(International Print/Online Journal)

SJIF IMPACT FACTOR: 5.565
PUBMED-National Library of Medicine ID-101739732

ISSN (Print): 2209-2870
ISSN (Online): 2209-2862

International Journal of Medical Science and Current Research (IJMSCR)
Available online at: www.ijmscr.com
Volume 4, Issue 5, Page No: 296-307
September-October 2021

IJMSCR

Expression of p16 and Ki67 in squamous intraepithelial lesions and squamous cell carcinoma of cervix

Ritika Hooda[1], Kulwant Singh[2] Swaran Kaur[3] Parveen Rana[4] Anjali Sindhu[5] Kunal Kadian[6]
[1]Post Graduate, [2,4]Professor, [3]Professor and Head [4]Post Graduate Resident, Dept of Pathology, BPS GMC(W) Khanpur Kalan, Sonepat Haryana, [5]Senior Resident, Dept of Pathology, PGIMS Rohtak, Haryana

***Corresponding Author:**
Dr. Anjali Sindhu
SR, PGIMS Rohtak

Type of Publication: Original Research Paper
Conflicts of Interest: Nil

Abstract

Background-
Cervical cancer is a public health problem in India accounting for 17% of all cancer deaths among women aged 30-69 years. Invasive cervical cancer is preceded by precursor lesions hence cervical cancer has the greatest potential for prevention. p16 and Ki67 positivity in cervical smears is a marker of cervical dyskaryosis. The grade of positivity of both the markers is indicative of the higher grade of lesion, hence predicting survival.

Design-
The aim of our study was to evaluate the expression of p16 and Ki67 in squamous intraepithelial lesions and squamous cell carcinoma of cervix. The study was conducted on 40 cervical biopsy and hysterectomy specimens received in the Department of Pathology, BPS GMC, Khanpur Kalan. H&E-stained sections were studied and classified. IHC assessment and grading for p16 and Ki67 was performed.

Results and Discussion-
Out of 40 cases, 36 cases were diagnosed as SCC and 4 cases as dysplasia. Staining for p16 was positive in all the cases of carcinoma (100%) and 50% of cases of dysplasia. ($p<0.001$) Ki67 was positive in all 40 cases (100%). A positive correlation between p16 and Ki67 was also found which is also statistically significant ($p<0.001$)

Conclusion-
The expression of p16 is upregulated with the increase in dysplastic cells to cervical carcinoma which indirectly indicates degree of malignancy. Ki67 which is usually positive in the lower third of the epithelium also covered almost entire thickness in cervical carcinoma. This finding could be useful to enhance diagnostic accuracy.

Keywords: NIL

INTRODUCTION

Cervical cancer is a public health problem in developing countries like India, so much so that India alone accounts for one-quarter of the worldwide burden of cervical cancers.[1,2] It is the third largest cause of cancer mortality, accounting for 10% of all cancer deaths among women.[3] The median age of cervical cancer is 38 years(ranging from 21-67 yrs).[4] Rural women have a larger incidence of cervical lesions as compared to urban females due to a poorer access to health facilities and lack of knowledge about hygiene practices.[5] The 5 year survival rate averages to 48.7%. The length of survival largely depends on the stage at which the disease is diagnosed.[6] Therefore, it is vital to understand the epidemiology of cervical cancer in India. Screening by pap smear has reduced the incidence of cervical cancer in developed countries, but implementation of this screening technique has not been successful in developing countries.[7] Cervical cancer is generally caused by persistent infections with High-Risk- Human

Papilloma Virus (HR-HPV. HPV-16 and 18 are the two most common highly oncogenic types found in invasive cervical cancer.[8]

Cervical cancer has the greatest potential for prevention as the causative agent is identified and the latency period between the initial infection and development of disease is long. Several cervical screening tests such as Visual Inspection with Acetic acid [VIA], magnified VIA [VIAM], Visual Inspection with Lugol's Iodine [VILI], HPV DNA testing and the Papanicolaou test are available. Cervical biopsy, which is used in conjunction with Papanicolaou cytology testing, HPV DNA testing and colposcopy, has an important role in the evaluation and management of women with cervical dysplastic lesions. Thus, cervical biopsy is crucial for the prevention and early detection of cervical cancer. Since HPV infections supersede cell cycle controls, the immune detection of cell proteins that are differentially expressed in infected cells is currently being considered for use as tumor and prognostic marker, as well as for application in different modalities of cervical cancer screening. Up to 90% of HPV infections regress spontaneously, even without treatment, after a few months.[9] If the viral infection persists, however, the risk of developing a precancerous lesion increases as well as the risk of developing an invasive carcinoma.[10]

p16INK4A: The E6 and E7 proteins of the high-risk HPV inhibit the p53 and Rb proteins respectively (cell cycle regulatory proteins). They phosphorylate retinoblastoma (Rb) protein which induces the release of a transcription factor E2F from its bound form with pRb. The released E2F then stimulates the expression of genes which are involved in G1-S transition. p16INK4a prevents the phosphorylation of RB, keeping it in the hypophosphorylated form. HPV E7 protein expression leads to inactivation of Rb gene, cells uncontrollably pass to the S- cell cycle stage. Thus, in the p16/cyclin D1/cdk4/pRB cell cycle regulatory cascade, the correlation between pRB and p16INK4a is obvious in various cancers. These increased levels of p16INK4a in the nucleus and cytoplasm of affected cells can be detected by means of immunostains.[11]

Over expression of p16INK4a is characteristic of dysplastic and neoplastic cervical epithelium. It can serve as a biomarker that is independent of the individual HR-HPV type and indicative of the cervical cancer disease process in action.[12]

p16INK4a expression is present in almost all high-grade squamous and glandular lesions and rarely in benign conditions. Presence of p16INK4a positivity in cervical smears is a marker of cervical dyskaryosis.

Material & Methods: This was a cross sectional study conducted in the department of Pathology at Bhagat Phool Singh Government Medical College for Women, Khanpur Kalan, Sonepat for a duration of one year. In this study, a total of 80 cervical biopsies and hysterectomy specimens were taken. Specimen were fixed in 10% formalin, grossed and processed as per the standard protocol and the slides were stained with hematoxylin and eosin stain. Immunohistochemical stains for p16INK4a was applied and slides were graded according to the percentage of cells stained.

Scoring criteria: p16 is mainly expressed in the nucleus and cytoplasm.[13] p16 expression was considered negative when it was expressed in less than 5% of dysplastic squamous cells, weak positive when expressed in 5-25% of cells, moderate positive when expressed in 26-50% and intense positive when expressed in more than 50% of dysplastic squamous cells.

Data was analysed using the statistical package SPSS version 22. Chi-square test was used to analyse the data and p value was calculated wherever required. p value of 0.05 or less was considered as statistically significant.

Observations and Results:

A cross sectional study was conducted in the department of Pathology at Bhagat Phool Singh Government Medical College for Women, Khanpur Kalan, Sonepat for a duration of one year. In this study a total of 80 cervical biopsies and hysterectomy specimens were taken. Specimens were fixed in 10% formalin and then subjected to hematoxylin and eosin staining after routine processing of the tissue. Immunohistochemical stains for p16INK4a was applied and slides were graded according to the percentage of cells stained.

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Age group(Years)	Number of patients	Percentage
20-29	2	2.5%
30-39	11	13.75%
40-49	20	25%
50-59	16	20%
60-69	18	22.5%
70-79	10	12.5%
80-89	3	3.75%
TOTAL	80	100%

Table 1: Age wise distribution of patients included in the study

A total of 80 cases were studied. Age of the patients ranged from 26-80 years (Mean age = 53.4 years; median age =54 years). Maximum premalignant lesions were seen in the age groupof 50-59 years old. For premalignant lesions, age range varied from 26-70 years (Mean age = 52.8 years, median age= 55 years).

Histopathological Diagnosis	Number of subjects (n=80)	Percentage
CIN I	6	7.5%
CIN II	1	1.25%
CIN III	8	10%
Squamous cell carcinoma	65	81.25%
Total	80	100%

Table 2: Histopathological diagnosis of cases

Total CIN cases were 15 (18.75%) whereas the SCC cases comprised 81.25%(65/80)

*

Cervical lesion	p16 INK4a Positive cases (percentage)	p16 INK4a Negative cases (percentage)	Total (percentage)
CIN I	2 (33.33%)	4(66.67%)	6(100%)
CIN II	1(100%)	0(0%)	1(100%)
CIN III	8(100%)	0(0%)	8(100%)
Squamous cell carcinoma (SCC)	65(100%)	0(0%)	65(100%)
TOTAL	76(95%)	4(5%)	80(100%)

Table 3: Immunohistochemically expression of p16INK4a in different cervical lesions

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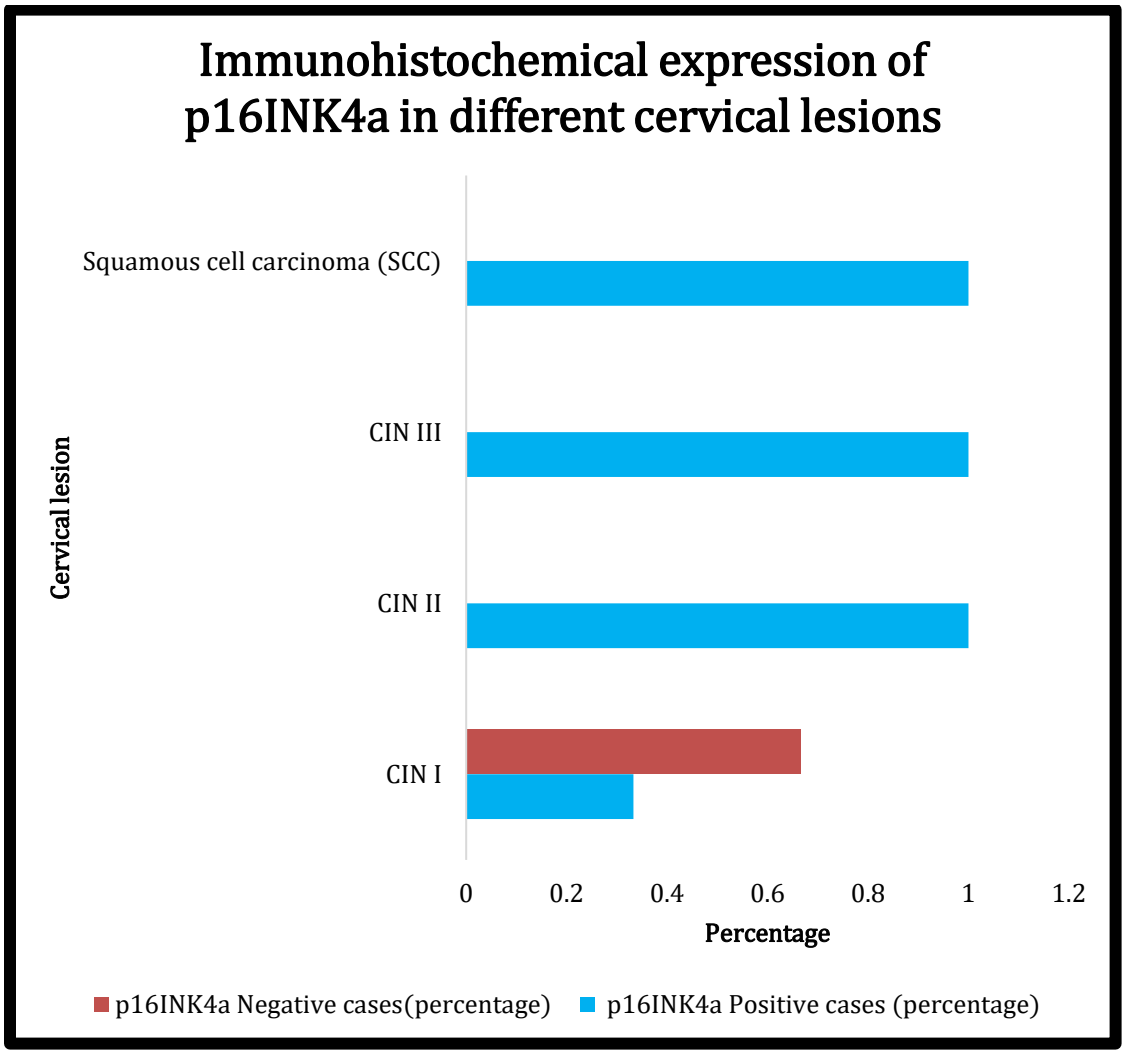
Majority of cases of CIN I showed negative expression. All cases of CIN II, CIN III and squamous cell carcinoma were positive for p16INK4a.



Histopathological diagnosis	Grades of p16INK4a Expression				Total
	Grade 0 (negative)	Grade 1	Grade 2	Grade 3	
	No. of cases (%)	No. of cases (%)	No. of cases (%)	No. of cases (%)	
CIN I	4(66.67%)	2(33.33%)	0(0%)	0(0%)	6(100%)
CIN II	0(0%)	1(100%)	0(0%)	0(0%)	1(100%)
CIN III	0(0%)	0(0%)	3(37.50%)	5(62.50%)	8(100%)
Squamous cell carcinoma (SCC)	0(0%)	0(0%)	7(10.77%)	58(89.23%)	65(100%)
TOTAL	4	3	10	63	80

Table 4: Correlation of grade of p16INK4a expression in different cervical lesions

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Grade of p16 INK4a expression increased from CIN I to SCC.

Cervical lesion	p16INK4a negative	p16INK4a positive	Total
CIN I (LSIL)	4	2	6
CIN II and CIN III (HSIL)	0	9	9

Table 5. p16INK4a expression in premalignant intraepithelial lesions

A statistically significant difference between p16INK4a expression in CIN I and CIN II/III was observed (p value= 0.011)

Discussion: Cervical cancer is second commonest malignancy of females with SCC constituting majority of the cases.[3] In most of the cases, cervical SCC is preceded by various stages of cervical intraepithelial neoplasia. Screening for these lesions is carried out by cervical pap smear examination and the diagnosis is confirmed by histopathological examination of cervical biopsy specimen. Many immunohistochemical markers have been studied to aid in the diagnosis and categorization of cervical squamous lesions. p16INK4a is a tumor suppressor protein that is encoded by the CDKN2A gene and has a major role in down regulation of cell cycle by inhibiting the progression of cell cycle from G1 to S phase. p16INK4a is a marker of productive HPV infection and has been used as surrogate marker of HR-HPV infection.[14,15]

In our study, a total of 80 consecutive cases with histopathologic diagnosis of cervical SIL/ SCC were studied. Age range of the patients was 26-80 years. Mean age for squamous intraepithelial lesions (CIN I, II and III) was slightly higher in present study compared to other studies and requires further study.[16-20] Mean age for SCC compared well with other studies.[16,17,19-21]

	Makaju et al[16]	**Zhang et al[17]**	**Sharma et al[18]**	**Gupta et al[19]**	**Rana et al[20]**	**Raju et al[21]**	**Present study**
CIN I (LSIL)	43.5	36.8	36.67	38.9	-	-	53.2
CIN II	48	40.1	39.33	41.3	-	-	**58**
CIN III	43.4	41	-	46.4	-	-	52
Squamous cell carcinoma	53.97	43.09	-	48.5	54.0	54.2	53.5

Table 5. Comparison of mean ages for cervical lesions among different studies

Cases of SCC appear to be more in present study. It may perhaps be due to less utilization of preventive cervical pap smear screening in our rural setting and requires further workup. Measurers like patient education regarding routine pap smear examination may be helpful.

Cervical intraepithelial neoplasia constituted 18.75% of total cases in present study compared to 30.4 %[22,] 31.4%[23]and 36.5%[19] and 52.8%[24]in other studies. The correspondingly a smaller number of CIN cases in the present study may be due to late presentation of females in the hospital in rural setting.

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	Krishnappa et al[22]	Kumari et al[23]	Gupta et al[19.]	Saravanan et al[24]	Present study
Squamous cell carcinoma	69.6%	68.6%	63.5%	47.2%	81.25%
CIN	30.4%	31.4%	36.5%	52.8%	18.75%

Table 6: Proportion of squamous lesions

In the present study, proportion of CIN I was higher in younger age groups whereas proportion of SCC was more in older age groups. Most cases of CIN II and CIN III were seen in the age groups in between. (Table 5) However, SCC was the predominant diagnosis across all age groups due to reasons mentioned previously. These findings suggest that proportion of higher-grade lesions and SCC increases with increasing age. Similar findings have been observed in other studies. [17,23]

In the present study, Immunohistochemistry showed p16INK4a expression in 95% of all cases whereas negative expression was observed in the rest of cases (5%). p16INK4a positivity was seen in 33.33% of CIN I, 100% cases each of CIN II, III and squamous cell carcinoma cases. 66.67% of CIN I cases were negative for p16INK4a expression. These findings compare well with most of other studies. In other studies, variation in values has been seen. However, it was observed in all these studies that p16INK4a positivity increases with increasing grade of the lesions like the observations in present study.

Study	CIN I	CIN II	CIN III	Squamous cell carcinoma
Shi et al[25]	9.09%	-	-	95.65%
Zhong et al[13]	67.32%	98.85%	99.38%	100%
Castle et al[26]	26.6%	71.9%	90.7%	94.5%
Pandey et al[27]	37.5%	-	-	96%
Mandal et al[28]	33.3%	58.1%	73.8%	-
Sarma et al[29]	32	52.3	100	100
Present study	33.33%	100%	100%	100%

Table 7: p16INK4a positive expression (percentage) in different studies

Evaluation of grade of p16INK4a expression showed that there was increased grade of expression with increase in grade of lesions from CIN I to SCC. CIN I showed predominantly grade 0 (negative) expression. CIN III and squamous cell carcinoma showed predominance of grade 3 expression. Similar findings have been reported in other studies.[27,29] These observations suggest that positive expression and increased grade of expression is observed with increase in severity of cervical lesions (from CIN I to SCC).

CONCLUSION: To conclude, p16INK4a expression in the present study showed increased expression with increasing grade (severity of cervical lesions) from CIN I to SCC. It suggests that this immunohistochemical marker can be of help in making diagnosis when it is not possible to obtain unequivocal diagnosis on hematoxylin and eosin stained sections. Its routine use in such cases may be helpful and can help in planning appropriate treatment decisions by the treating clinician.

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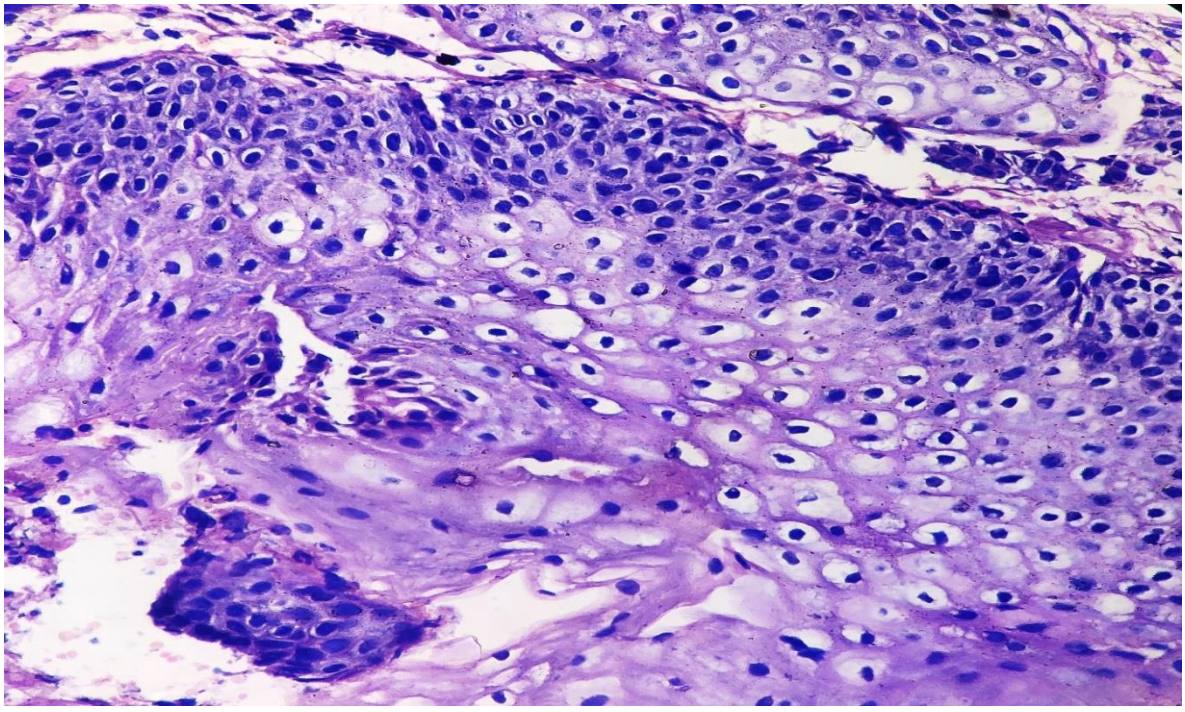

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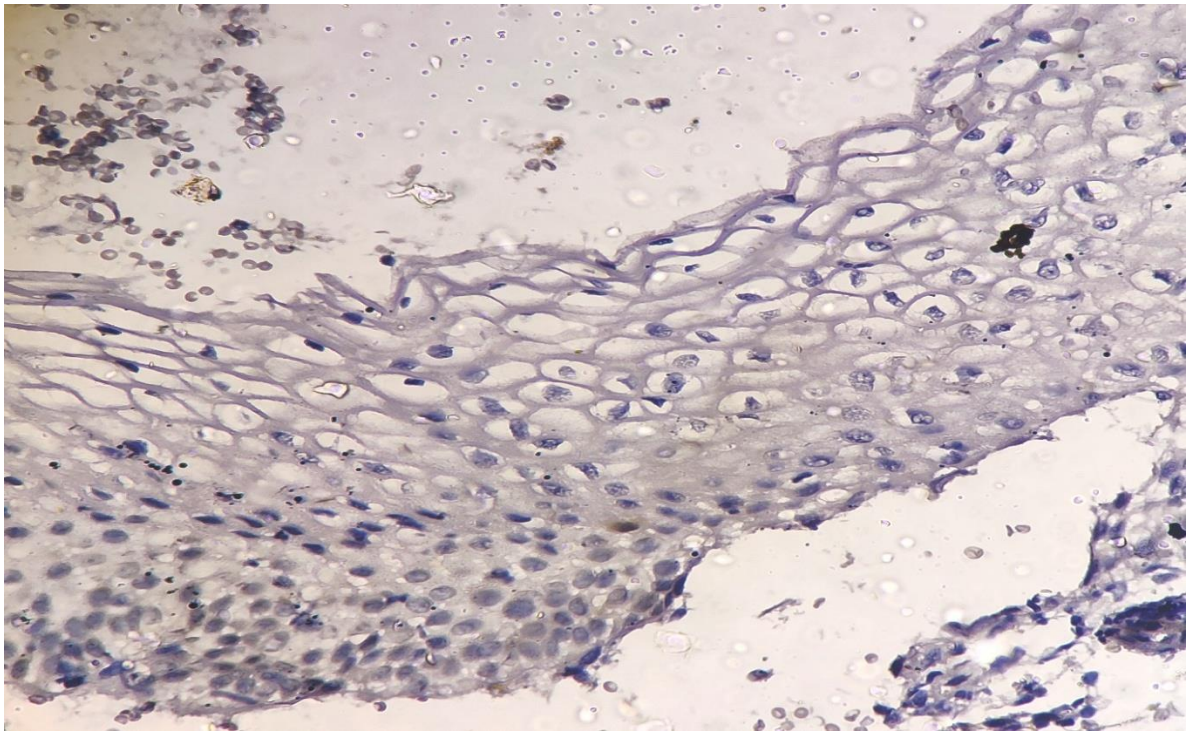
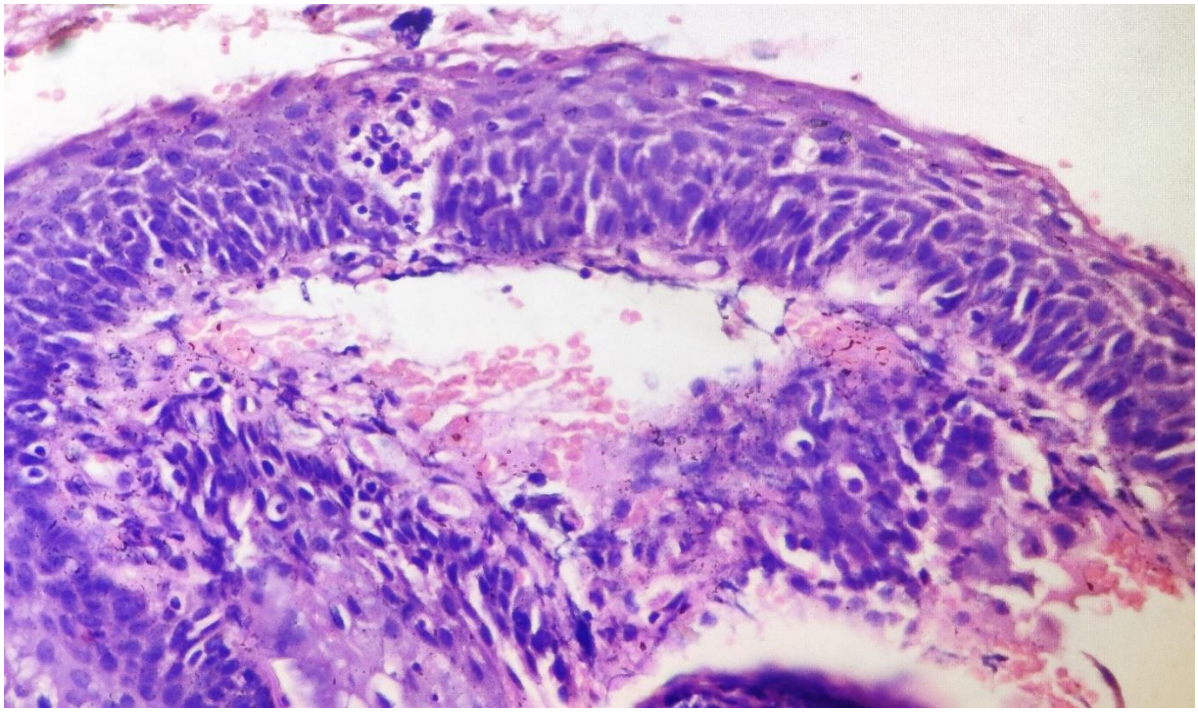
Photomicrograph 1: Tissue section of mild dysplasia showing proliferation confined to basal third along with koilocytic changes (H & E, 400X)



Photomicrograph 2: Tissue section of a case of mild dysplasia showing absence of positivity of p16INK4a (grade 0) in stratified squamous epithelium (IHC, 400X)



Photomicrograph 3: Tissue section of a case of moderate dysplasia showing proliferation of epithelium confined to lower two- third of epithelium (H & E, 400X)

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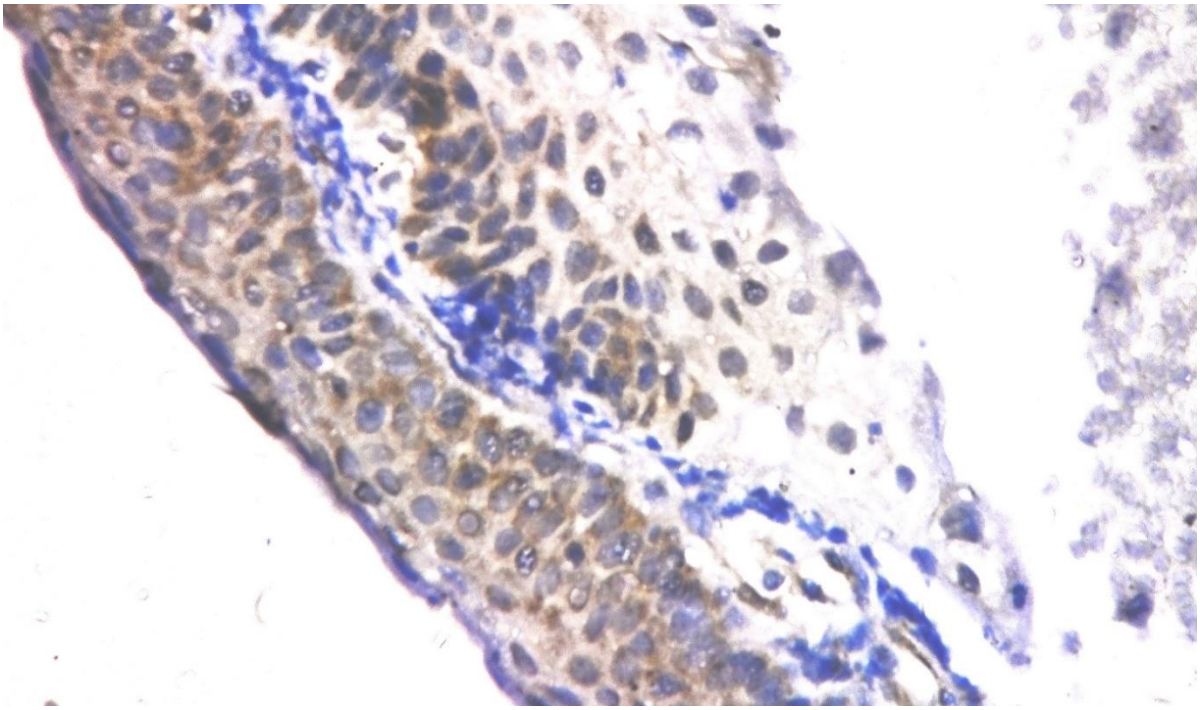
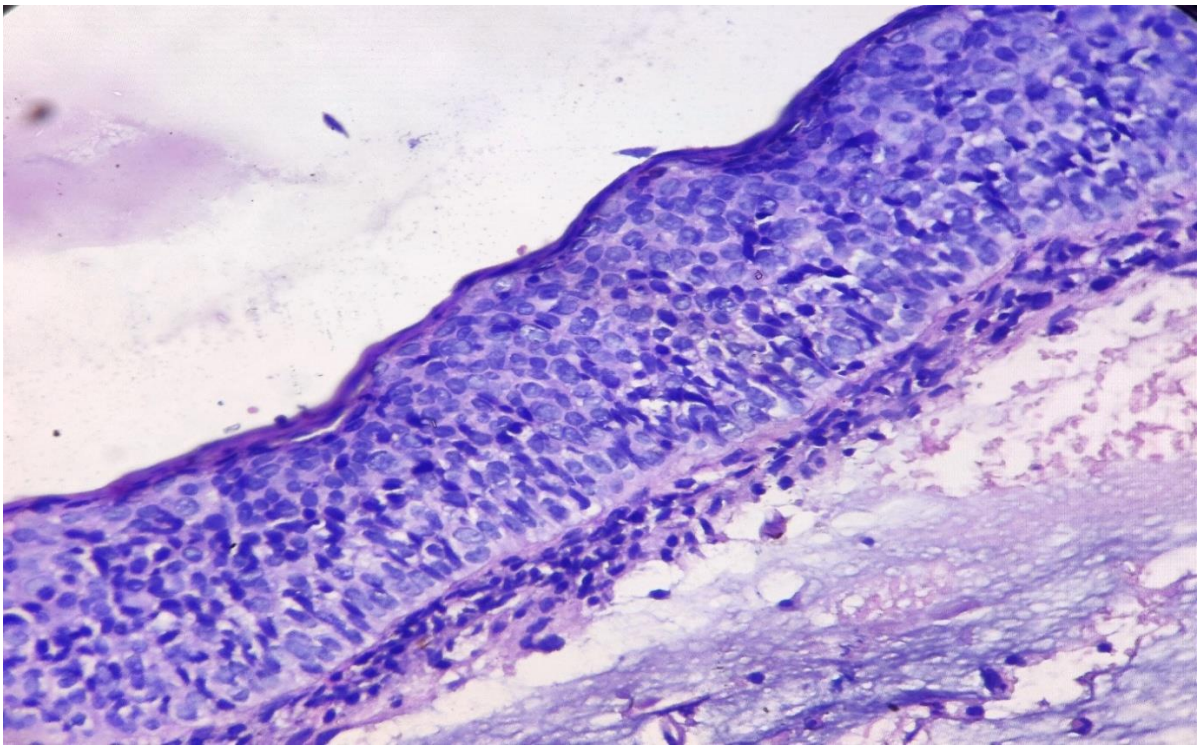


Photomicrograph 4: Case of moderate dysplasia showing grade 1 positivity of p16INK4a expression in stratified squamous epithelium (IHC, 400X)



Photomicrograph 5: Tissue section from a case of severe dysplasia showing epithelial proliferation including all the layers along with nuclear atypia (H & E, 400X)

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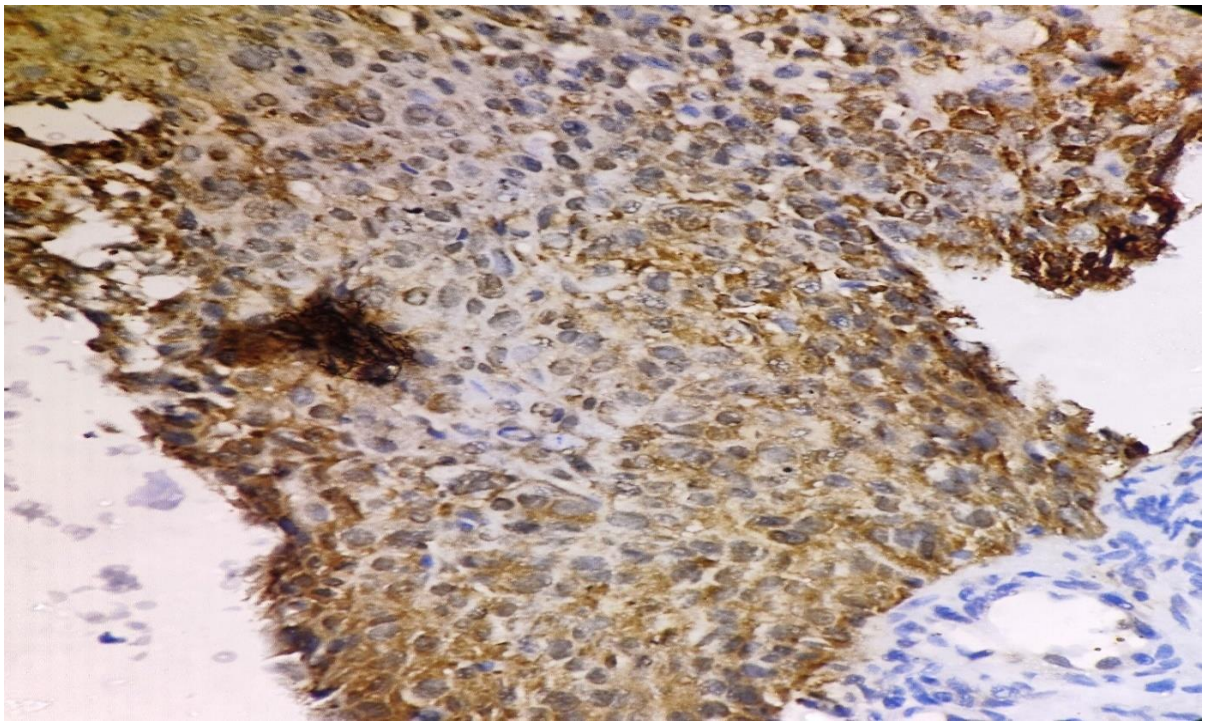
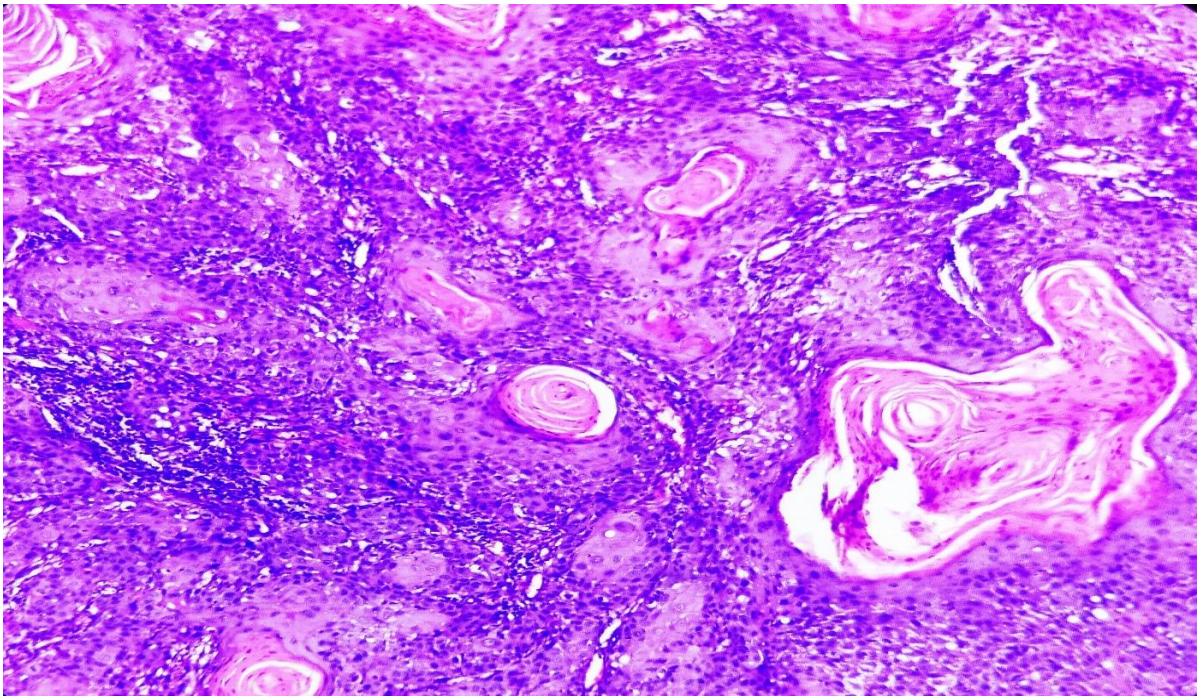


Photomicrograph 6: Tissue section of a case of severe dysplasia showing 2+ positivity for p16INK4a in stratified squamous epithelium (IHC, 400X)



Photomicrograph 7: Squamous cell carcinoma showing sheets of malignant squamous cells with interspersed inflammatory stroma. Many keratin pearls are also noted (H & E, 100X)

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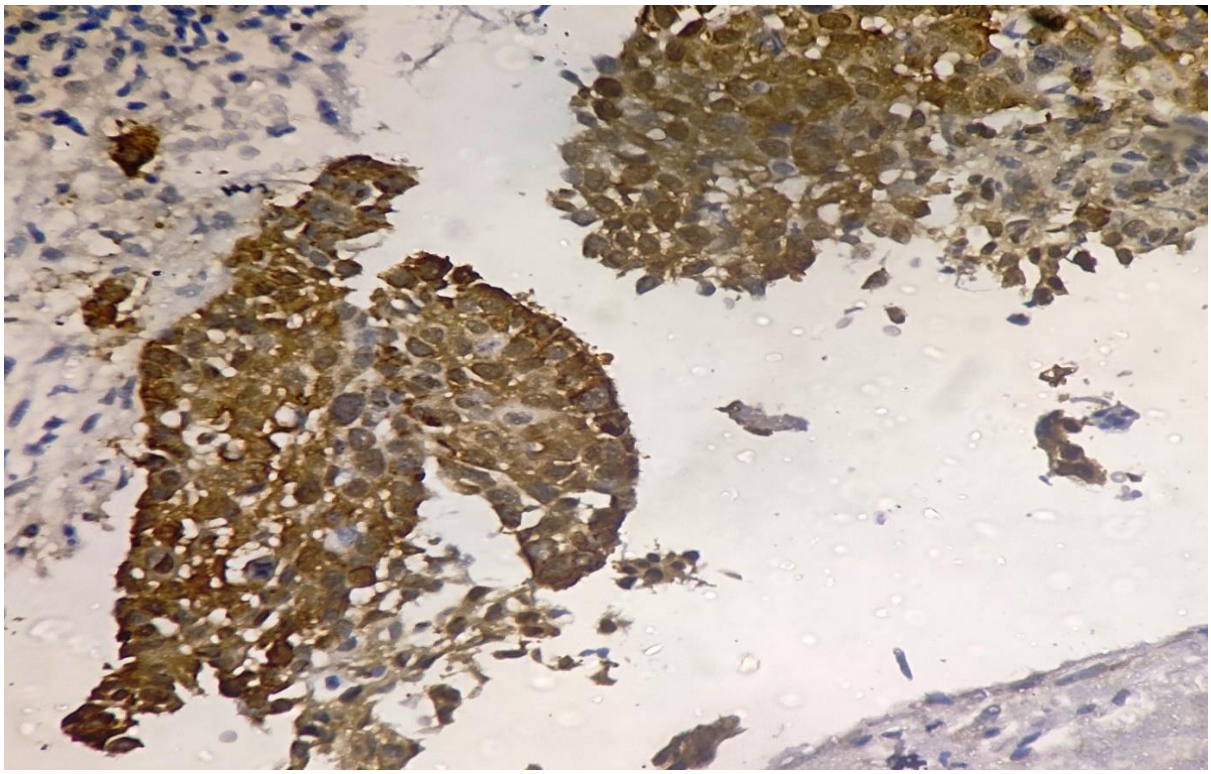

Photomicrograph 8: Tissue section of a case of Squamous cell carcinoma showing 3+ positivity of p16INK4a (IHC, 400X)

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