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Research Article

Expression of P16^{ink4a} In Cervical Intraepithelial Neoplasia And Invasive Squamous Cell Carcinoma Of Uterine Cervix

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ABSTRACT

Carcinoma's of cervix remains a major killer in women in the developing world including India. According to recent data provided by the atlas of cancer in India project by the ICMR, cervical cancer is the second most common cancer in women after breast cancer .In our country , cervical cancer shows an incidence of 25 per 1,00,000 women. The association of cervical cancer with high risk HPV infection has long been established. The protein p16 serves as a surrogate marker for the oncogenic activities of HPV in replication – competent cells of cervical epithelia and its over expression is well established in CIN and invasive squamous cell carcinoma by many studies.

SUMMARY: P16 can be used as a marker for determining HPV infection in Cervical intraepithelial neoplasms (CIN) and Squamous cell carcinoma (SCC) of cervix where there is non-availabitlity of HPV-PCR method in a tertiary care Hospital.

Key Words: *Cervical Cancer, Human Papilloma Virus (HPV), P16, Cervical intraepithelial neoplasms (CIN), Squamous cell carcinoma (SCC)*

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INTRODUCTION

Carcinoma's of cervix remains a major killer in women in the developing world including India. According to recent data provided by the atlas of cancer in India project by the ICMR, cervical cancer is the second most common cancer in women after breast cancer. In our country, cervical cancer shows an incidence of 25 per 1,00,000 women. The association of cervical cancer with high risk HPV infection has long been established. However, in a substantial number of females, HPV produces transient infection which gets cleared off by host immune responses. It has recently been shown that viral persistence is necessary to bring about a morphological phenotypic change in the squamous cell and subsequently development of high grade cervical intraepithelial neoplasia. Genomic integration of these viral genes can disrupt several cellular proteins resulting in up regulation of a tumour suppressor gene p16which is a cyclin dependent kinase(CDK4) inhibitor. The disruption of Rb by HPV E7 results in accumulation of the E2F and increased levels of p16 through negative feedback regulation. Thus, over expression of p16 indicates already advanced interference of the viral oncoproteins with cellular proteins involved in cell cycle regulation. The protein p16 serves as a surrogate marker for the oncogenic activities of HPV in replication – competent cells of cervical epithelia and its over expression is well established in CIN and invasive squamous cell carcinoma by many studies.

HPV infection can be detected by HPV-PCR method as well as by Immunohistochemistry (IHC) method by P16^{INK4a} marker. But the detection of HPV by PCR method is not possible in the tertiary care set up henceforth the use of P16 IHC can be done in those scenarios.

The aim of the present study is to determine the p16^{INK4a} expression in cervical intraepithelial neoplasia (CIN) and invasive squamous cell carcinoma(SCC) of cervix.

MATERIAL AND METHODS:

The present study was across sectional study done in the department of Pathology, Chalmeda Anand Rao Institute of Medical sciences, Karimnagar over a period of two years.

Already diagnosed preinvasive and invasive squamous cell carcinomas of cervix a total 84 cases were included in the present study.

The samples were fixed in 10% formalin and embedded in paraffin.

- Routine haematoxylin and eosin staining was done in the paraffin embedded blocks.
- WHO classification for Cervical Intraepithelial Neoplasia-CIN1,CIN2,CIN3

BRODER'S MICROSCOPIC GRADING:

- Well differentiated carcinoma(grade 1)
- Moderately differentiated carcinoma(grade2)
- Poorly differentiated carcinoma(grade3)

IHC STAINING

- Expression of p16 protein in 5µM FFPE sections on poly-L-lysine coated slides were evaluated using G175-405 clone(Biogenix, USA).
- After epitope retrieval, sections were incubated with prediluted primary mouse antihuman p16INK4a antibody for 30 min followed by chromogenic substrate diaminobenzidine and counterstaining with Harris hematoxylin.
- P16 expression in all immunostained slides were evaluated and scored independently by two of the pathologists. Difference in interpretation were reviewed jointly to obtain a consensus.

EVALUATION FOR P16^{INK4a} IMMUNOSTAINING

- P16 immunoreactivity was evaluated taking into account of, the percentage positivity of tumour cells and staining intensity.
- Positivity was seen as a brown reaction product staining the nucleus(N) or cytoplasm(C) or both.

IHC SCORING[1,2]

Samples were assigned an immune histological score (0 – 8) according to the intensity of staining and the proportion of stained cells in the cervical epithelium. The total score was the sum of score for stain intensity (0 – 3 points: 0 – no staining; 1 – weak staining; 2 points – moderate staining; and 3 – strong staining) and the score for proportion of epithelial cells stained negative (grade 0) when no cells stained, positive cells > 0-10 % (grade 1), positive cells >10-50% (grade 2), positive cells > 50-80% (grade 3) and positive cells > 80% (grade 4).

OBSERVATIONS AND RESULTS:

The present study included all the cervix biopsy specimens that are reported in the Department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar over a period of two years. A total of 84 cases were obtained.

Table 1: Age Distribution

Age group (in years)	No. of cases	Percentage (%)
< 40	08	9.5
41 – 50	48	57.2
51 – 60	12	14.3
61 – 70	08	9.5
<70	08	9.5
Total	84	100

In our study we noticed the maximum number of cases were in the age group of 41-50 years constituting 57.2% (48/84) and least number of cases were below 40 years age and more than 70 years of age each constituting 9.5% (8/84).

Table 2: Histomorphology of cases

Histomorphology	No. of cases	Percentage (%)
CIN 1	08	9.5
CIN 2	16	19.0
CIN 3	24	28.6
SCC	36	42.9
Total	84	100

In our study, the maximum histomorphology of cases were squamous cell carcinoma constituting 42.9% (36/84) and least cases were CIN 1 constituting 9.5% (8/84).

Table 3: Expression of IHC P16

P16 IHC Staining	CIN 1	CIN 2	CIN 3	SCC
Total positive cases (66)	4	10	16	36
Only Cytoplasmic positivity (14)	2	2	6	4
Only Nuclear positivity (22)	0	2	8	12
Both Cytoplasmic and nuclear positivity (30)	2	6	2	20
Negative stain (18)	4	6	8	0
Total Cases	8	16	24	36

Nuclear or cytoplasmic or both immune reactivity was taken as positive for p16 immunostaining. Variable degree of p16 staining was seen in the cervical stromal fibroblasts which served as an internal positive control. There was a progressive increase in the percentage positivity as well as the staining intensity through increasing grades of cervical dysplasia and invasive squamous cell carcinoma.

In our study we noticed that all the squamous cell carcinomas showed P16 positivity constituting 100% (66/66) and out of 24 cases of CIN 3, 16 were positive for P16 constituting 66.7%.

The study also showed 10 out of 16, positive cases for P16 in CIN 2 cases constituting 62.5% and 4 out of 8 cases showed P16 positivity in CIN 1 constituting 50%. And we observed that the P16 expression increased with the increased grading of the cervical intraepithelial neoplasms and carcinoma.

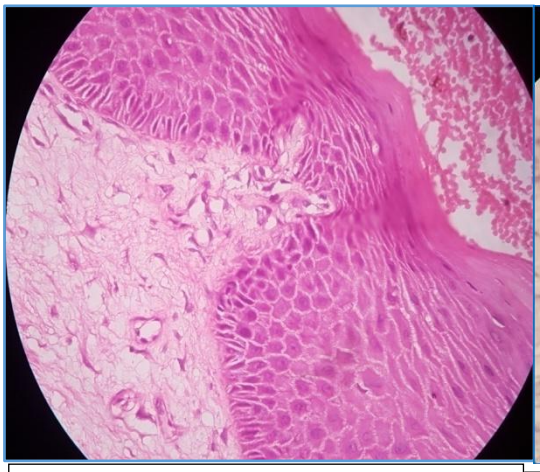

Fig 1: H & E 40X CIN 1- showing mild dysplasia

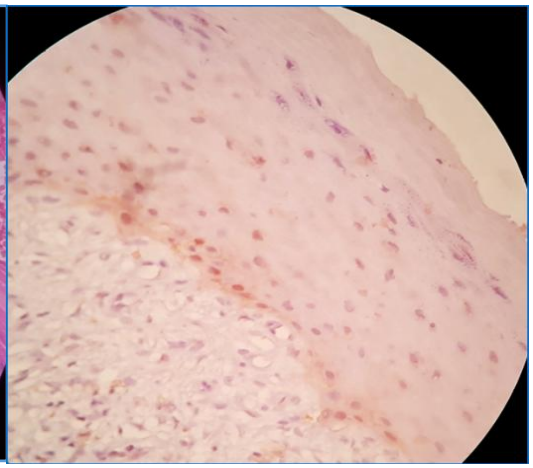

Fig 2: CIN 1-P16 immunoexpression showing focal weak cytoplasmic staining

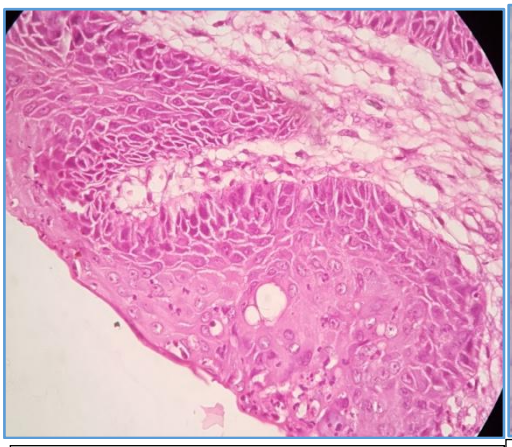

Fig 3: H & E 40X CIN 2- showing moderate dysplasia

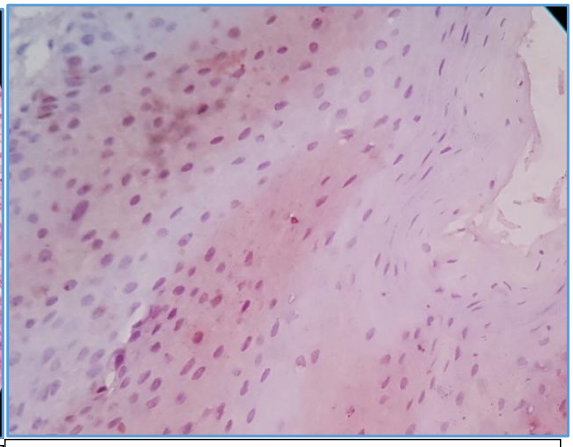

Fig 4: CIN 2- P16 immunoexpression showing moderate nuclear cytoplasmic staining.

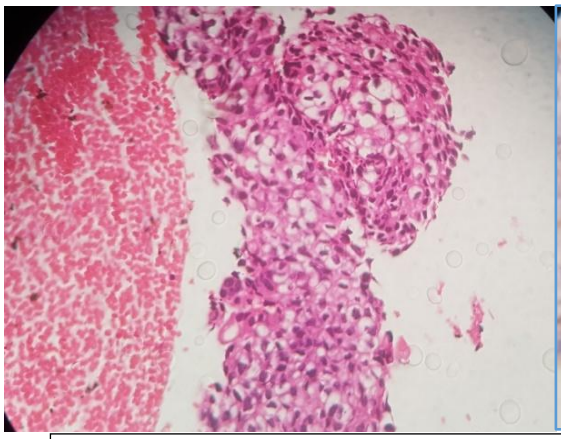

Fig 5: H & E 40X CIN 3- showing severe dysplasia

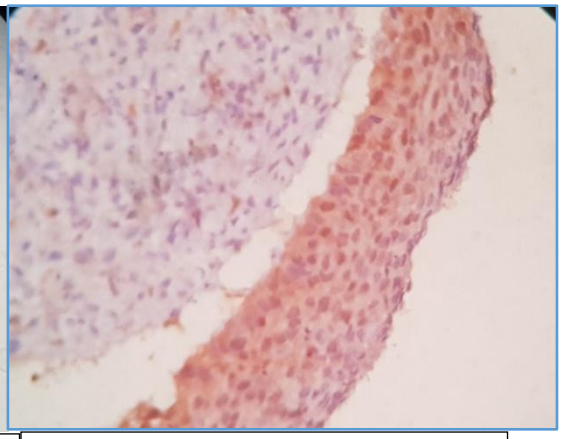

Fig 6: CIN 3- P16 immunoexpression showing strong nuclear cytoplasmic positivity

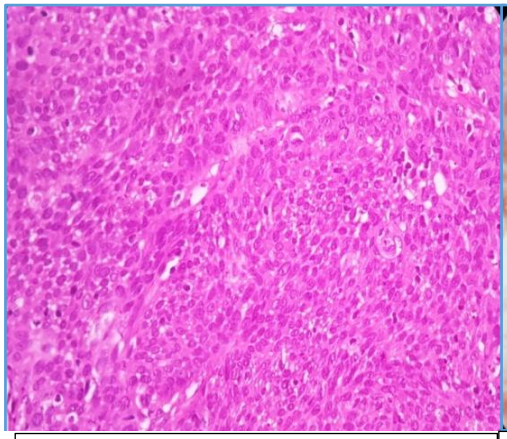

Fig 7: H & E 40 X Squamous Cell Carcinoma Section showing moderate pleomorphic squamous cells

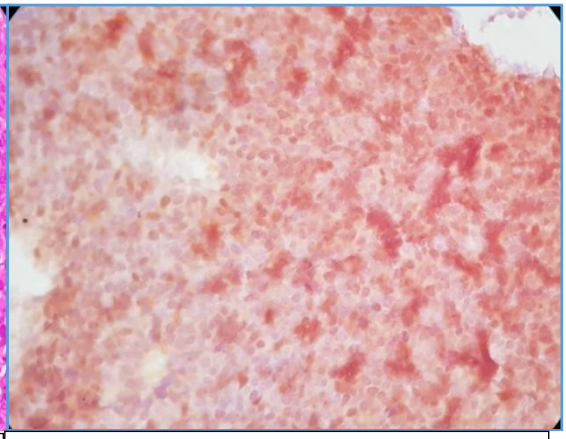

Fig 8: Squamous Cell Carcinoma-P16 immunoexpression showing strong nuclear cytoplasmic positivity

DISCUSSION

Cervical carcinoma has a well recognized precancerous state, morphologically as cervical intraepithelial neoplasia (CIN),which is graded from CIN 1 to 3.Thus, the alterations of cellular proteins at different levels can be analyzed at every step of progression to carcinoma. It is now recognized that the higher grade CIN(2 and 3) is related to the persistence of HPV infection with its integration into the cellular genomic DNA. Although there are several previous

reports on the role of p16 in cervical carcinogenesis there is paucity of them in Indian literature, inspite of the fact that cervical carcinoma is one of the most common cancer among females in India.

In the present study most common affected age group was 41-50yrs(57%) followed by 51-60 yrs(14.2%).Out of total 84 cases CIN 1 were 8 cases, CIN 2 were 16 cases, CIN3 were 24 cases and SCC were 36cases.P 16 immunostaining was positive for 66 cases out of 84 in which CIN 1 were 4 cases(50%), CIN 2 were 10 cases(62.5%), CIN3 were 16 cases(66.7%) and SCC were 36 cases(100%).

These results were similar to the study carried out by Gupta et al[23] which reported that 50% , 60% and 70% were CIN1, CIN2 and CIN3 cases respectively positive for p16 expression. Similarly Agoff et al[4] reported that 57% ,75% and 91% were CIN1, CIN2 and CIN3 cases respectively positive for p16 expression .

The p16 expression of SCC(100%) in the present study was correlating with Murphy et al[5], which reported 100% p16 expression in SCC. The immuneoreactivity for p16 in squamous cell carcinoma is unequivocally shown by all studies till date and has been confirmed by this study. Over expression of p16 appears to correlate with the degree of cervical neoplasia, which may improve the histopathological diagnosis and hence the management of cervical lesions.

Table 4: Comparison table on Studies on $p16^{INK4a}$ expression

Study	**CIN 1% (No.positive/ No.analyzed)**	**CIN 2% (No.positive/ No.analyzed)**	**CIN 3% (No.positive/ No.analyzed)**	**SCC% (No.positive/ No.analyzed)**
Agoff et al[4],2003	57% (43/76)	75% (60/80)	91% (103/113)	92%(42/46)
Murphy et al[5],2004	100% (38/38)	100% (33/33)	98% (45/46)	100% (10/10)
Gupta et al[3],2010	50% (10/20)	60% (12/20)	70% (14/20)	95% (19/20)
Present study	50% (4/8)	62% (10/16)	66% (16/24)	100%(36/36)

CONCLUSION

Maximum number of cases were in 41-50 years of age. All the squamous cell carcinomas were strongly positive for P16. As the grading of cervical intraepithelial neoplasms increases the positivity of P16 was also increased. Strong nucleocytoplasmic p16 expression were seen in a small proportion of CIN1 cases. Some reports have suggested that p16expression in CIN1 might have a correlation with viral persistence, which proves that the low grade lesions(CIN1) have a tendency to progress to higher ones(CIN3 & SCC) and thus allow appropriate and aggressive follow up and management in such patients. However with a limited follow up period, it was not possible to derive any conclusion from p16 positivity in CIN1 lesions in the present study. Thus p16 expression acts as a surrogate marker for cervical intraepithelial neoplasia and should be incorporated into routine surgical practice.

CONFLICTS OF INTEREST: NONE

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