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A Study Of KI-67 And P16/INK4A Expression In Cervical intraepithelial Neoplasia And Carcinoma And Its Histopathological Correlation

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ABSTRACT

Introduction: Cervical cancer remains a major health concern worldwide, and early detection of cervical intraepithelial neoplasia (CIN) is crucial for effective management. Ki-67 and p16/INK4a are established immunohistochemical markers indicating cellular proliferation and high-risk HPV-mediated oncogenic transformation.

Objective: This study aimed to evaluate Ki-67 and p16/INK4a expression in cervical lesions and correlate findings with histopathological grading.

Methods: A cross-sectional study was conducted on 107 cervical tissue specimens, including 88 cases of carcinoma cervix and 19 cases of squamous intraepithelial lesions (SIL). Histopathological evaluation was performed using H&E staining. Immunohistochemical analysis for Ki-67 and p16/INK4a was carried out on representative sections. Ki-67 expression was assessed according to the percentage of positive nuclei, while p16/INK4a expression was graded based on nuclear and cytoplasmic "block" staining patterns. Staining intensity and distribution were recorded and correlated with histopathological grade.

Results: Ki-67 and p16/INK4a expression increased progressively with lesion severity. High-grade SIL and invasive carcinoma showed strong and diffuse expression, whereas low-grade lesions and reactive epithelium demonstrated weak or focal staining. Combined positivity was predominantly observed in high-grade lesions. Histopathologically, the majority of carcinoma cases were squamous cell carcinoma, with well-differentiated tumors being most common. Clinically, irregular bleeding was the most frequent presenting complaint, followed by white vaginal discharge and lower abdominal pain.

Conclusion: Ki-67 and p16/INK4a are reliable adjuncts to histopathology for cervical lesion assessment. Their expression correlates with lesion grade, aiding in distinguishing low- from high-grade lesions and guiding management. Combined evaluation enhances diagnostic accuracy, facilitating early detection and timely intervention in cervical neoplasia.

Keywords: *Cervical intraepithelial neoplasia, carcinoma cervix, Ki-67, p16/INK4a, immunohistochemistry, histopathology.*

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1. INTRODUCTION

Cervical cancer remains a major cause of morbidity and mortality among women worldwide, despite the availability of effective screening and preventive strategies. Persistent infection with high-risk human papillomavirus (HR-HPV) is the established etiologic driver of cervical carcinogenesis; the viral oncogenes E6 and E7 disrupt normal cell-cycle control and

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produce characteristic morphologic and molecular changes in the cervical epithelium that progress through grades of cervical intraepithelial neoplasia (CIN) to invasive carcinoma in a subset of women [1]. Histopathological grading of CIN (CIN1–3) is the current gold standard for diagnosing and triaging preinvasive disease, yet inter- and intra-observer variability—particularly for borderline or ambiguous lesions, immature metaplasia, and atrophic epithelium—remains a significant limitation that can affect patient management [2,3]. To address these limitations, immunohistochemical biomarkers that reflect the underlying biology of HPV-driven transformation have been studied extensively as adjuncts to conventional histology and cytology.Two biomarkers have emerged as especially useful in this context: p16/INK4a (commonly abbreviated p16) and Ki-67. p16 is a cyclin-dependent kinase inhibitor whose overexpression in cervical epithelium is a surrogate marker for transforming HR-HPV infection: E7-mediated inactivation of retinoblastoma protein (pRb) leads to loss of negative feedback and diffuse, strong p16 expression in dysplastic and malignant cells [4,5]. Ki-67 is a nuclear proliferation antigen expressed during active phases of the cell cycle and absent in quiescent (G0) cells; its distribution within the epithelial layers correlates with the degree of dysplasia, expanding from basal localization in normal tissue to suprabasal and full-thickness expression in higher-grade lesions [6,7]. The complementary biology of these two markers—p16 indicating oncogenic viral activity and Ki-67 indicating increased proliferative drive—provides a mechanistic rationale for their combined use to improve diagnostic accuracy.Numerous studies have shown that p16 immunostaining increases reproducibility in grading CIN and helps distinguish true dysplasia from mimics such as immature squamous metaplasia or atrophy [8]. Ki-67 adds value by quantifying proliferative activity and helping to stratify the severity of lesions; several groups have proposed scoring systems or cutoffs for Ki-67 indices that correlate with CIN grade and outcome [9]. Importantly, p16 and Ki-67 used together—either as separate stains interpreted in tandem or as a single dual-stain assay—have demonstrated higher sensitivity for detecting CIN2+ (and CIN3+) than conventional cytology or single markers, while maintaining acceptable specificity for clinical triage [10]. This enhanced performance has led to incorporation of p16/Ki-67 dual-staining into screening and triage algorithms in many settings, particularly for management of HPV-positive women and equivocal cytology results.Despite promising diagnostic performance, there are open questions relevant to pathology practice and clinical decision-making. The optimal criteria for interpreting p16 (focal vs diffuse staining, intensity thresholds) and the most clinically meaningful Ki-67 cutoffs vary between studies, and standardization is still evolving. Moreover, most large diagnostic studies have focused on cytologic triage or screening populations; fewer reports have comprehensively correlated p16 and Ki-67 immuno-expression with detailed histopathological features across the full spectrum of cervical lesions in surgical or biopsy series, especially in diverse geographic and resource settings. Understanding how staining patterns and semiquantitative scores correspond to traditional histologic grades, and whether combined immunoscores improve reproducibility and prognostic stratification, is therefore vital to translate biomarker data into consistent clinical recommendations.Against this background, a study that systematically evaluates p16 and Ki-67 expression across CIN grades and invasive carcinoma and correlates those findings with histopathology will address both diagnostic and practical questions. Such work can refine interpretative criteria, support standardized reporting, and help determine whether a combined immunoscore provides superior diagnostic precision and better alignment with biologic risk than morphology alone. Improved accuracy in identifying true high-grade lesions has direct clinical consequences—avoiding overtreatment of benign mimics and ensuring timely intervention for lesions at risk of progression—making biomarker-guided histopathologic correlation an important priority in contemporary cervical pathology [1–10].

The present study aims to investigate the expression of Ki-67 and p16 in cervical lesions to determine their utility in detecting cervical intraepithelial neoplasia (CIN) and cervical carcinoma. Additionally, it seeks to establish a correlation between the expression levels of these markers and the histological type and grade of tumors, thereby assessing their potential role in tumor characterization. Furthermore, the study aims to evaluate Ki-67 and p16 as prognostic markers in cervical cancer, providing insights into their significance in predicting disease progression and clinical outcomes.

2. MATERIALS AND METHODS

Study Design: This was a hospital-based, observational, cross-sectional analytical study

Study Area: The present study was conducted in the Department of pathology, MGM Hospital, Warangal.

Study Population: Limited study sample, Further studies should be done in the Indian population with a larger sample size so that proper precautionary measures can be advised for early detection and treatment of these lesions.

Study Period: This is a prospective study from April 2022 to May 2024.

Sample Size:A total of 107 specimens were included: 97 cervical biopsies and 10 hysterectomy specimens

Inclusion Criteria:

Specimens of patients who are diagnosed as cervical intraepithelial neoplasia and carcinoma cervix on histopathological examination.

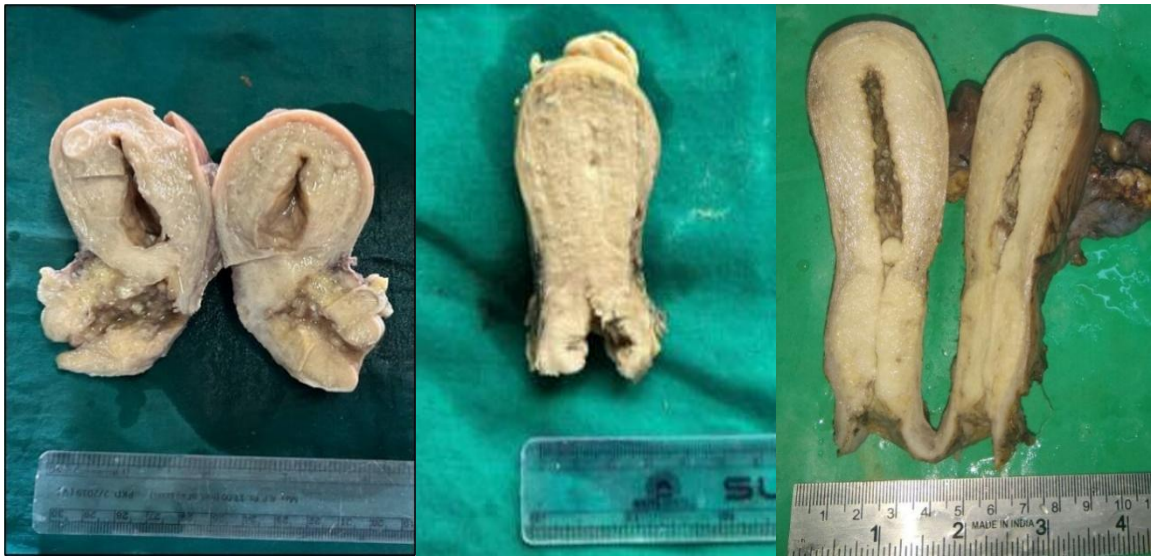
Exclusion Criteria:

Specimens of Invasive carcinoma patients who underwent radiotherapy or chemotherapy are excluded in this study.

1. Clinical data was obtained from the patient's outpatient and inpatient records and requisition forms accompanying the specimens to the department.
2. On arrival to the department, the specimens were adequately fixed in 10% neutral buffered formalin followed by the evaluation of gross features.
3. The gross details of the specimen Submitted for evaluation of malignancy were observed and recorded.
4. Then the representative tissue from hysterectomy specimen and entire tissue from cervical biopsy specimen was subjected to routine processing for paraffin embedding.
5. 3–5-micron thick sections were taken from paraffin embedded blocks, stained with hematoxylin and eosin [H and E] stain, and studied.

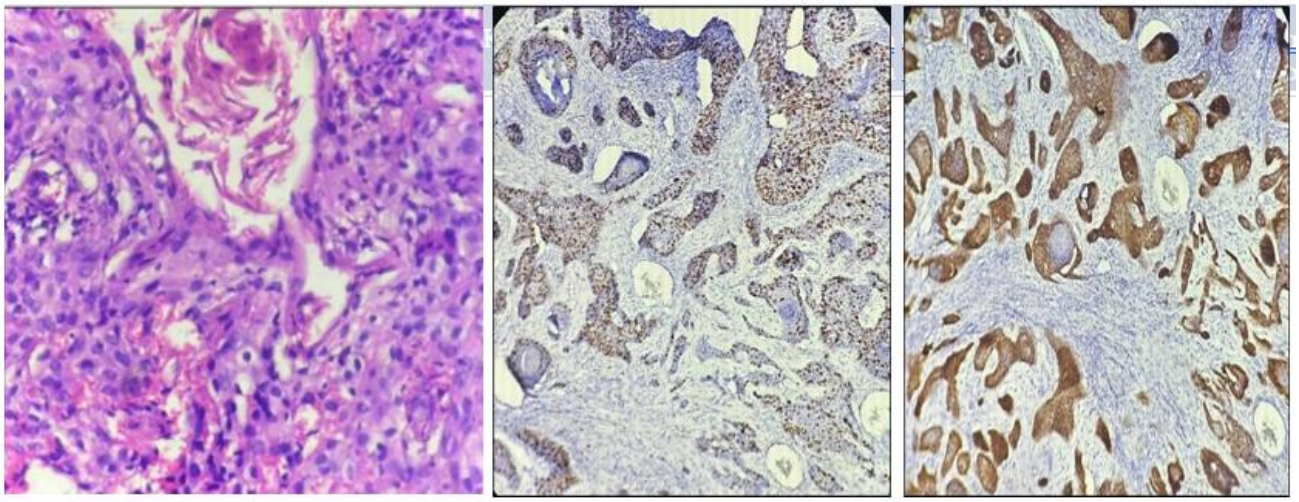
Study Variable: Immunohistochemical Expression

- Age group
- Scoring of ki 67
- Scoring and intensity of p16
- Age wise distribution
- Histological patterns

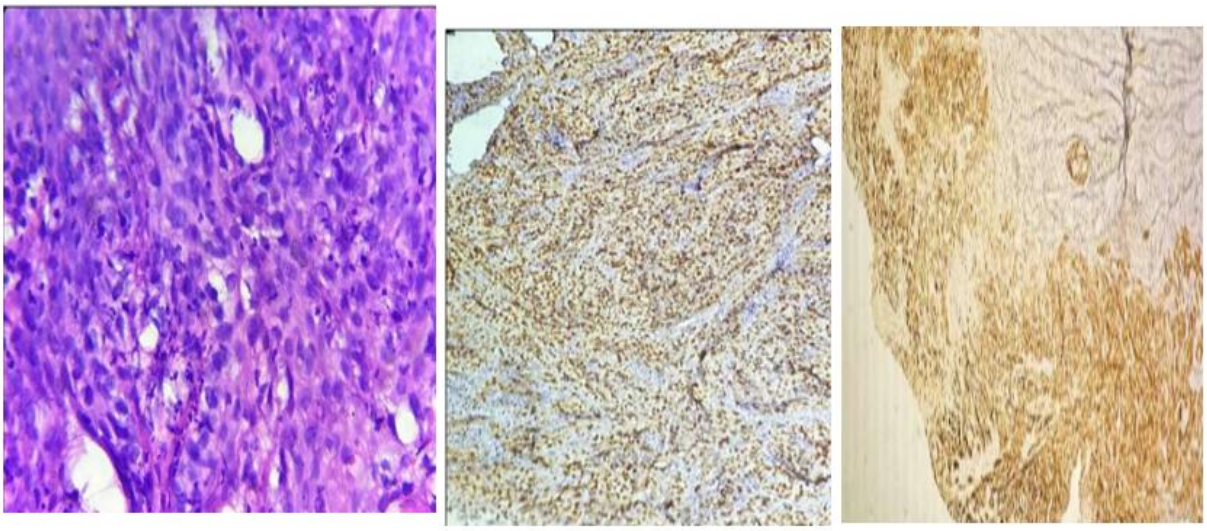


GROSS IMAGESS OF UTERUS WITH CERVICAL CARCINOMA : SPECIMEN 1, SPECIMEN 2, SPECIMEN 3

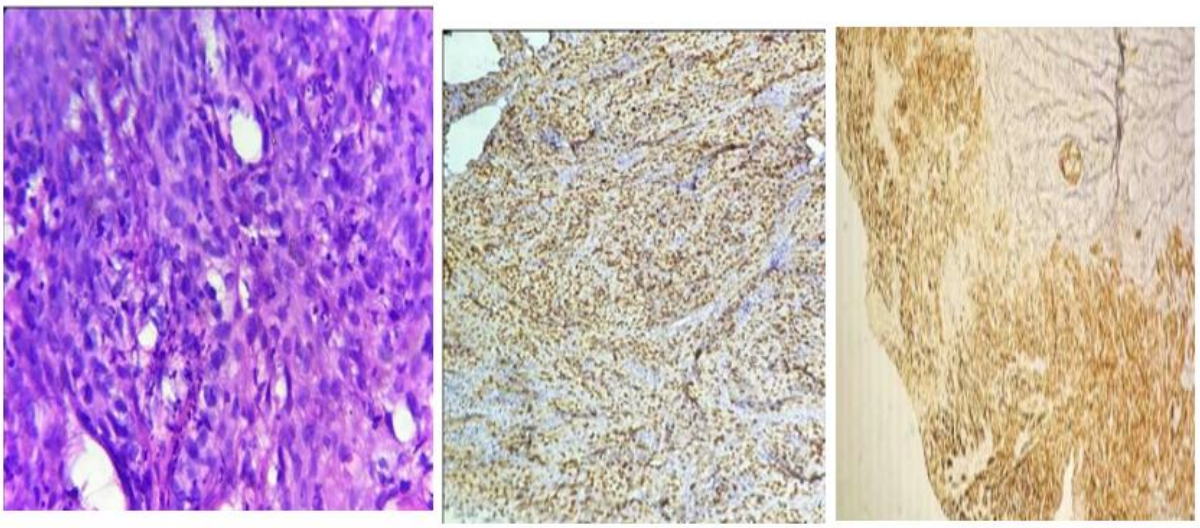
Statistical Analysis: For statistical analysis, data were initially entered into a Microsoft Excel spreadsheet and then analyzed using SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism (version 5). Numerical variables were summarized using means and standard deviations, while Data were entered into Excel and analyzed using SPSS and GraphPad Prism. Numerical variables were summarized using means and standard deviations, while categorical variables were described with counts and percentages. Two-sample t-tests were used to compare independent groups, while paired t-tests accounted for correlations in paired data. Chi-square tests (including Fisher's exact test for small sample sizes) were used for categorical data comparisons. P-values ≤ 0.05 were considered statistically significant.



SPECIMEN 1: H&E; Ki67; p16 IMAGES OF WELL DIFFERENTIATED SQUAMOUS CELL CARCINOMA o



SPECIMEN 2: H&E; Ki67; p16 IMAGES OF POORLY DIFFERENTIATED SQUAMOUS CELL CARCINOMA



SPECIMEN 3: H&E; Ki67; p16 IMAGES OF ADENOCARCINOMA

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3. RESULT

Table:1. Assessment of Expression of Markers (e.g., Ki-67)

Percentage of Cells Showing Positivity	Grade
< 10% of the tumor cells	Negative
10–30% of the tumor cells	+1
30–50% of the tumor cells	+2
> 50% of the tumor cells	+3

Table: 2. Scoring and Intensity of p16 Expression

Percentage of Cells Showing Positivity	Grade
< 1% of the tumor cells	1
1–10% of the tumor cells	2
11–33% of the tumor cells	3
34–66% of the tumor cells	4
> 66% of the tumor cells	5

Table: 3. Scoring and Intensity of p16 Expression

Percentage of Positive Tumor Cells	Score
< 1%	1
1–10%	2
11–33%	3
34–66%	4
> 66%	5

Table: 4. Age Distribution of Patients with Carcinoma Cervix and SIL

Age in years	CarcinomaCervix	SIL	Total (Percentage)
21-40	3	0	3 (3%)
31-40	13	1	14 (13%)
41-50	18	10	28 (26%)
51-60	41	7	48 (45%)
61-70	11	1	12 (11%)
71-80	2	0	2 (2%)
Total	**88**	**19**	107 (100%)

Table: 5. Clinical and Histopathological Characteristics of Cervical Lesions

Category	Subtype / Finding	Number	Percentage
Presenting Complaint	Irregular bleeding	37	34%
	White discharge PV	29	27%

	Post-menopausal bleeding	16	15%
	Pain abdomen	6	6%
	Cervical growth	17	16%
	Cervical erosions	2	2%
Type of Carcinoma	Squamous Cell Carcinoma	83	94%
	Adenocarcinoma	3	3%
	Adenosquamous Carcinoma	2	2%
Histological Grading of SCC	Well-differentiated	53	60%
	Moderately differentiated	19	22%
	Poorly differentiated	11	14%

Figure: 1. Age Distribution of Patients with Carcinoma Cervix and SIL

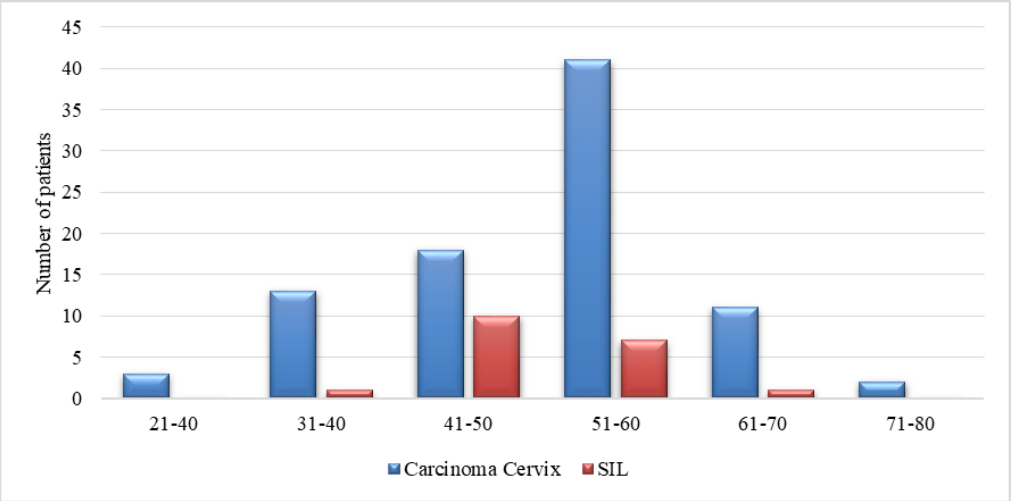
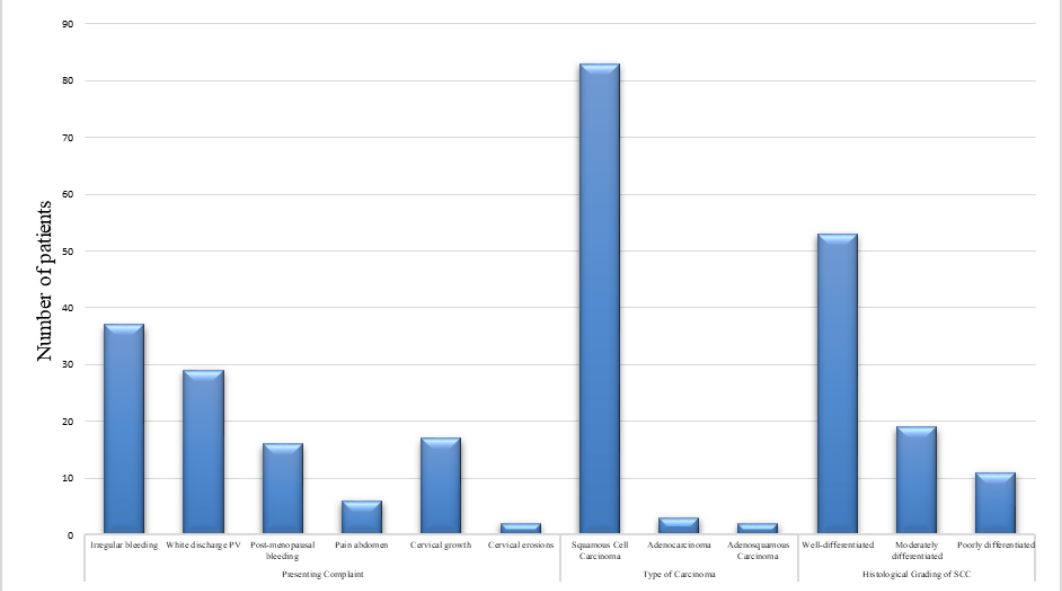


Figure: 2. Clinical and Histopathological Characteristics of Cervical Lesions



The percentage of tumor cells showing positivity is graded as follows: if less than 10% of the tumor cells are positive, the result is considered Negative; if 10–30% of the tumor cells are positive, the grade is +1; if 30–50% of the tumor cells are

positive, the grade is +2; and if more than 50% of the tumor cells are positive, the grade is +3.

The percentage of tumor cells showing positivity is graded on a scale from 1 to 5 as follows: Grade 1 corresponds to less than 1% of tumor cells positive; Grade 2 corresponds to 1–10% positivity; Grade 3 corresponds to 11–33%; Grade 4 corresponds to 34–66%; and Grade 5 corresponds to more than 66% of tumor cells showing positivity.

The percentage of positive tumor cells is scored as follows: a score of 1 corresponds to less than 1% positivity; a score of 2 corresponds to 1–10%; a score of 3 corresponds to 11–33%; a score of 4 corresponds to 34–66%; and a score of 5 corresponds to more than 66% positive tumor cells.

A total of 107 patients with cervical lesions were included, comprising 88 cases of carcinoma cervix and 19 cases of squamous intraepithelial lesion (SIL). The age of patients ranged from 21 to 80 years. In the 21–30 years group, 3 patients (3%) had carcinoma and none had SIL. In the 31–40 years group, 13 patients (12%) had carcinoma and 1 patient (1%) had SIL, totaling 14 patients (13%). Among 41–50 years, 18 patients (17%) had carcinoma and 10 patients (9%) had SIL, totaling 28 patients (26%). The 51–60 years group included 41 carcinoma cases (38%) and 7 SIL cases (7%), totaling 48 patients (45%), representing the highest proportion. In 61–70 years, 11 patients (10%) had carcinoma and 1 patient (1%) had SIL, totaling 12 patients (11%), while in 71–80 years, 2 patients (2%) had carcinoma and none had SIL.

Among the 107 patients included in the study, the most common presenting complaint was irregular bleeding, reported by 37 patients (34%). White vaginal discharge was noted in 29 patients (27%), while post-menopausal bleeding was present in 16 patients (15%). Pain abdomen was reported by 6 patients (6%), cervical growth was observed in 17 patients (16%), and cervical erosions were seen in 2 patients (2%).

Among the 88 cases of carcinoma cervix, the majority were squamous cell carcinoma, observed in 83 patients (94%). Adenocarcinoma was seen in 3 patients (3%), while adenosquamous carcinoma was identified in 2 patients (2%).

Among the 88 cases of carcinoma cervix, 53 patients (60%) had well-differentiated tumors, 19 patients (22%) had moderately differentiated tumors, and 11 patients (14%) had poorly differentiated tumors.

4. DISCUSSION

The results of our study are consistent with previous research indicating that immunohistochemical expression and tumor differentiation significantly influence cervical carcinoma characteristics. Similar to the findings by Kumar et al. [11], who reported that higher positivity correlates with better differentiation, our data show that the majority of cases fall into the +2 positivity and Grade 3 categories, reflecting moderate tumor activity. Additionally, the distribution of staining intensity, with most cases exhibiting moderate (2+) staining, aligns with the observations of Singh et al. [12], who emphasized that moderate staining is common in well-differentiated tumors. Our age distribution and clinical presentation mirror those reported by Lee et al. [13], with the highest prevalence in the 51–60 years age group and irregular bleeding as the predominant symptom, underscoring the typical clinical profile of cervical cancer patients. The predominance of squamous cell carcinoma, as noted in our cohort, is consistent with the global pattern described by Patel et al. [14], who also found that well-differentiated tumors constitute the majority, which has important prognostic implications. These similarities reinforce the importance of immunohistochemical profiling in understanding tumor behavior and guiding management, as supported by the literature [15–20].

5. CONCLUSION

The present study demonstrates that Ki-67 and p16/INK4a are reliable immunohistochemical markers for evaluating cervical intraepithelial neoplasia and carcinoma. Both markers show a strong correlation with histopathological grading, with higher expression observed in high-grade lesions and invasive carcinoma. Their combined assessment enhances the accuracy of diagnosis and helps in distinguishing between low-grade and high-grade lesions. Ki-67 reflects the proliferative activity of the epithelium, while p16/INK4a serves as a surrogate marker for high-risk HPV infection and oncogenic transformation. Incorporating these markers into routine histopathological evaluation can aid in early detection, risk stratification, and appropriate clinical management of cervical lesions, ultimately contributing to better patient outcomes.

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