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Expression of p16 in cervical intraepithelial neoplasm and invasive cervical carcinoma: An immunohistochemical study

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Abstract
Background: According to WHO-Cervical Cancer is the fourth most common cancer in women. This study evaluated expression of P16 in cervical intraepithelial lesion and cervical carcinoma and its correlation with tumour grade, age, etc.
Material and Methods: 106 histopathologically proven Cervical Intraepithelial lesions (CIN) and invasive cervical carcinoma cases were subjected to IHC for p16.
Result: Most common age group involved was 51-60 years. Bleeding per vaginum was most frequent complaint in Invasive Cervical carcinoma and discharge per vaginum was most frequent complaint in cervical carcinoma patients. P16 showed positivity in only 7.10% of CIN I cases, 30% of CIN II cases, 57.10% of CIN III cases and majority (81.30%) of invasive cervical carcinoma.
Conclusion: The expression of P16 positivity increases with increasing grades of dysplasia (Carcinoma>CIN III> CIN II> CIN I).

Keywords: Per vaginum was most frequent, intraepithelial lesions

Introduction
India accounts for nearly one-fourth of the world's cervical cancer deaths, with 60,078 deaths and 96,922 new cases in 2018. This largely preventable disease is the second most common cause of cancer mortality among Indian women [1]. Despite the availability of effective low cost screening options in India, limited access to screening and treatment services, diagnosis at a later stage, and low investment in health care infrastructure all contribute to the higher number of deaths [2].

Histological variants of cervical cancer
1. Squamous cell carcinoma – 70-80% of the cervical carcinoma are of squamous type.
2. Adenocarcinoma – 20% of the cervical carcinoma are of this type [3].

Types of Premalignant cervical Lesions
1. CIN 1- the undifferentiated cells are confined to the lower one-third of the epithelium.
2. CIN II- the undifferentiated cells occupy the lower two-third of the epithelial thickness
3. CIN III– The entire thickness of the epithelium is replaced by abnormal cells. The basement membrane, however, is intact and there is no stromal infiltration.

The risk factors associated with cervical cancer are high risk sexual behaviour, multiple full-term pregnancies, use of oral contraceptive pills, low socioeconomic status, exposure to diethylstillbestrol, chlamydia infection, etc.
The main presenting symptoms of cervical carcinoma are bleeding per vaginum, leucorrhoea, metorrhagia, dyspareunia, cervical growth, etc.

Materials and Methods
This study was conducted on 106 histopathologically proven cases of Cervical Intraepithelial lesion and Cervical Carcinoma. Blocks after cutting were stained with Hematoxylin and Eosin stain and were studied for classification and histopathological grading. Immunohistochemistry of the tumours was done for P16. Positive and negative controls were run with every batch of the IHC.

Test sections showed positivity in the form of specific color. P16 Scoring system- Both nuclear and cytoplasmic brownish coloration are taken as positive for p16 [5].

Table 1: P16 Scoring system

Percentage positivity	Score
0	Negative
1 - 25%	1+
26 - 50%	2+
51 - 75%	3+
76 – 100%	4+

Result

On H&E staining done on 106 paraffin blocks, 75 were reported to be Invasive cervical carcinoma (71%) and 31 were reported to be cervical intraepithelial neoplasms (29%). According to menstrual status, 71 (75%) cases were premenopausal whereas 35 (25%) cases were postmenopausal. Low-grade preneoplastic lesions (CIN I) are seen most commonly in 51-60 years of age, high grade lesions (CIN II, III) are most commonly seen in 41 – 60 years of age and invasive cervical carcinoma are most commonly seen in 51-60 years of age. The commonest presenting complaint was white discharge in patients with all grades of CIN and post-menopausal bleeding in patients with invasive cervical carcinoma cervix. Out of the 106 specimens, 31(29.20%) cases were of cervical intraepithelial neoplasm and 75 (70.80%) were of invasive cervical carcinoma.

Out of 75 cases, 71 (94.67%) cases were of squamous cell carcinoma type and 4 (5.33%) cases were of adenocarcinoma type. Among the 71 squamous cell carcinoma cases (SCC), 60(84.51%) cases were diagnosed as Large Cell Non- Keratinizing SCC, 7(9.86%) cases were diagnosed as Large Cell Keratinizing SCC, and 4 (5.63%) cases as Small Cell Non-Keratinizing SCC. Patients were also categorized according to their grades. In SCC, 3(4.2%) cases were of the well-differentiated type, 63(88.7%) were moderately differentiated and 05(7.0%) cases were poorly differentiated. All four cases of adenocarcinoma were moderately differentiated. No significant statistical correlation of tumour grade, stage, parity and age of the the patient, histomorphological variant of SCC with p16 expression was found in our study.

Results of P16 Immunostaining in premalignant and malignant cervical carcinoma.

P16 showed positivity in only 7.10% of CIN I cases, 30% of CIN II cases, 57.10% of CIN III cases and majority (81.30%) of invasive cervical carcinoma. A highly significant correlation between histological grade and p16 positivity is seen, i.e, with the increase in tumour grade, p16 positivity increased. Few of the CIN III (43%) and invasive cancer (19%) cases showed p16 negativity.

Table 2: Results of P16 Immunostaining in premalignant and malignant cervical carcinoma

	p16 Negative		p16 Positive		Total	Chi Value	p value
Grade	N	% age	N	% age			
CIN-I	13	92.90%	1	7.10%	14	35.012	<0.001
CIN-II	7	70.00%	3	30.00%	10		
CIN-III	3	42.90%	4	57.10%	7		
Invasive cervical carcinoma	14	18.70%	61	81.30%	75		
	37	34.90%	69	65.10%	106		

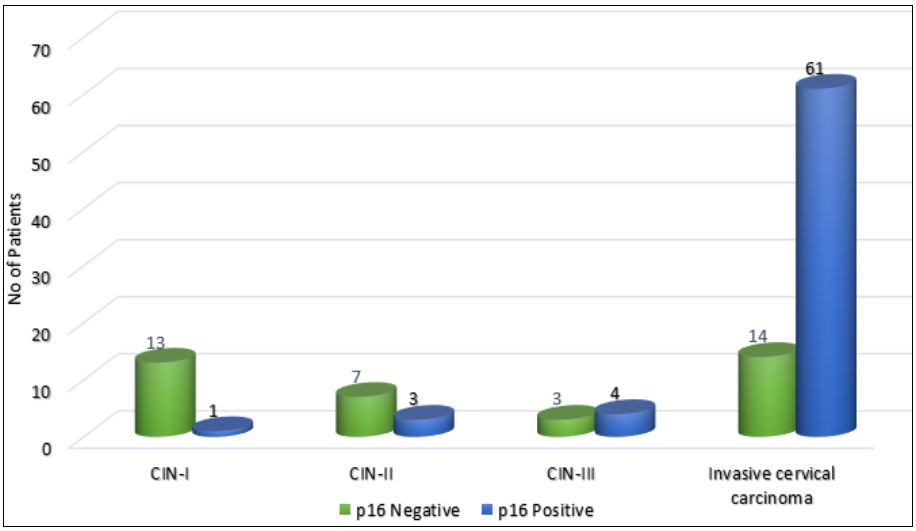

Fig 1: P16 Immunostaining in premalignant and malignant cervical carcinoma

Correlation of percentage of cells positive with p16 with severity of cervical lesion

Most of CIN show negative staining, while most of the CIN II and CIN III showed grade 1+ and 2+ scoring respectively and majority of the invasive Cervical Carcinoma showed grade 4+ scoring. Therefore it is seen that with there is increase in the percentage of cells positive with p16 with increasing grade of CIN and invasive cervical carcinoma. This correlation was found to be highly significant.

Table 3: Correlation of percentage of cells positive with p16 with severity of cervical lesion

Histological Grading	Percentage Positivity					Total	Chi Value	p value
	Negative	Positive						
	0%	1 - 25%	26 - 50%	51 - 75%	76 – 100%			
CIN I	13	1	0	0	0	14	40.735	<0.001
CIN II	7	2	1	0	0	10		
CIN III	3	0	2	2	0	7		
Invasive cervical carcinoma	14	15	16	14	16	75		
TOTAL	37	18	19	16	16	106		

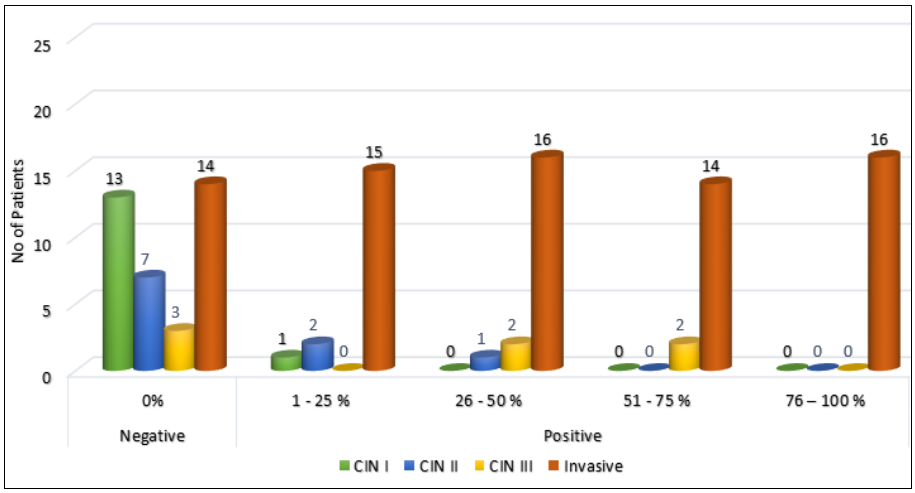

Fig 2: Correlation of percentage of cells positive with p16 with severity of cervical lesion

Discussion

Cervical cancer is one of the most common cancers in India. Screening for cervical cancer helps in the clear reduction of invasive cervical cancers.

The percentage of overall p16 positivity in this study was 65.10% which was close to that of Chaloob *et al*. (74.28%) and Supriya *et al*. [6, 7] (76.1%). In the present study, P16 showed positivity in only 7.10% of CIN I cases, 30% of CIN II cases, 57.10% of CIN III cases and majority (81.30%) of invasive cervical carcinoma. A highly significant correlation between histological grade and p16 positivity is seen, i.e, with the increase in tumour grade, p16 positivity increased. These findings were similar to those of study by Umar *et al*. (HSIL-88.81%, Carcinoma-88.52%) which also found that p16 expression was directly related to increasing grade of CIN [8].

Few of the CIN III (43%) and invasive cancer (19%) cases showed p16 negativity. P16 negative high-grade lesions also exist as reported by Valgareva *et al*. (2004). The possible explanation for the absence of p16 expression in these high-grade lesions could be methylation of the p16 promoter resulting in silencing of the p16 gene [9].

Many other studies have also emphasized the significance of using p16 immunostain as a marker for identifying dysplastic and neoplastic lesions caused by high-risk HPV.

It has been seen in our study that with increasing severity of the cervical lesion, a higher percentage positivity of p16 is seen. A similar study was done by Kishore V *et al*. which found the overexpression of p16 in increasing frequency in the sequence: CIN1 (25%) –CIN2 (50%) –CIN3 (75%) – invasive carcinoma (100%) [10].

Another study done by Pari P *et al*. also reiterated with the findings of our study showing increased expression of p16 with increasing grade of dysplasia [11].

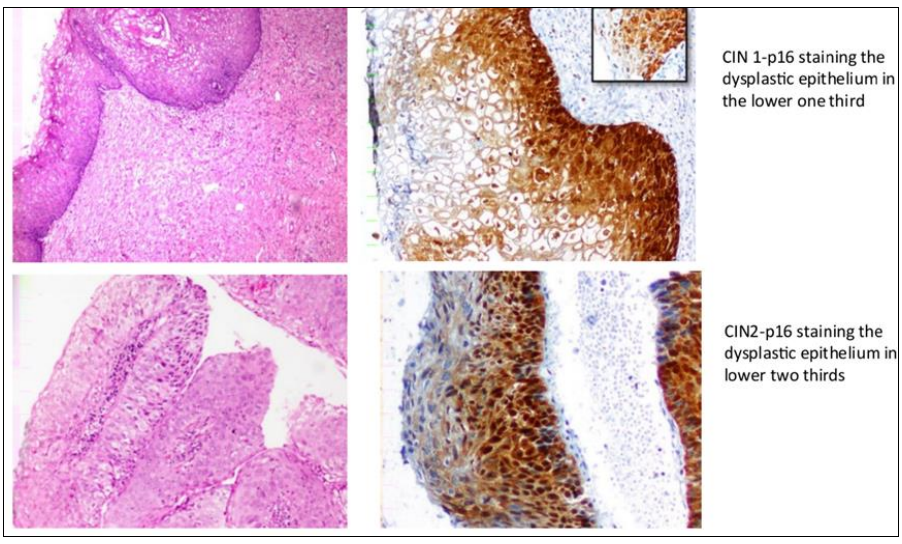

Fig 3: The percentage of overall p16 positivity

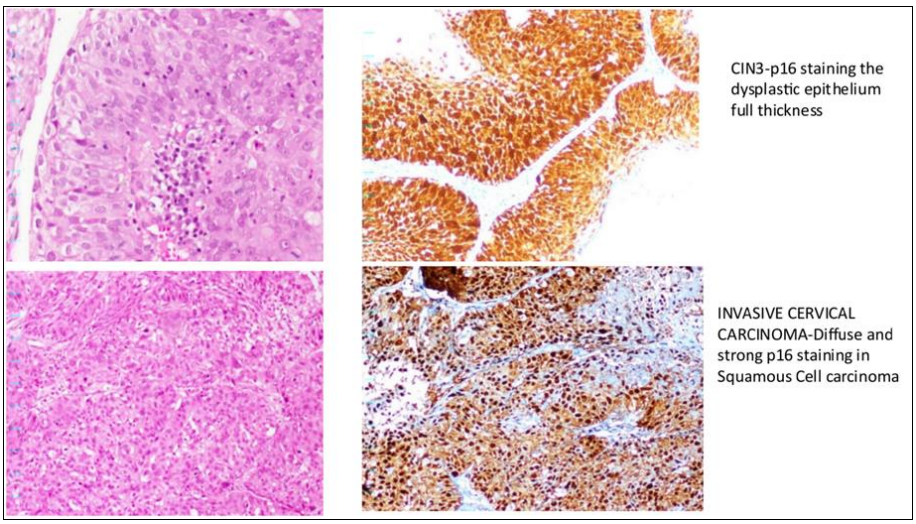

Fig 4: Few of the CIN III (43%) and invasive cancer (19%) cases showed p16 negativity

Conclusion

The neoplastic transformation of cervical epithelial cells by HPV can be detected by p16 expression. Overexpression of the protein p16 is an important feature of dysplastic and neoplastic cervical epithelial cells. The expression of P16 positivity increases with increasing grades of cervical dysplasia (Carcinoma>CINIII<CIN II<CIN I). Henceforth, our study reiterated that the protein p16 can be used as a specific diagnostic marker of cervical intraepithelial lesions and cervical carcinoma.

Conflict of Interest

Not available

Financial Support

Not available

References

1. Reichheld A, Mukherjee PK, Rahman SM, David KV, Pricilla RA. Prevalence of Cervical Cancer Screening and Awareness among Women in an Urban Community in South India – A Cross Sectional Study. Ann. Glob. Health. 2020;86(1):30.
2. Van Dyne EA. Establishing baseline cervical cancer screening coverage- India, 2015–2016. MMWR. Morbidity and mortality weekly report. 2019;68:14-9.
3. Oh CM, Jung KW, Won YJ, Shin A, Kong HJ, Jun JK, *et al*. Trends in the incidence of in situ and invasive cervical cancer by age group and histological type in Korea from 1993 to 2009. PloS one. 2013;8(8):72012.
4. Chopra SJ, Mathew A, Maheshwari A, Bhatla N, Singh S, Rai B, *et al*. Natio. Padubidri VG, Daftary SN, editors. Howkins & Bourne, Shaw's Textbook of Gynecology, -EBOOK 16th edition; Published by Reed Elsevier India Private Limited; c2014. p. 486.
5. Lewis JS Jr, Thorstad WL, Chernock RD, Haughey BH, Yip JH, Zhang Q, *et al*. p 16 positive oropharyngeal squamous cell carcinoma: an entity with a favorable prognosis regardless of tumor HPV status. Am J Surg Pathol. 2010;34(8):1088-96.
6. Chaloob MK, Hussein AG, Qasim BJ. Correlation of P16 (Ink4a) and CK17 to HPV (16E6 + 18E6) in Premalignant and Malignant Lesions of Uterine Cervix: A Clinicopathologic Study. Iran J Pathol. 2016 Fall;11(4):377-390. PMID: 28855930; PMCID: PMC5563936.
7. Supriya Srivastava. p16INK4A and MIB -1: An immunohistochemical expression in preneoplasia and neoplasia of cervix. Indian journal of pathology and microbiology. 2010;53(3):518-524.
8. Umar Immunohistochemical expression of p16INK4A protein in cervical dysplasia and carcinoma in patients attending federal teaching hospital Afr. J Cell Pathol20167416
9. Volgareva G, Zavalishina L, Andreeva Y, Frank G, Krutikova E, Golovina D, *et al*. Protein p16 as a marker of dysplasia and neoplastic alterations in cervical epithelial cells. BMC Cancer. 2004;4:58.
10. Kishore V, Patil AG. Expression of p16INK4A Protein in Cervical Intraepithelial Neoplasia and Invasive Carcinoma of Uterine Cervix. J Clin Diagn Res. 2017;11(9):17-20. 17.
11. Pari PV, Bhat R. A study on expression of p16INK4a by immunohistochemistry in cervical dysplasia and Invasive carcinoma of cervix. IP Arch Cytol Histopathol Res. 2020;4(4):310-4.

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