological factor for cervical cancer is persistent infection with high-risk types of human papillomavirus (HPV), which is responsible for the majority of cervical intraepithelial neoplasia (CIN) cases and invasive cervical carcinoma (ICC) [3]. Early detection and accurate classification of cervical lesions are crucial for effective clinical management and improving patient outcomes [4].

The tumor suppressor protein p16 has emerged as a significant biomarker associated with HPV-related carcinogenesis. Its overexpression is indicative of the dysregulation of the cell cycle, primarily driven by

the HPV E7 oncoprotein. Immunohistochemistry for p16 is increasingly recognized as a valuable tool for differentiating high-grade cervical lesions from benign and low-grade changes [5]. However, despite its potential, data on the utility of p16 immunohistochemistry in the context of cervical cancer in Tanzania remain limited.

This study evaluated the expression of p16 in uterine cervix biopsies at Muhimbili National Hospital, focusing on its role in distinguishing between CINI, CINII, CINIII as well as benign lesions. By assessing the diagnostic accuracy of p16 immunohistochemistry, this research provides insights that could enhance cervical cancer screening and management strategies in Tanzania. Furthermore, the findings contribute to the growing body of evidence supporting the integration of p16 testing into routine diagnostic protocols, ultimately improving patient care and outcomes in cervical cancer management [6], [7].

2. Methods

2.1 Study Area

This study was conducted at Muhimbili National Hospital (MNH), a tertiary care and teaching hospital located in Dar es Salaam, Tanzania. As the national referral center, MNH receives patients from tertiary zonal hospitals across the country. The Department of Anatomical Pathology, a pivotal diagnostic unit at MNH, processes approximately 20,000 tissue specimens annually, including biopsies and surgical excisions. MNH also collaborates with the Ocean Road Cancer Institute (ORCI) for cervical cancer management, providing comprehensive diagnostic and therapeutic services. Integrating clinical service delivery with medical education and research, MNH serves as a hub for specialized care in Tanzania.

2.2 Study Design

This was a cross-sectional, prospective, laboratory-based study conducted over one year (2017–2018) to evaluate p16 expression in uterine cervical biopsies.

2.3 Study Population

The study involved cervical biopsies and specimens collected between 2017 and 2018 that were submitted for histological evaluation.

2.4 Inclusion and Exclusion Criteria

The inclusion criteria comprised hysterectomy specimens from women aged 18 years and older with no gross cervical lesions. Exclusion criteria included cases where the cervix was received separately from the hysterectomy specimen, indicating that gross cervical changes were observed clinically, necessitating separate processing. Additionally, specimens where the uterus lacked ectocervical and/or endocervical tissue were excluded, as this signified an absence of cervical representation. Finally, uteri with clinically significant cervical lesions were also excluded from the study.

2.5 Data Collection

Hysterectomy specimens meeting the inclusion and exclusion criteria were identified from the anatomical pathology register. Demographic and clinical data were extracted from patient records. Eligible specimens underwent grossing, with additional cervical blocks sampled as necessary. To ensure confidentiality, specimens were de-identified and assigned unique research study numbers.

2.6 Microtomy and Tissue Preparation for Staining

Tissue sections, 4 µm thick, were cut from selected blocks and mounted on charged slides for immunohistochemistry. Simultaneously, hematoxylin and eosin (H&E)-stained slides were prepared alongside those designated for p16 immunohistochemistry.

2.7 Antigen Retrieval

Heat-induced epitope retrieval was performed using a citrate buffer solution (pH 6.0) to enhance antigen exposure and optimize antibody binding.

2.8 Blocking of Endogenous Peroxidase Activity

To minimize non-specific staining, endogenous peroxidase activity was blocked with a 3% hydrogen peroxide solution.

2.9 Immunohistochemical Staining

A p16-specific primary antibody was applied to the tissue sections, followed by a secondary antibody and visualization using diaminobenzidine (DAB) chromogen.

2.10 Immunostaining Evaluation

The immunostained slides were independently assessed by two pathologists. p16 positivity was defined as strong, diffuse nuclear and cytoplasmic staining in more than 75% of cells. Histopathological diagnoses and p16 immunohistochemistry results were systematically recorded for all slides.

2.11 Data Analysis

Data were analyzed using SPSS version 27 (IBM Corp., Armonk, NY, USA). Associations between p16 expression and lesion grades were determined using chi-square and Fisher’s exact tests.

2.12 Ethical Considerations

Ethical approval

for this study was obtained from the Institutional Review Board (IRB) of Muhimbili University of Health and Allied Sciences (MUHAS) under approval number REF. NO MU/PGS/SAEC/VOL.X/. Patient confidentiality was maintained throughout the study.

3. Results

3.1 Demographics

During the study period from November 2017 to February 2018, a total of 270 hysterectomy specimens were received at the MNH histopathology laboratory. Out of these, 172 specimens were excluded based on the established exclusion criteria, resulting in 98 eligible specimens. Ultimately, 92 hysterectomy specimens were included in the analysis. The age of the patients ranged from 22 to 72 years, with the majority falling within the 41–50-year age group. The mean age of the participants was 47 years (SD = 9.8) (Fig. 1).

A histogram showing age distribution and percentage of the 92 patients

3.2 Histopathological Diagnosis

The histopathological evaluation of the specimens revealed various diagnoses, including high-grade cervical intraepithelial neoplasia (CIN III) (Fig. 6G, Fig. 7H), low-grade CIN I, Koilocytosis (Fig. 5C, Fig. 6E) and Reactive cervicitis (Fig. 5A). The distribution of diagnoses is illustrated in Fig. 2, which summarizes the frequency of each histological category.

A bar diagram showing a number of the H&E histological diagnosis on H&E examination.

3.3 p16 Immunohistochemistry Results

Immunohistochemical analysis for p16 expression was performed on all specimens. The results revealed p16 overexpression in 93% of HSIL cases (Fig. 6H, Fig. 7I) and 71% of LSIL cases (Fig. 5D, Fig. 6F). In contrast, minimal or absent p16 expression was noted in 15% of Reactive Squamous Metaplasia cases (Fig. 5B) and 0% of Normal cases. The correlation between p16 expression and histopathological diagnoses is illustrated in the graph (Fig. 4), emphasizing the diagnostic utility of p16 in distinguishing high-grade lesions from benign or reactive changes.

A clustered bar diagram showing the distribution of numbers and percentages of the H&E histological diagnosis by age.

3.4 Statistical Analysis

Statistical analysis revealed a significant association between p16 expression and the severity of cervical lesions ($p < 0.05$). The detailed statistical outcomes are presented in Table 1, which outlines the relationship between p16 positivity and the various histopathological categories.

Table 1 Association between p16 expression and lesion grades in cervical biopsies. The table highlights the distribution of p16-negative and p16-positive cases across different lesion categories, with a statistically significant association (Pearson chi-square, $p < 0.001$).

	p16						
	Negative		Positive				
	n	%	n	%	TOTAL(n)	t-test	P value
Normal	15	100	0	0	15	Pearson chi-square	<0.001
Reactive	11	85	2	15	13		
LSIL	10	29	24	71	34		
HSIL	2	7	28	93	30		
TOTAL	38	41	54	59	92		

4. Discussion

The expression of p16 as a biomarker for high-risk human papillomavirus (HR-HPV) infection in cervical lesions has been a focal point in understanding cervical pathology. In our study, we observed that more than half of the cases (59%) were p16 positive, indicating a significant prevalence of HR-HPV infection among the studied population. This finding aligns with previous research, such as that conducted by Murphy et al. (2003), which demonstrated that all HR-HPV-positive cervical carcinomas exhibited positive p16 expression [8],[9],[10],[11]. Our results support the notion that p16 can serve as a reliable surrogate biomarker for HR-HPV infection, reinforcing its utility in clinical diagnostics.

The histological diagnosis revealed that the most common findings were low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL), comprising 37% and 33% of cases, respectively. Notably, among the LSIL cases, 71% were p16 positive, while an overwhelming 93% of HSIL cases were p16 positive. This stark contrast underscores the potential of p16 immunohistochemistry in differentiating between various grades of cervical lesions, as higher p16 expression correlates with more severe dysplastic changes [7],[12].

Furthermore, our analysis of age distribution indicated that the frequency of cervical intraepithelial neoplasia (CIN) was notably higher in the 41–50 age group, suggesting that this demographic is the reflection of prior HR-HPV infection at young age that has cultivated this significant cervical pathology. This finding that CIN frequency in the 41–50 age group is fully supported by the provided context [13]. In fact, the papers present somewhat supporting information regarding age and CIN risk: [14]Zhang et al. (2010) indicates that age less than 45 years old is a related risk factor for CIN (95% CI: 1.069–1.828). This suggests that younger women, rather than those in the 41–50 age group, may be at higher risk. Chowdhury et al. (2017) reports that women between the ages of 20–30 years had the highest HPV positive rate (50.0%), followed by the 31–40 years age group (43.6%). This again suggests that younger women may be at higher risk for HPV infection, which is strongly associated with CIN [13]. However, it is

important to note that age alone is not the sole determinant of CIN risk. Other factors play significant roles, such as HR-HPV infection, which is consistently identified as a major risk factor across multiple studies [15],[16],[14]. Additionally, factors like smoking [16], childbirth [16], and bacterial vaginosis [17] have been associated with increased CIN risk in women with persistent HPV infection.

The correlation between H&E histological diagnosis and p16 immunohistochemistry further emphasizes the importance of integrating these diagnostic modalities. Our findings indicate that all normal and unremarkable cases were p16 negative, while reactive squamous metaplasia showed a low rate of p16 positivity (15%). This distinction is crucial for pathologists in accurately diagnosing and recommendation on managing cervical lesions.

Mandal et al. (2019) reports that 0% of women with normal histology showed p16 overexpression, while 33.3% of women with CIN 1 were p16 positive [18]. This supports the notion that normal cervical tissue tends to be p16 negative. Goyal et al. (2019) presents an interesting contradiction, noting that p16 positivity can sometimes be observed in squamous metaplasia lacking morphological characteristics of atypical squamous metaplasia or squamous intraepithelial lesion (SIL). This study found that all cases of p16-positive bland squamous metaplasia were positive for HPV E6/E7 mRNA, suggesting these lesions may be early SILs and not yet morphologically evident SILs [19]. Majority generally support the usefulness of p16 IHC in aiding accurate cervical lesion diagnosis, and emphasize that p16 expression increases with lesion severity and is associated with high-risk HPV infection. However, some cases of apparently benign metaplasia may show p16 positivity, warranting further investigation.

5. Conclusion

Our study reinforces the role of p16 as a valuable biomarker for HR-HPV infection and highlights its significance in the histopathological assessment of cervical lesions. The integration of p16 immunohistochemistry into routine diagnostic practice may enhance the accuracy of cervical cancer screening and improve patient management strategies.

Therefore, we highlight the significant utility of p16 immunohistochemistry in the diagnostic evaluation of cervical lesions and our findings demonstrate that p16 expression is a reliable biomarker for identifying high-grade cervical intraepithelial neoplasia (CINII and CIN III), Low grade intraepithelial lesions/Neoplasia (CIN 1, Koilocytosis) and effectively in distinguishing these lesions from benign changes. The ability of p16 to enhance diagnostic accuracy is particularly crucial in resource-limited settings like Tanzania, where access to advanced diagnostic tools may be limited.

The results also underscore the importance of incorporating p16 immunohistochemistry into routine diagnostic algorithms and protocols for cervical biopsies. By doing so, healthcare providers can optimize patient care, reduce the risk of overtreatment, and ensure that appropriate interventions are directed towards those with high-grade lesions. Furthermore, this study paves the way for future research aimed at exploring the prognostic value of p16 in predicting treatment outcomes and recurrence rates in cervical cancer patients.

The implementation of p16 immunohistochemistry in cervical cancer diagnostics holds promise for improving clinical management and outcomes, particularly in regions with high cervical cancer prevalence.

Lastly, while aging may contribute to cervical pathology risk, the provided context does not support the claim that CIN is most frequent in the 41–50 age group. The relationship between age and CIN risk appears to be complex, with various studies pointing to different age ranges as potentially higher risk. The persistent HR-HPV infection remains a critical factor in CIN development across age groups.

6. Recommendations

Based on the findings of this study, it is recommended that p16 immunohistochemistry be implemented as a routine diagnostic tool for cervical biopsies in Tanzania. The integration of p16 testing into clinical practice can significantly enhance the accuracy of cervical cancer diagnoses, particularly in distinguishing high-grade lesions from benign or low-grade changes. This is crucial in a resource-limited setting where misdiagnosis can lead to inappropriate treatment and increased morbidity.

Additionally, larger multicenter studies should be conducted to validate these findings across diverse populations and to assess the cost-effectiveness of p16 immunohistochemistry in routine diagnostics. Such studies would provide valuable data to inform healthcare policies and improve cervical cancer screening programs.

Furthermore, it is essential to explore the prognostic value of p16 in predicting treatment outcomes and recurrence rates, as this could lead to more personalized management strategies for patients.

Finally, establishing a comprehensive database on the prevalence of high-risk HPV types in Tanzania will be vital for evaluating the effectiveness of prophylactic HPV vaccination programs and for monitoring changes in the incidence of cervical cancer over time. By addressing these recommendations, the healthcare system in Tanzania can improve early detection and management of cervical cancer, ultimately reducing its burden on women's health.

Declarations

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Figures

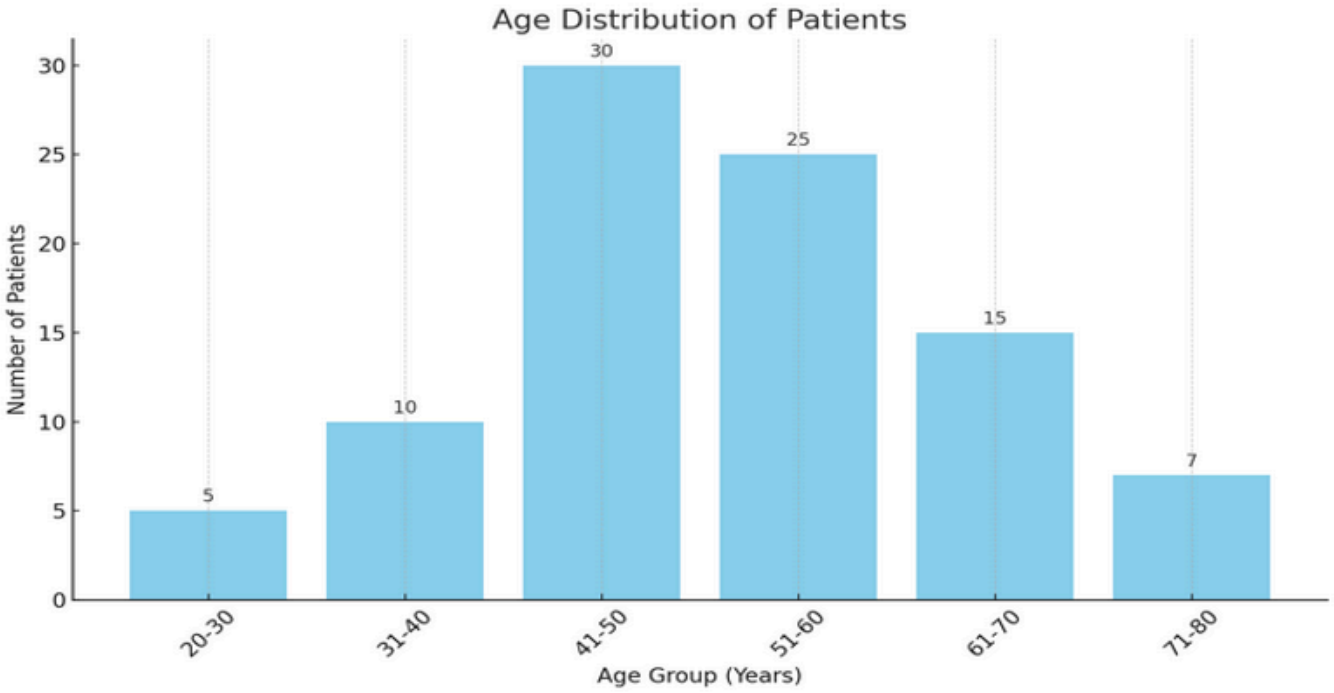

Figure 1

Age distribution a

A histogram showing age distribution and percentage of the 92 patients

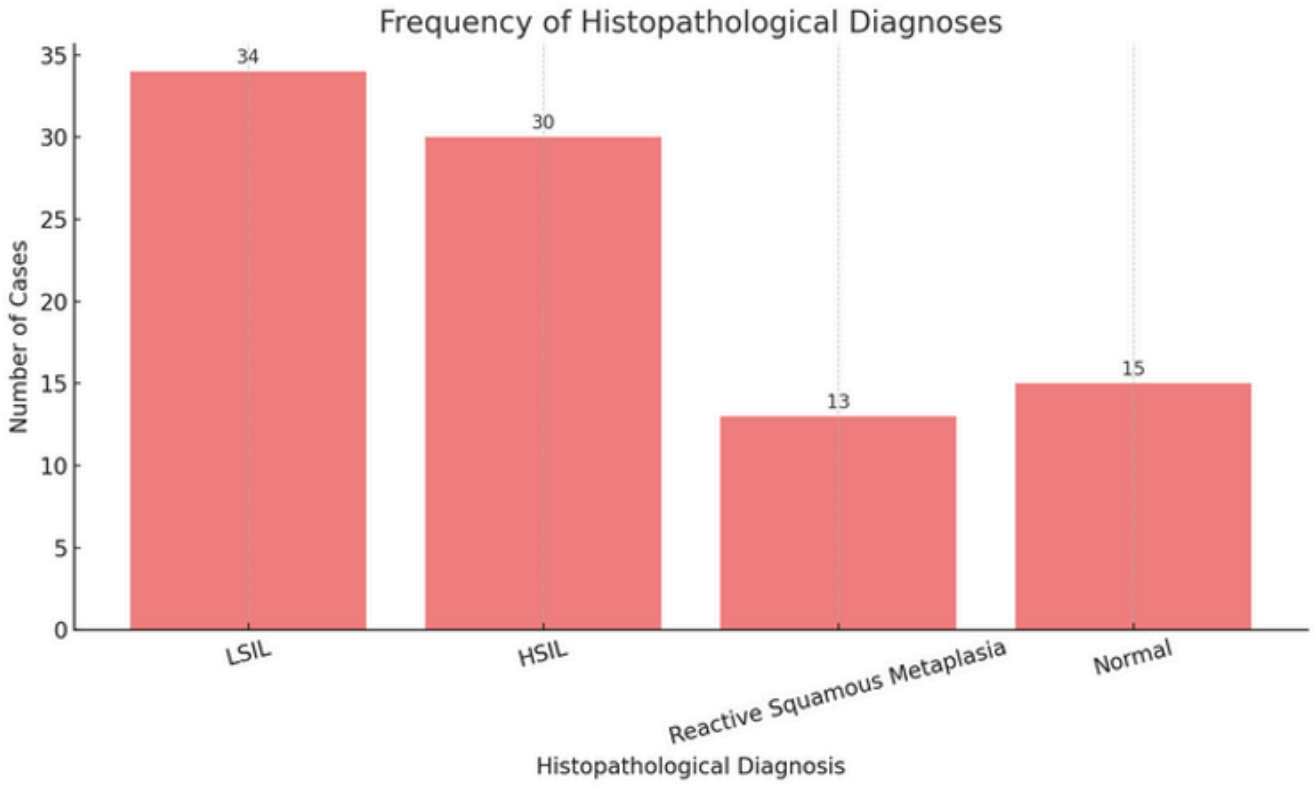

Figure 2

H&E histological diagnosis

A bar diagram showing a number of the H&E histological diagnosis on H&E examination.

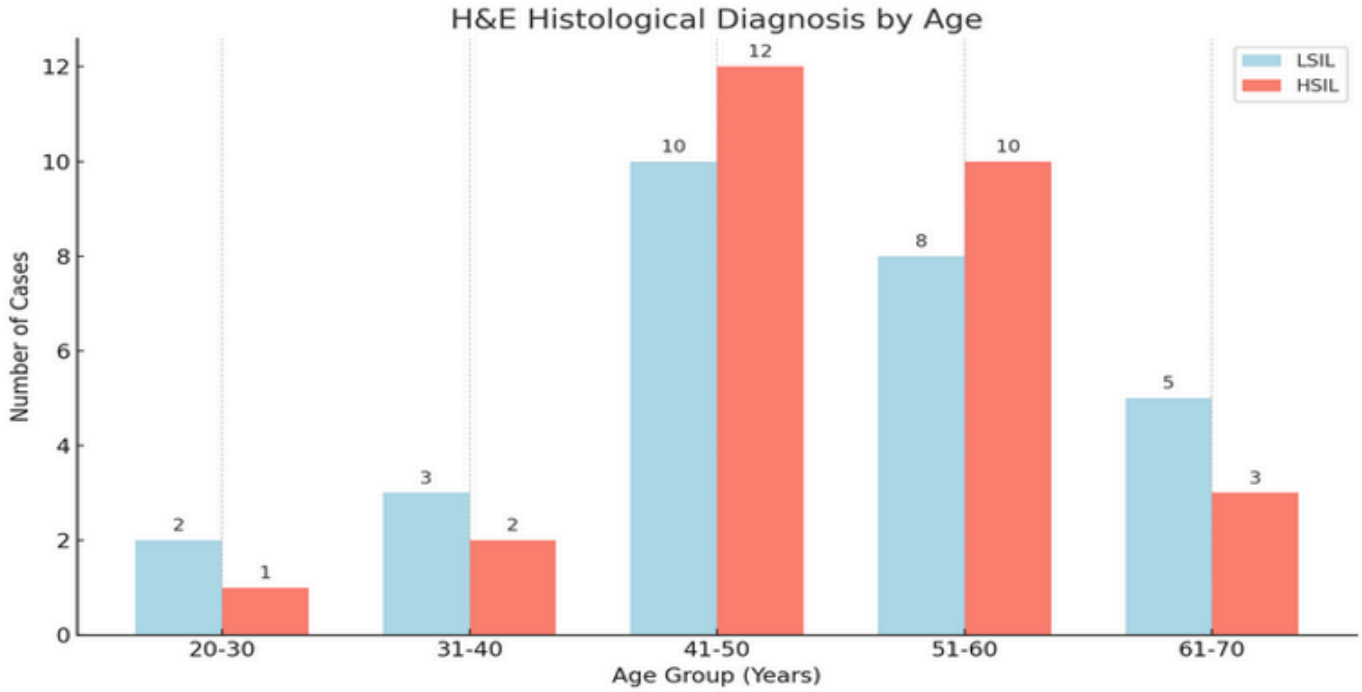

Figure 3

H&E histological diagnosis by age

A clustered bar diagram showing the distribution of numbers and percentages of the H&E histological diagnosis by age.

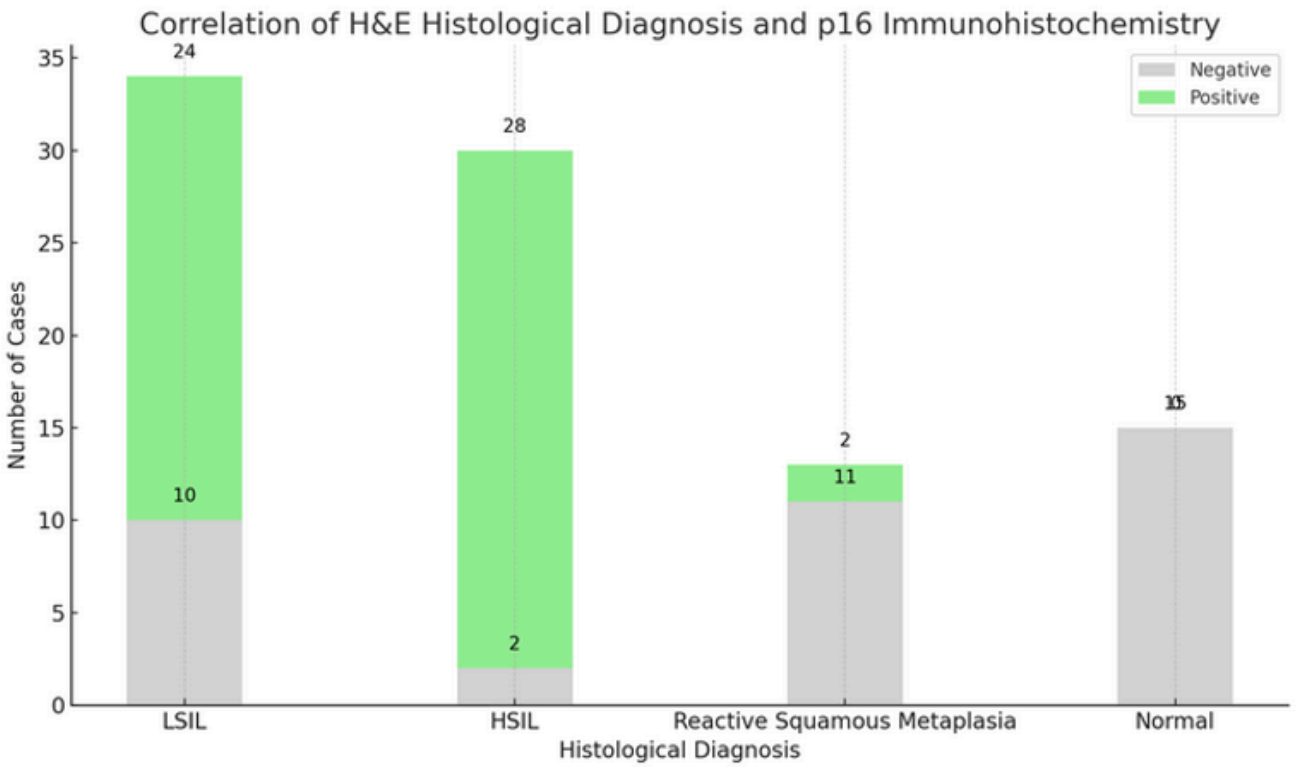

Figure 4

H&E histological diagnosis and p16 immunohistochemistry Correlation of H&E histological diagnosis and p16 immunohistochemistry

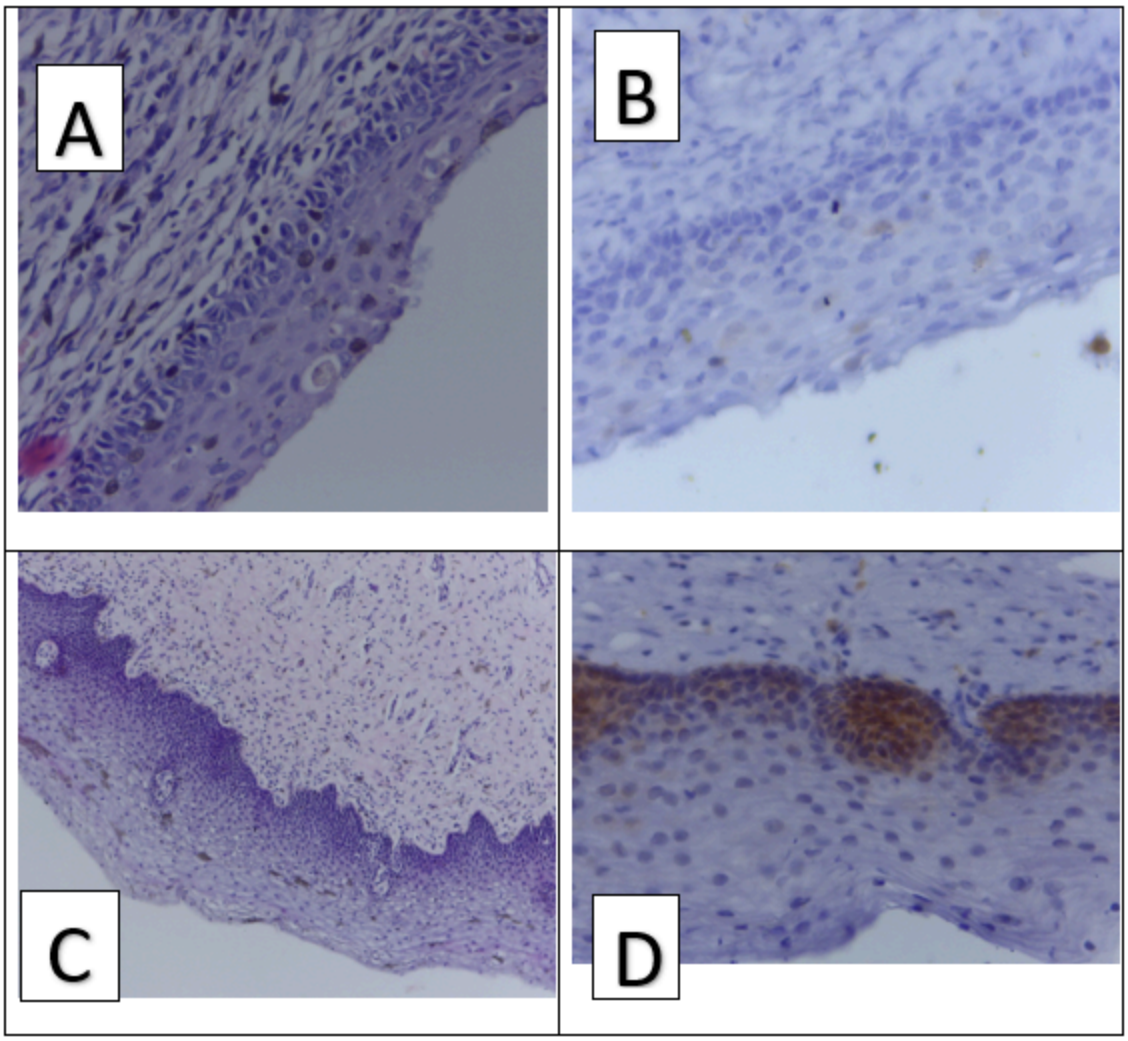

Figure 5

Photomicrographs of ectocervix stained with hematoxylin and eosin stain A and C; and immunohistochemistry for p16 (B&D). A: Reactive cervicitis showing inflammatory cells. Original magnification X4. B: shows lack of p16 expression in reactive cervicitis. C: Low grade intraepithelial lesion showing and D: is a Low-grade intraepithelial lesion showing p16 expression.

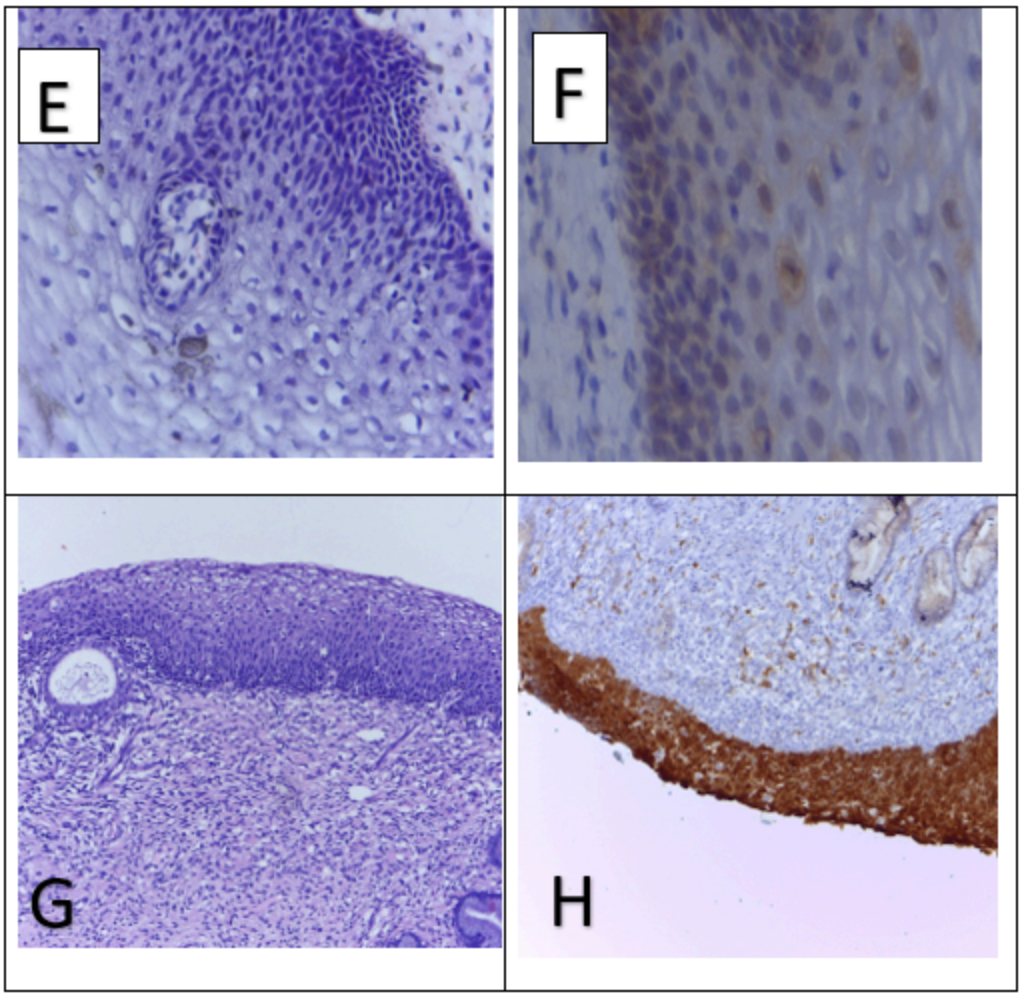

Figure 6

Photomicrographs of ectocervix stained with hematoxylin and eosin stain E and G; and immunohistochemistry for p16 (F&H). F: Low grade intraepithelial lesion with koilocytosis. Original magnification X40. B: shows p16 expression in LSIL. G: High grade intraepithelial lesion and D: is a high-grade intraepithelial lesion showing p16 expression.

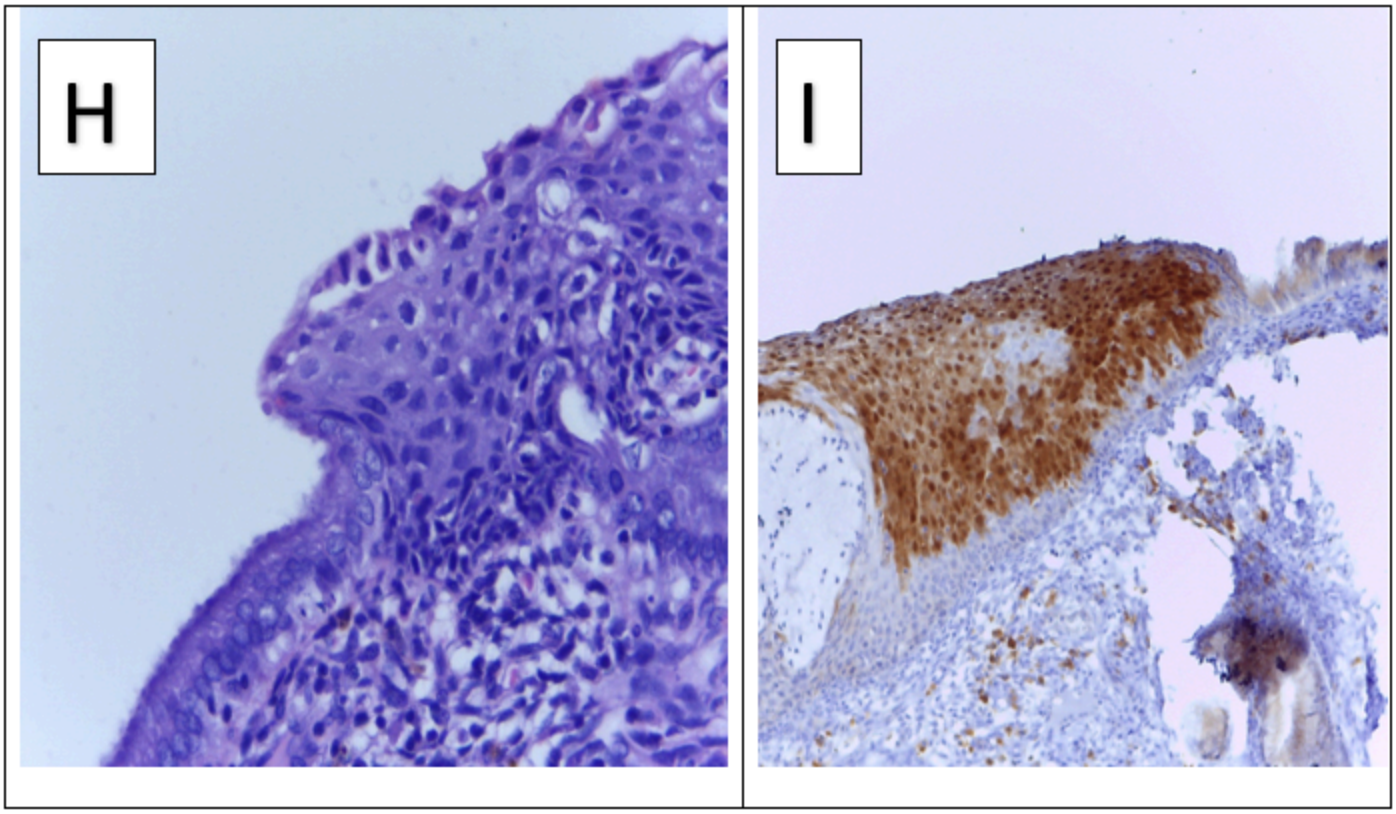

Figure 7

Photomicrographs of ectocervix stained with hematoxylin and eosin stain H; and immunohistochemistry for p16 (I). H: High grade intraepithelial lesion at squamocolumnar junction. Original magnification X40. I: shows p16 expression in HSIL. Original magnification X40.