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"ROLE OF P16 IMMUNOCYTOCHEMICAL MARKERS IN EPITHELIAL CELL ABNORMALITIES OF CERVIX

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Abstract

Background: The prevalence of cervical cancer patients in India has varied from 87.8% to 96.67%. It has been studied that HPV-16 and 18 are the highly oncogenic types found in invasive cervical cancer and out of these two HPV-16 has been found more commonly.

Materials and methods: This study is a hospital based cross-sectional study carried out in the Department of Pathology of Integral Institute of Medical Sciences and Research, Hospital, Lucknow. The institutional ethical committee approved the study. From March 2021 to September 2022, pap smears were collected from 47 patients attended OPD as well as IPD in Gynaecological department.

Results: In the present for study the sensitivity, specificity, PPV and NPV for P16 was 95.0%, 42.89%, 35.29% and 92.31% respectively.

Conclusion: The present study is undertaken to evaluate the diagnostic performance and Utility of p16 immunocytochemistry for triaging patients with epithelial cell abnormality of the cervix as the per 2014 Bethesda reporting system and identify premalignant and malignant lesions of the cervix and then follow up those cases and correlate with histopathological findings.

Keywords: Immunocytochemistry, malignant lesions.

Introduction

Cervical cancer is the third most common cancer in women worldwide. With continuing improvement in screening methods and vaccination programs in developed countries, the disparity of burden between women in developed countries and women in resource-poor settings becomes even more profound. Currently, more than eighty-five percent of cervical cancer deaths occur in low and middle-income countries. Tragically, cervical cancer is the leading cause of cancer deaths in women in the developing world.[1] High-risk subtypes of the human papillomavirus (HPV) are the cause of the disease in most cases.[2]

There are three screening modalities for cervical cancer i.e., cytology, visual inspection, and HPV test. Pap smear in cytology has several drawbacks, such as high false-negative rates, low

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sensitivity, subjective interpretation, and low predictive value, as one-third of women who progressed to cervical cancer had a normal Pap smear [3]. The p16 protein immunocytochemistry has been mentioned as an effective approach for triage in women with cytological abnormalities, such as atypical squamous cells of undetermined significance (ASC-NOS)[4] . The p16 protein, a cyclin-dependants inhibitor, decelerates the cell cycle by facilitating retinoblastoma protein (Rb) and E2F transcription factor re-binding. The E7 oncoprotein, which is the product of E7 oncogene of hr-HPV interrupts the linkage between Rb and E2F transcription factor, resulting in disruption of Rb/E2F pathway[4].Therefore, overexpression of p16 protein in a dysplastic cervical epithelial cell is indicative of hr-HPV- induced transformation[5]. These studies showed the importance of dual immunostaining in precancerous minor cervical abnormalities. A systematic review and meta-analysis showed that p16 immunocytochemistry has higher specificity than hr-HPV testing to detect underlying high-grade squamous intraepithelial lesions in the triage of ASC-NOS or low-grade squamous intraepithelial lesions (LSIL).[6]

Material & Methods

This study is a hospital based Prospective, observational study carried out in the Department of Pathology of Integral Institute of Medical Sciences and Research, Lucknow during March 2021 to September 2022. Cervical samples of all patients attending gynaecological O.P.D. and I.P.D. patients was collected and stained with Papanicolaou stain. All patients reported as having epithelial abnormality as per the Bethesda reporting system was immunostained with p16.

Approval from the Institutional Research and Ethical Committee was taken before the start of the study. An informed verbal, as well as written consent, was taken from the patient as well as the attendant if present A detailed examination of All women of age 25 to 65 years attending gynaecology O.P.D. as well as I.P.D. of our institute was taken. It included the demographic data; presenting chief complaints with Menstrual history including Last Menstrual Period, Gynaecological history, Obstetric history, whether Pregnant or not, Past medical history, Past treatment history, Socioeconomic status, Personal history, Also Per vaginal and Per Speculum Examination were done.

Statistical analysis

All statistical analyses were performed using Microsoft EXCEL. Chi-square test for independence for evaluating the association of p16 staining with cervical epithelial abnormality. All the categorical data were compared by Fisher's exact test. A P value < 0.05 was taken as statistically significant. Sensitivity, specificity, positive predictive value, and negative predictive value of p16 INK4a in detecting pre-neoplastic lesions and neoplastic lesions of the

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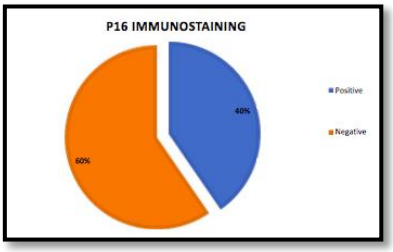
cervix were calculated.

Results

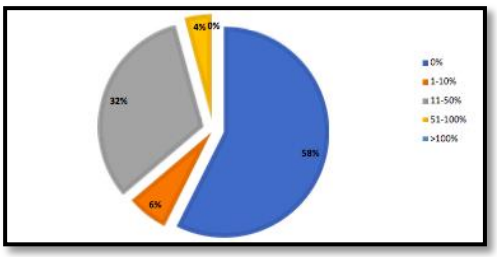
Out of a total of 47 patients, 18 (38.3%) patients fell into the 41–50 age range, followed by 14 (29.8%) patients in the 31–40 range, 8 (17.0%) patients in the 51–60 range, 4 (8.5%) patients greater than the age of 60, and 3 (6.4%) patients were seen to be 30 years or below. Mean of the age of the total studied patients was 44.68 ± 10.06 years. (Table 1)

Table 1: Association between age group & cytological diagnosis

Age group	ASC-NOS (n=25)	AGC-NOS (n=13)	LSIL (n=3)	HSIL (n=5)	ASC-H (n=1)
<30	3 (12%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
31-40	8 (32%)	4 (30.8%)	0 (0%)	2 (40%)	0 (0%)
41-50	8 32%)	7 (53.8%)	1 (33.3%)	1 (10%)	1 (100%)
51-60	5 (20%)	2 (15.4%)	1 (33.3%)	0 (0%)	0 (0%)
>60	1 (4%)	0 (0%)	1 (33.3%)	2 (40%)	0 (0%)



Graph 1: Distribution of patients according to p16 immunostaining



Graph 2: Grading of p16 immunostaining

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Table 2: Association between p16 immunostaining grading & cytological diagnosis

P16 Immunostaining	ASC-NOS (n=25)	AGC-NOS (n=13)	LSIL (n=3)	HSIL (n=5)	ASC-H (n=1)
0%	18 (72%)	6 (46.2%)	2 (66.7%)	0 (0%)	1 (100%)
1-10%	2 (8%)	1 (7.6%)	0 (0%)	0 (0%)	0 (0%)
11-50%	5 (20%)	6 ((46.2)	1 (33.4%)	3 (60%)	0 (0%)
51-100%	0 (0%)	0 (0%)	0 (0%)	2 (40%)	0 (0%)

Table 3: Association between p16 immunostaining grading & histological diagnosis

P16 Immunostaining	Chronic Cervicitis (n=13)	CIN -I (n=26)	CIN -II (n=5)	SCC (n=2)	Adenocarcinoma (n=1)
0%	12 (92.3%)	14 (53.8%)	1 (20%)	0 (0%)	0 (0%)
1-10%	0 (0%)	2 (7.7%)	0 (0%)	0 (0%)	1 (100%)
11-50%	1 (7.7%)	10 (38.5%)	4 (80%)	0 (0%)	0 (0%)
51-100%	0 (0%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)

Table 4: p16 immunostaining comparison b/w cervical cytology and cervical biopsy samples for intraepithelial lesions

Specimen	P16 positivity in cervical cytology	P16 positivity in cervical biopsy
Sensitivity (%)	55.88	95.0
Specificity (%)	92.31	42.89
PPV (%)	44.44	35.29
NPV (%)	72.34	92.31

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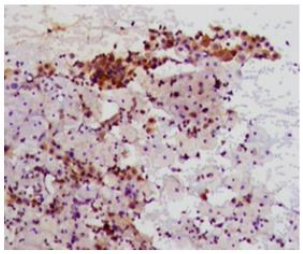
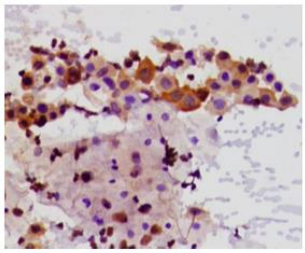
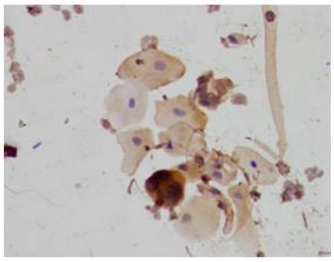
Figures : P16 Staining



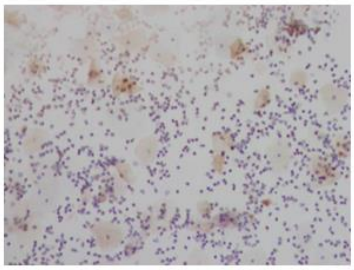
ASC-NOS [20 X]



ASC-NOS [40X]



LSIL [40X]



P16 -ve [10X]

Discussion

Cervical cytology has a low sensitivity for detecting precancerous lesions. When compared to Pap smear screening, the high-risk HPV DNA test demonstrated excellent sensitivity but poor specificity. It is critical to identify better triaging for hr HPV positive women with mild cytological abnormalities in order to limit the frequency of unnecessary colposcopies and biopsies. Under normal physiological conditions, p16 is a protein that causes cell cycle arrest. p16 functions as a CDK inhibitor, preventing Rb phosphorylation by cyclin D-CDK4/6, which causes cell cycle arrest. HPV oncoproteins E6 and E7 may explain the molecular mechanism of p16 in HPV- related neoplasms. The integration of viral DNA into host DNA results in the overexpression of viral oncoproteins. The E6 protein attaches to and degrades p53, while E7 promotes cell cycle advancement by displacing E2F from the RB protein.[7.8] This inactivation of the RB protein frees p16INK4a from its negative feedback regulation, resulting in a paradoxical rise in p16 levels.

High viral loads were discovered in a few benign instances and low-grade squamous lesions, which may have been caused by elevated episomal levels unrelated to HPV's carcinogenic potential. This aspect supported the use of the cancer cell biomarker p16 as a more accurate auxiliary tool for predicting which patients will experience disease

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progression than viral copy quantity. hr-HPV testing of ASC-NOS smears is a presently accessible method for determining the risk in a patient with an aberrant cytological smear. But since 83.4% of the LSIL smears were positive for hr-HPV, this was not a cost-effective strategy there. Histopathology only revealed CIN 2,3 in a small percentage of ASC-NOS/hr-HPV-positive and LSIL/hr-HPV-positive patients, which suggests that a significant portion of the population receives unwarranted colposcopy referrals. Patients who get an ASC-H or HSIL diagnosis on a cervical PAP smear screening result in an increase in the referral of colposcopies.[9]

In the present study when the cases were distributed on the basis of cytology findings it was observed that the majority of the studied cases were having ASC-NOS (53.2%) followed by AGC-NOS (27.7%), HSIL (10.6%), LSIL (6.4%) and ASC-H (2.1%).

In our study, the association of the cytological findings was done on the basis of age and it was found that the abnormal cytology findings were higher in the age group ranging from 41 to 50 years followed by 31 to 40 years but the difference was statistically insignificant (p=0.323). Our findings were consistent with the findings of **Kumar GV et al**[10] who reported that ASC-NOS was the most typical intraepithelial lesion seen in women of all ages. The median age was 42.55 years (standard deviation of 11.02 years) for overall abnormal pap smears. The oldest patient was 71 years old, while the youngest lady was 24 (p>0.05). **Prigenzi KCK et al** [11] shows accordance to the Bethesda classification, the distribution of cytology findings. No patient had invasive or glandular lesions. There was no association between age and Bethesda categorization of cytology data (p>0.05).

Conclusion

It has been well established that the success of cervical cancer screening programs might be improved by biomarkers that specifically reflect the pathogenesis of HPV infections and proliferation of the cancerous cells in cervical intraepithelial lesions through markers p16INK4a. These biomarkers usually aim to designate the expression of E6 and E7 oncogenes in basal keratinocytes affected by HPV. Overexpression of p16, which occurs as a consequence of inactivation of tumour suppressor proteins due to HPV derived E7 protein, acts as a screening marker for dysplasia due to HPV infection.

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