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Original Article

An Analytical Study Evaluating P16 Expression in Premalignant and Malignant Cervical Lesions and its Correlation with Clinicopathological Parameters

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ABSTRACT

Background: Cervical cancer remains a major global health burden, particularly in developing countries, and is strongly associated with persistent infection by high-risk human papillomavirus (HPV). p16, a cyclin-dependent kinase inhibitor, has emerged as a valuable surrogate biomarker for HPV-associated cervical neoplasia. This study aimed to evaluate p16 expression in premalignant and malignant lesions of the uterine cervix and to correlate its expression with clinicopathological parameters and severity of lesions.

Materials and method: This prospective study was conducted on 30 histopathologically confirmed cases, including cervical intraepithelial neoplasia (CIN I, II, III), squamous cell carcinoma (SCC), and adenocarcinoma, over a two-year period. Formalin-fixed paraffin-embedded tissue sections were subjected to hematoxylin and eosin staining and immunohistochemical analysis for p16 expression. Staining intensity and extent were scored to derive an immunoreactive score (IRS), with a score ≥4 considered positive. Statistical analysis was performed using chi-square tests.

Results: Results demonstrated that SCC was the most common diagnosis (63.33%), followed by CIN (23.33%) and adenocarcinoma (13.33%). A significant increase in p16 expression was observed with increasing severity of lesions. All cases of CIN II, CIN III, and SCC showed 100% positivity, whereas CIN I cases were negative. Adenocarcinoma showed variable expression. The association between p16 expression and lesion severity was statistically significant ($p < 0.001$). No significant correlation was observed with age.

Conclusion: In conclusion, p16 expression correlates strongly with the severity of cervical lesions and serves as a reliable diagnostic biomarker for distinguishing low-grade from high-grade lesions and malignancy.

Keywords: *Cervical cancer, p16 expression, HPV, Cervical intraepithelial neoplasia, squamous cell carcinoma.*

INTRODUCTION

Cervical cancer is prevalent among women worldwide and has been reported to be largely associated with human papilloma virus (HPV) [16]. Cervical cancer was the fourth most common cancer and the fourth leading cause of cancer deaths in women worldwide in 2020 [11]. In India, cervical cancer is the second most common cancer among women and second leading cause of cancer deaths in women during 2020 [8]. The progression of cervical cancer involves many oncogenes and cancer suppressor genes which finally lead to abnormal tumor proliferation in cervix [16].

P16 has been indicated as a useful biomarker of cervical neoplasia [14]. P16 is an inhibitor of cyclin dependent kinase 4/6 (CDK4/6) in cell cycle G1 progression. Even though p16 act as a tumor suppressor, as oncogene E7 expressed by human papilloma virus (HPV) mediates the degradation of retinoblastoma protein (RB) which is a target of CDK4/6 kinase, the

tumor suppressor role of p16 is abolished in HPV transformed cervical cancers. Surprisingly, it has been reported that p16 has oncogenic activity in cervical carcinoma cell lines, suggesting that p16 is not only the diagnostic marker of cervical neoplasia, but also it is necessary for the survival of cervical carcinoma cells. [9]

MATERIALS AND METHODS

Study Design and Setting

This prospective observational study was conducted in the Department of Pathology of a tertiary care teaching hospital in India over a period from August 2022 to August 2022. Permission from the ethical committee was obtained prior to the commencement of the study. Aim and objectives of the study were to analyze expression of p16 in premalignant & malignant lesions of uterine cervix and its correlation with clinicopathological parameters.

Study Population

A total of 30 cases of premalignant and malignant lesions of uterine cervix.

Inclusion & Exclusion Criteria

Inclusion criteria consisted of all histologically confirmed cases of Cervical Intraepithelial Neoplasia (CIN I, II, and III), Squamous Cell Carcinoma (SCC), and Adenocarcinoma of the cervix. Exclusion criteria comprised patients with prior neoadjuvant chemotherapy or pelvic radiotherapy, benign cervical lesions such as cervicitis or polyps, and rare histological variants including adenosquamous, mucoepidermoid, and adenoid basal carcinomas. Additionally, carcinosarcomas, mixed epithelial-mesenchymal tumors, primary germ cell tumors, and all secondary metastatic malignancies were excluded from the study.

Sample collection

Clinical data of patients like clinical history, radiological investigations were collected. Cervical biopsies and Radical hysterectomy specimens received were fixed in 10% neutral buffered formalin overnight. An examination was done on the next day and tumor mass if present was identified. Site, size, extent, color, consistency of the tumor mass was recorded. Bits were taken from representative areas. The tissues were then processed and Haematoxylin and eosin-stained slides of all samples were reviewed and classified according to criteria outlined by the World Health Organization. p16 immunohistochemical stains were conducted by using ready to use mouse monoclonal antibodies.

Data Collection and Analysis

The study reviewed 30 histopathological slides categorized by epithelial dysplasia and histological subtype. Pre-malignant cases included Cervical Intraepithelial Neoplasia I, II, and III. Squamous Cell Carcinoma cases were classified as Grade I well-differentiated, Grade II moderately differentiated, or Grade III poorly differentiated. Adenocarcinoma of the cervix was also included as a glandular malignancy.

p16 Interpretation and Scoring System

p16 expression was evaluated based on the intensity and distribution of nuclear or cytoplasmic staining. Staining intensity was scored as 0 for no staining, 1 for weak, 2 for moderate, and 3 for strong staining. The extent of positivity was scored based on the percentage of stained cells, with 0 for 0%, 1 for 1–10%, 2 for 11–50%, 3 for 51–80%, and 4 for 81–100%. A composite immunoreactive score was then calculated using these two parameters.

Final Score Calculation

The final immunoreactive score (IRS) was calculated by multiplying the staining intensity by the percentage of positive cells (extent), yielding a total score range of 0 to 12 [3]. For statistical analysis, a cut-off value was established where scores ≥ 4 were categorized as "positive" for p16 expression, while scores of 0 to 3 were classified as "negative."

Data was compiled and evaluated using MS-Excel and Statistical Package for the Social Sciences version 29 software. Correlation between histopathological diagnosis and immunohistochemical results of P16 was calculated by chi – square tests. The probability value of <0.05 was considered statistically significant.

RESULTS

Age wise distribution of cervix carcinoma

The age of patients diagnosed with cervical carcinoma ranged from 35 to 75 years, with a mean age of 54.7 years. The highest incidence was observed in the 40–60 age range, which represented 60% of the study population (Table 1, Figure 1).

Table 1: Age wise distribution of cervix carcinoma

Age group	No. Of cases	Percentage
31-40	3	13.04%
41-50	7	30.43%
51-60	7	30.43%
61-70	4	17.39%

>70	2	8.70%
Total	23	100.00%

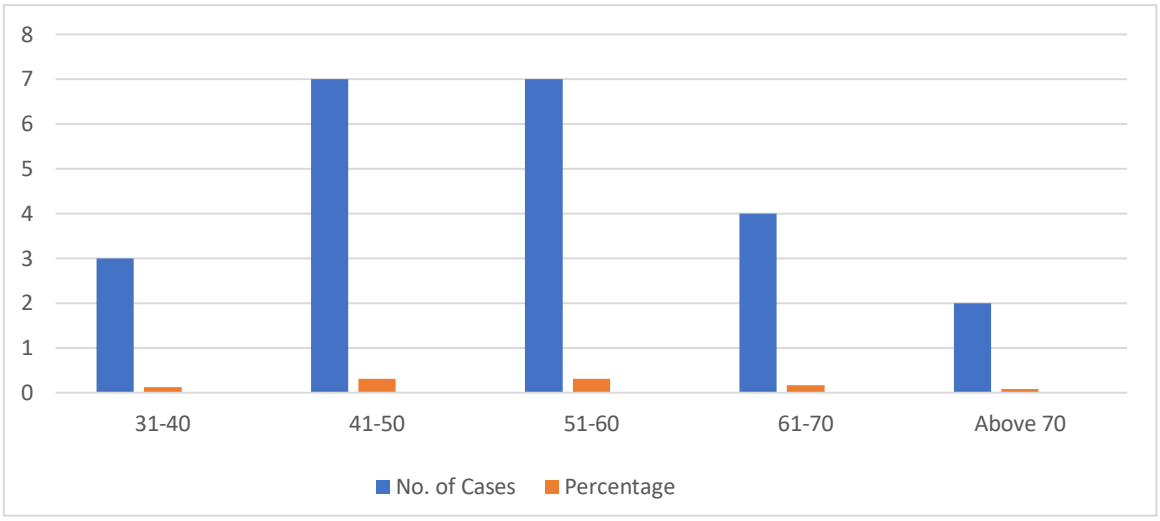

Figure 1: Age wise distribution of cervix carcinoma

Menopausal Status

Regarding reproductive status, cervical carcinoma was most frequently diagnosed in post-menopausal women, accounting for 60.87% of cases. In contrast, 39.13% of the affected patients were pre-menopausal (Table 2, Figure 2).

Table 2: Incidence of carcinoma with respect to menstrual status

Menstrual status	No. Of cases	Percentage
Menstruating	9	39.13%
Postmenopausal	14	60.87%
Total	23	100.00%

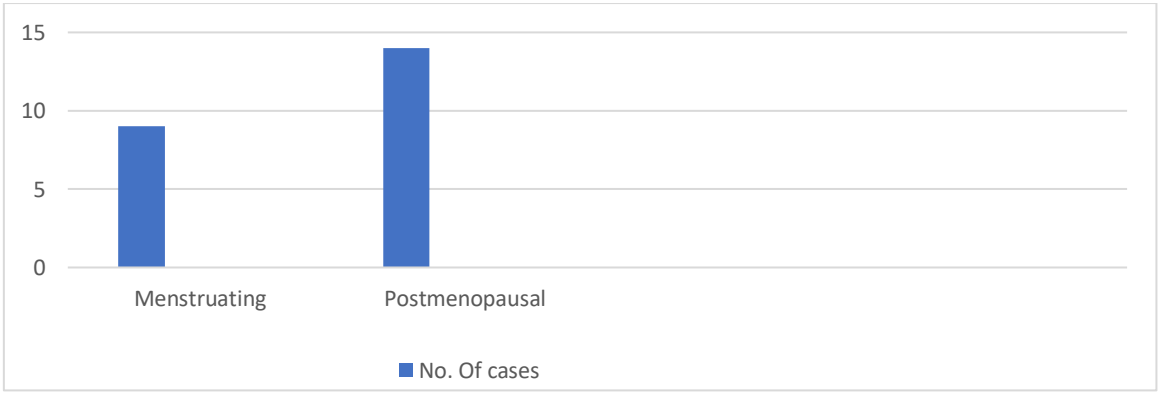

Figure 2: Incidence of carcinoma with respect to menstrual status

In our study, the most common complaint in patients with CIN was leukorrhea and in patients with squamous cell carcinoma was post-menopausal bleeding (Table 3, Figure 3).

Table 3: Incidence of various symptoms in cervical intraepithelial neoplasia, squamous cell carcinoma and adenocarcinoma

Diagnosis	Symptoms				
	Abnormal uterine bleeding	Leucorrhea	Postmenopausal bleeding	Pus discharge	Total
Adenocarcinoma	1 (3.33%)	2 (6.67%)	1 (3.33%)	0 (0.0%)	4 (13.33%)
CIN	2 (6.67%)	4 (13.33%)	1 (3.33%)	0 (0.0%)	7 (23.33%)
SCC	5 (16.67%)	3 (10.0%)	10 (33.33%)	1 (3.33%)	19 (63.33%)
Total	8 (26.67%)	9 (30.0%)	12 (40.0%)	1 (3.33%)	30 (100.00%)

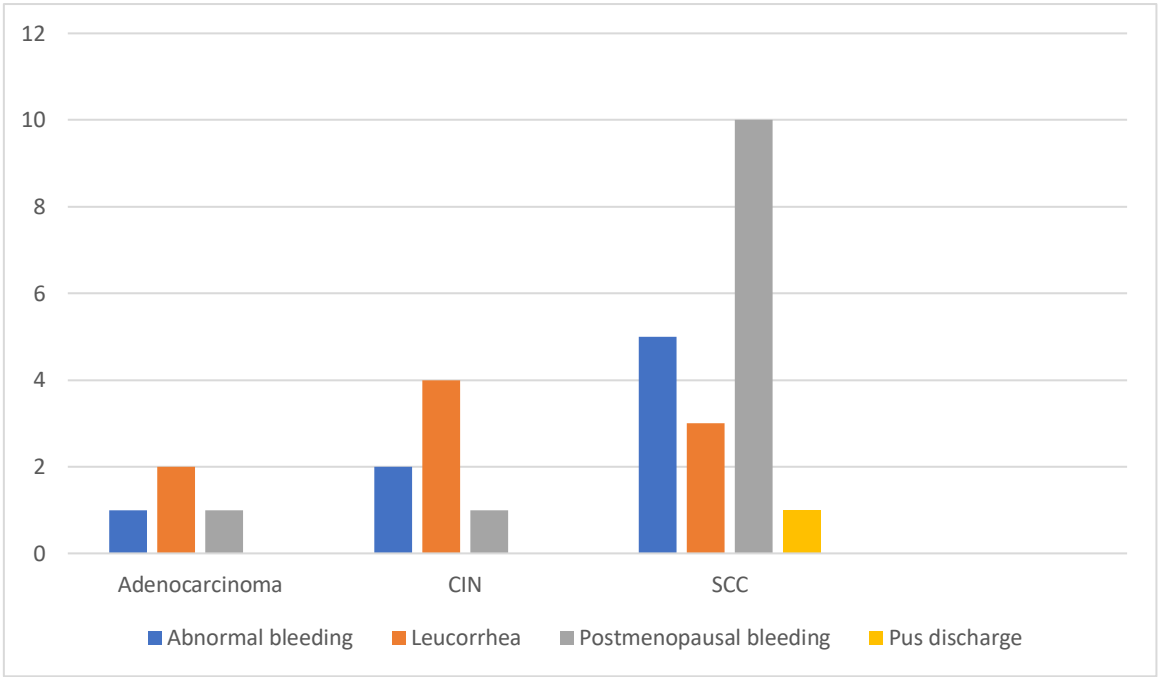

Figure 3: Incidence of various symptoms in cervical intraepithelial neoplasia, squamous cell carcinoma and adenocarcinoma

Histopathological Diagnosis

As illustrated in Table 4 and Figure 4, Squamous Cell Carcinoma (SCC) was the most prevalent diagnosis, comprising 19 cases (63.33% of the total cohort). Cervical Intraepithelial Neoplasia (CIN) accounted for 7 cases (23.33%), while Adenocarcinoma was identified in 4 cases (13.33%).

Table 4: Histological subtypes of premalignant and malignant lesions of cervix

HPE diagnosis	No. Of cases	Percentage
SCC	19	63.33%
CIN	7	23.33%
Adenocarcinoma	4	13.33%
Total	30	100.00%

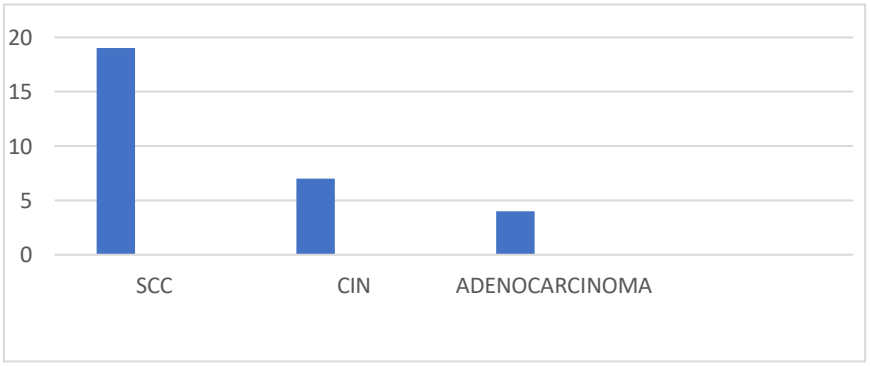

Figure 4: Histological subtypes of premalignant and malignant lesions of cervix

Correlation between p16 expression and age

Regarding age distribution of p16 positivity, the 41–50 and 51–60 age groups were the most represented, each accounting for 30% of the total cohort as detailed in Table 5 and Figure 5. In the 31–40 group, there were three positive cases and one negative, while the 41–50 group contained six positive and three negative cases. Notably, every patient aged 51 and older—including those in the 51–60, 61–70, and over 70 categories—demonstrated 100% p16 positivity. Despite this trend of universal positivity in older patients, statistical analysis indicates no significant association between age and p16 expression within this dataset, suggesting that p16 expression levels are independent of patient age in this study population.

Table 5: Correlation between p16 expression and age

P16 expression	Age group					
	31-40	41-50	51-60	61-70	Above 70	Total
Negative	1 (3.33%)	3 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (13.33%)

Positive	3 (10.0%)	6 (20.0%)	9 (30.0%)	6 (20.0%)	2 (6.67%)	26 (86.67%)
Total	4 (13.33%)	9 (30.0%)	9 (30.0%)	6 (20.0%)	2 (6.67%)	30 (100.00%)

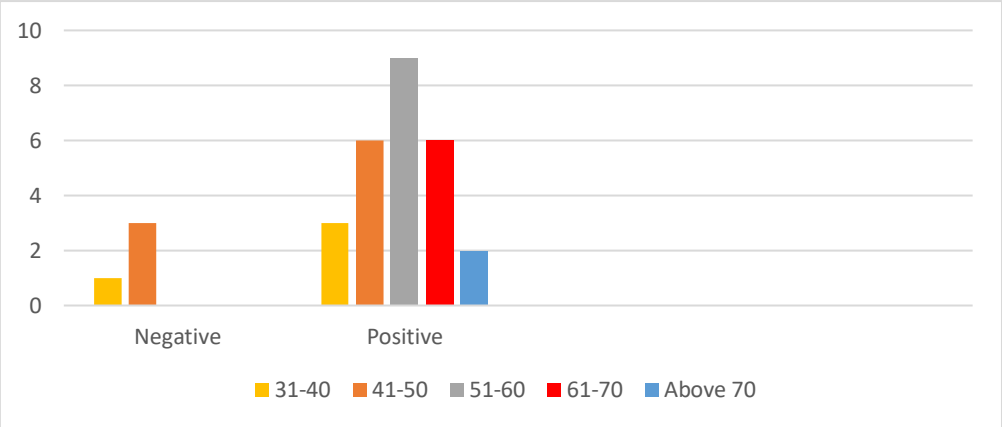

Figure 5: Correlation between p16 expression and age

Correlation between p16 expression and histological diagnosis

The relationship between histological diagnosis and p16 expression across the 30 cases is summarized in Table 6 and Figure 6, revealing a clear trend where p16 positivity increases significantly with the severity of the lesion. High-grade lesions and malignancies, including all 19 cases of Squamous Cell Carcinoma and all cases of CIN II and CIN III, demonstrated 100% p16 positivity. In contrast, adenocarcinoma showed a balanced distribution with two cases testing positive and two testing negatives, while all CIN I cases were negative for p16 expression.

Table 6: Results of p16 expression in cervical intraepithelial neoplasia, squamous cell carcinoma and adenocarcinoma

HPE diagnosis	P16 expression Negative	P16 expression Positive	Total
Adenocarcinoma	2 (6.67%)	2 (6.67%)	4 (13.33%)
CIN I	2 (6.67%)	0 (0.0%)	2 (6.67%)
CIN II	0 (0.0%)	2 (6.67%)	2 (6.67%)
CIN III	0 (0.0%)	3 (10.0%)	3 (10.0%)
SCC	0 (0.0%)	19 (63.33%)	19 (63.33%)
Total	4 (13.33%)	26 (86.67%)	30 (100.00%)
Chi-square statistic: 21.35 / p-value: 0.00027			

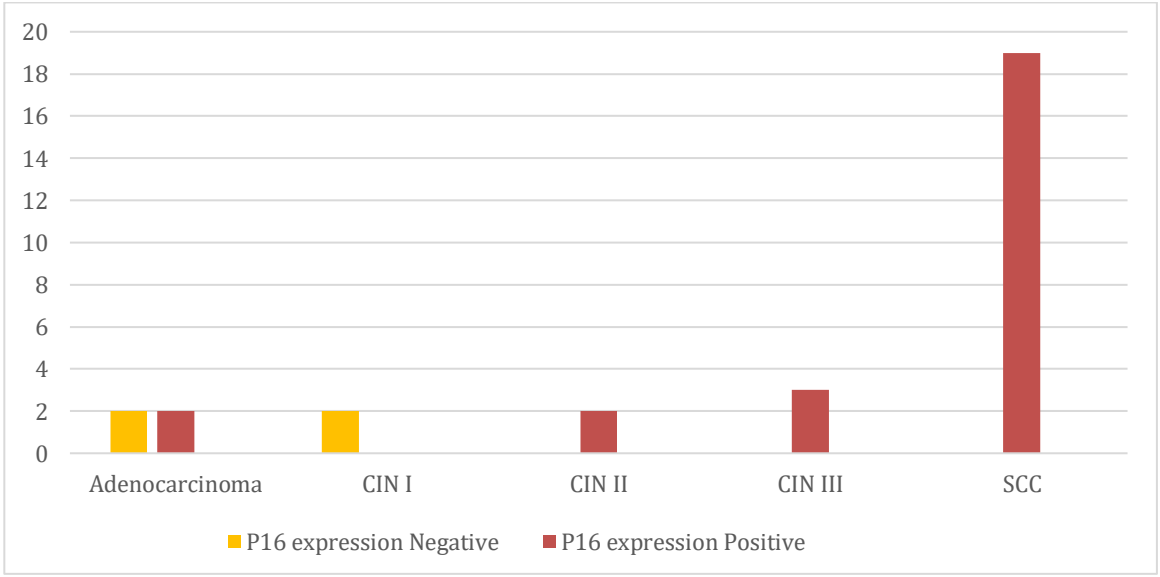

Figure 6: Results of p16 expression in cervical intraepithelial neoplasia, squamous cell carcinoma and adenocarcinoma

Correlation between diagnosis and intensity of p16 staining

Moderate Intensity was the most frequent finding, accounting for 14 cases (46.67%) (Table 7, Figure 7). This group was primarily composed of 8 cases of Squamous Cell Carcinoma (SCC), alongside 2 cases each of Adenocarcinoma, CIN II, and CIN III. **Strong Intensity** followed closely with 12 cases (40.0%), dominated almost entirely by SCC (11 cases) with

only a single case of CIN III. Finally, **Negative Intensity** was the least common, representing 4 cases (13.33%) split equally between Adenocarcinoma (2 cases) and CIN I (2 cases). Overall, SCC was the most prevalent diagnosis across the sample, showing a strong correlation with higher intensity levels.

Table 7: Correlation between diagnosis and intensity of p16 staining

HPE diagnosis	Moderate	Negative	Strong	Total
Adenocarcinoma	2 (6.67%)	2 (6.67%)	0 (0.0%)	4 (13.33%)
CIN I	0 (0.0%)	2 (6.67%)	0 (0.0%)	2 (6.67%)
CIN II	2 (6.67%)	0 (0.0%)	0 (0.0%)	2 (6.67%)
CIN III	2 (6.67%)	0 (0.0%)	1 (3.33%)	3 (10.0%)
SCC	8 (26.67%)	0 (0.0%)	11 (36.67%)	19 (63.33%)
Total	14 (46.67%)	4 (13.33%)	12 (40.0%)	30 (100.00%)
Chi-squared statistic: 25.76 / p-value: 0.001				

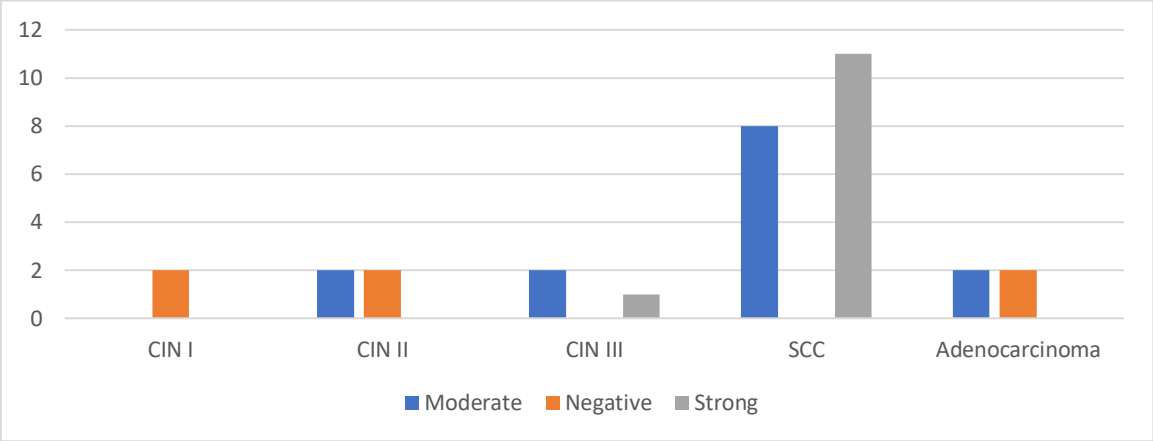

Figure 7: Correlation between diagnosis and intensity of p16 staining

DISCUSSION

Cervical carcinoma is the fourth most common cancer in women worldwide. Infection with Human Papilloma Virus has been a well-established etiological factor for development of cervical carcinoma. Identifying Human papilloma virus infection can be done using p16 which is used as a diagnostic marker by immunohistochemical staining method [1].

In the present study, the age of patients diagnosed with carcinoma of the cervix ranged from 35 to 75 years, with a mean age of 54.7 years. The demographic distribution was primarily concentrated in the 40 to 60-year age bracket, which accounted for 60% of the total cases. Notably, no cases were observed in patients younger than 35 or older than 75 years.

These findings are consistent with the study by Geetha et al., which similarly observed a lower incidence of cervical carcinoma in women younger than 30 and older than 70 years [4]. This age distribution reflects the typical oncogenic progression of high-risk HPV, which often manifests as invasive disease several decades after initial exposure.

Furthermore, the majority of patients in our cohort were post-menopausal (60.87%), while 39.13% were still menstruating. This high prevalence in post-menopausal women underscores the importance of continued cervical cancer screening beyond the reproductive years, as physiological changes in the cervix during menopause can often mask early symptoms or complicate clinical diagnosis.

Regarding histological distribution, Squamous Cell Carcinoma (SCC) was the most prevalent subtype identified, followed by Adenocarcinoma. This distribution pattern is consistent with the findings reported by Usha Sarma et al [13]., reinforcing SCC as the predominant malignancy of the cervix.

Clinical presentation varied significantly based on disease progression. In patients diagnosed with Cervical Intraepithelial Neoplasia (CIN), the most frequent presenting complaint was leukorrhea. In contrast, patients with Squamous Cell Carcinoma most commonly presented with post-menopausal bleeding. These clinical observations align with the study by Kamna Gupta et al [5]., which also noted a high correlation between advanced cervical malignancy and abnormal post-menopausal uterine bleeding.

In the present study, p16 expression demonstrated a high degree of sensitivity for high-grade lesions. All cases of Squamous Cell Carcinoma (SCC) and CIN III (100% respectively) exhibited p16 positivity, findings that are consistent with the observations of Tae Hun Kim et al [8]. Gupta *et al* described p16 positivity in 50%, 60%,70%, and 95% cases of CIN I, CIN II, CIN III, and SCCs respectively [6]. Queriroz et al [12]., showed p16 positivity in 9.1% of metaplasia, 66.6% of CIN I, 93.4% of CIN II/CIN III and 100% of carcinoma cases. Studies by Brown et al [2]. and Murphy et al [10]., also

observed p16 immunopositivity in 100% cases of CIN I/CIN III/ SCC and in 98% cases of CIN II. Similarly, 100% of CIN II cases were positive for the marker in this study. In contrast, all CIN I cases were negative for p16 expression. This is in accordance with Bharadwaj et al [1]., where the majority of CIN I cases were negative—likely due to low viral loads in latent/subclinical HPV infections or the presence of low-risk HPV types that do not trigger the p16-overexpression pathway.

This association is statistically highly significant, with a chi-squared value of 21.35 and a p-value of 0.00027, suggesting that p16 expression is a reliable biomarker for differentiating between low-grade and high-grade cervical pathologies. Among adenocarcinoma cases, p16 positivity was observed in 50% of the cohort.

A progressive increase in staining intensity was observed as the severity of the lesion advanced. While CIN I showed no expression, CIN II cases consistently displayed moderate staining. In the CIN III group, 75% showed moderate staining and 25% exhibited strong positivity. A study by Zhang et al. have reported the HPV positivity rates 72.4% for CIN I, 81.4% for CIN II and 88.1% for CIN III [15] Study by Kishore et al [7]., found p16 positivity in 25% CIN1, 50%CIN2, and 75% cases of CIN3. Yan et al [14]. showed positive p16 immunoexpression in 24.4% of CIN1 and 87.5% of CIN2/3.

p16 expression peaked in the SCC group, where 57% of cases demonstrated strong positive staining and 43% showed moderate staining.

The correlation between the increase in the staining intensity of p16 expression and the progression from low-grade intraepithelial lesions to invasive squamous cell carcinoma was statistically significant ($p = 0.001$). Gupta et al., also concluded that p16 is significantly up-regulated in the high-grade lesions (CIN2/CIN3 - SCC) [5].

These results underscore the utility of p16 as a reliable diagnostic marker for identifying high-grade cervical disease and predicting oncogenic potential

CONCLUSION

Our study concludes that cervical carcinoma most frequently affects women aged 40–60 years, with a notably higher prevalence in the postmenopausal population compared to those who are menstruating. Histologically, squamous cell carcinoma remains the most dominant subtype. The high expression of p16 in approximately 86% of premalignant and malignant lesions underscores its value as a reliable surrogate marker for HPV infection. Furthermore, the progressive increase in p16 intensity and positivity alongside advancing grades of neoplasm suggests its utility as a diagnostic supplement for predicting disease progression. While limited by a small sample size and the absence of HPV DNA validation, this study supports the integration of p16 immunohistochemistry in routine histopathological evaluation. These findings highlight the urgent need for intensified health education and HPV vaccination programs to mitigate disease burden.

DECLARATION

Conflicts of interests: The authors declare no conflicts of interest.
Author contribution: All authors have contributed in the manuscript.
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