

p16, p63 AND ki67 Expression in Squamous Metaplasia, Cervical Glandular Hyperplasia, Cervical Intraepithelial Neoplasia and Cervical Cancer

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ABSTRACT:

Introduction: Persistent infection with human papilloma virus(HPV) has been the main cause of squamous metaplasia which in turn may lead to in situ changes and then cervical cancer. p16, p63 and ki67 immunohistochemistry (IHC) can be used as an ancillary method for the diagnosis of these lesions. **Aim:** To evaluate the expression of p16, p63 and ki67 in squamous metaplasia, cervical glandular hyperplasia, Cervical intraepithelial neoplasia (CIN), cervical cancer and correlate histological features with IHC patterns. **Settings and Design:** 100 histologically proven cases of above mentioned lesions were taken in a observational study conducted from august 2018 to 2020. **Methods and Material:** 100 formalin fixed paraffin embedded blocks from cervical tissue were subjected to IHC using p16, p63 and ki67. **Statistical analysis used:** Using the SPSS 10.0 statistical software program. P< 0.05 was considered statistically significant. **Results:** Positive rates of p16, p63 and ki67 expression in CIN III and cervical cancer were higher than those in CIN I and II but there was no significant difference between various grades of cervical squamous cell carcinoma. **Conclusions:** The p16, p63 and ki67 expression positivity increased with the increasing severity of cervical lesions. Thus, high expression of ki67, p63 indicates neoplastic progression and p16 identifies HPV 16 infection in the uterine cervix.

Key-words: p16, p63, ki67, HPV, IHC, CIN

INTRODUCTION

Cervical cancer is the fourth most common cause of cancer in women. The cervical squamous intraepithelial lesion is an important transitional stage of normal cervical tissue transforming into squamous cell carcinoma of the cervix over a period of time. Persistent infection with Human papilloma virus(HPV) has been the main cause of these lesions and HPV 16 is the most oncogenic, out of all.[1- 3] Therefore, expression of IHC markers p16, ki67 and p63 is a quick and effective method to detect precancerous and cancerous cervical lesions. P16 is a negative cell cycle regulator, ki67 marks cell proliferation and p63 is a marker of squamous differentiation.[4,5]

MATERIALS AND METHODS

The current observational study was conducted on histopathologically proven cases of squamous metaplasia, cervical glandular hyperplasia, cervical carcinoma and CIN. The study was approved by the ethical committee of the institute. A total 133 specimens were examined, out of which 33 could not be considered in the calculation due to loss of tissue in the processing or IHC failure. At the end, only 100 cases were finalized.

Inclusion criteria - Patients irrespective of age group, histologically diagnosed as squamous metaplasia, cervical glandular hyperplasia, CIN and cervical carcinoma were included in the study.

Exclusion criteria - Patients who have received radiotherapy/chemotherapy or coexistence of any other major illness were excluded from study.

Tissue blocks were prepared from samples of cervical tissue, were processed for hisopathological examination and IHC study. For tissue block preparation, firstly grossing of the formalin fixed specimens was done following the standard procedure. The grossed specimens were then exposed to tissue processing and paraffin blocks were prepared and samples were subjected to four micrometer thick sections, H & E stained slides were prepared and subjected to IHC staining.

The kits were obtained from Dako laboratories for p16, p63 and ki67. The staining was performed according to the manufacturer's protocol using peroxidise – antiperoxidase method.

Interpretation

P16 - Immunopositivity was considered when there was diffuse, intense, nuclear or cytoplasmic staining or both. Focal, moderate nuclear staining was also considered positive. Then, grading was performed by the number of positive cells as Grade 0, 1, 2, 3 and 4, based on the number of positive cells, <1%, 1-10%, 11-50%, 51-80% and >80%.

Ki67 - Immunopositivity was considered when there was mild, moderate and strong nuclear staining. Then, grading was performed by the number of positive cells as Grade 0, 1, 2, 3 and 4, based on the number of positive cells, <1%, 1-24%, 25-49%, 50-74% and >75%.

Statistical analysis was done using the SPSS 10.0 statistical software program. $P < 0.05$ was considered statistically significant.

P63 - Immunopositivity was considered when there was mild, moderate and strong nuclear staining. Then, grading was performed by the number of positive cells as Grade 0, 1, 2 and 3, based on the number of positive cells, <5%, 5-25%, 26-75% and >75%.

STATISTICAL ANALYSIS AND RESULTS

A total of 100 cases were selected. Out of 100 cases CIN and CGH was found to be present in 32 (32%) and 6 (6%) patients respectively. The most common entity in the present study was squamous metaplasia, accounting to 54%. 8(8%) cases were those of cervical carcinoma and all of them were squamous cell carcinoma (SCC). 45 percent of the patients belonged to the age group of 41 to 50 years while 32 percent and 16 percent of the patients belonged to the age group of 51 to 60 years and 30 to 40 years respectively. Mean age of the patients was 48.08 years.

Comparison of P16 expression in various lesions

While analysing expression of P16 in SCC patients, it was seen that mild and moderate expression was present in 12.5 percent and 50 percent of the patients respectively while strong expression was seen in 37.5 percent of the patients.

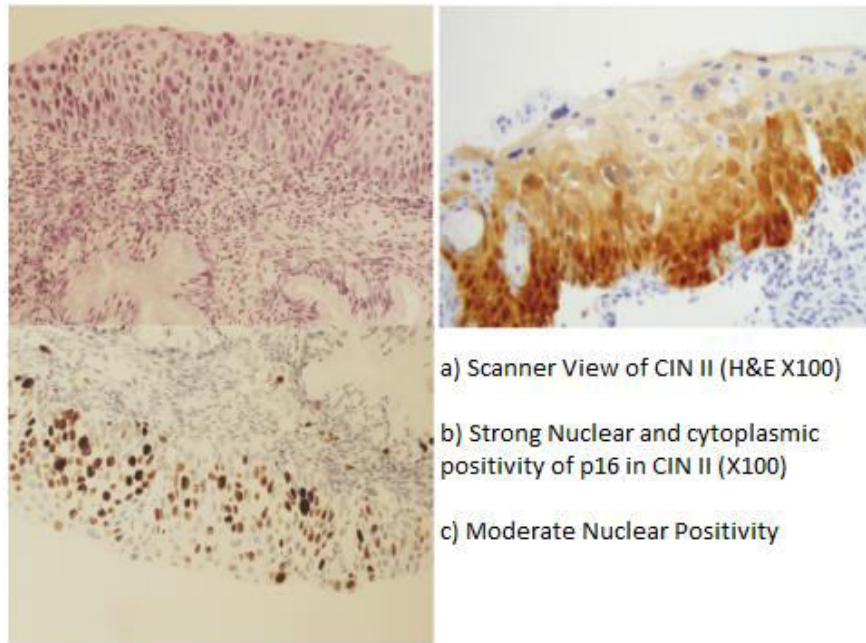
Expression	Carcinoma		CIS		CGH		Squamous metaplasia	
	Number of patient	Percentage of patients	Number of patients	Percentage of patients	Number of patients	Percentage of patients	Number of patients	Percentage of patients
Absent	0	0	15	46.88	4	66.67	39	72.22
Mild	1	12.5	9	28.12	2	33.33	13	24.07
Moderate	4	50	6	18.75	0	0	2	3.71
Strong	3	37.5	2	6.25	0	0	0	0
Total	8	100	32	100	6	100	54	100
Chi-square value	42.75							
p- value	0.0002 (Significant)							

[Table/Fig- 1] P16 expression in various lesions is summarised in the table below:

P16 expression - Out of 54 cases of squamous metaplasia, expression of p16 was mild and moderate in 24.07 percent (13 patients) while it was absent in 72.22 percent (39 patients) respectively.

Out of 12 cases of CIN I, expression of p16 was absent in 9 patients (75 percent) while expression was mild in 3 patients (25 percent). Out of 9 cases of CIN II, expression of p16 was absent in 4 patients (44.44 percent) while expression was mild and moderate in 4 patients (44.44 percent) and 1 patient (11.11 percent) respectively. Out of 11 cases of CIN III, expression of p16 was absent in 2 patients (18.18 percent) while expression were mild, moderate and strong expression was seen in 2 patients (18.18 percent), 5 patients (45.45 percent) and 2 patients (18.18 percent) respectively. Significant results were obtained while comparing the expression of p16 among different grades of CIN.

Out of 6 patients of CGH, mild expression of P16 was seen in 2 patients (33.33 percent) while expression was absent in 4 patients (66.67 percent).[Table/Fig- 1]



a) Scanner View of CIN II (H&E X100)

b) Strong Nuclear and cytoplasmic positivity of p16 in CIN II (X100)

c) Moderate Nuclear Positivity

[Table/Fig- 2]: a) S view of CIN II (H&E X100) b) Strong nuclear and cytoplasmic positivity of p16 in CIN II (X100) c) Moderate nuclear positivity

Comparison of expression of p63 among different pathologies

While analyzing expression of P63 in SCC patients, it was seen that mild and moderate expression was present in 12.5 percent and 37.5 percent of the patients respectively while strong expression was seen in 50 percent of the patients.

Expression	Carcinoma		CIS		CGH		Squamous metaplasia	
	Number of patient	Percentage of patients	Number of patients	Percentage of patients	Number of patients	Percentage of patients	Number of patients	Percentage of patients
Absent	0	0	11	34.38	4	66.67	28	51.85
Mild	1	12.5	8	25	2	33.33	22	40.74
Moderate	3	37.5	9	28.12	0	0	4	7.41
Strong	4	50	4	12.5	0	0	0	0
Total	8	100	32	100	6	100	54	100
Chi-square value	40.333							
p- value	0.00006 (Significant)							

[Table/Fig- 3]: Comparison of expression of p63 among different pathologies

P63 expression - Out of 54 patients of squamous metaplasia, expression of p63 was absent in 51.85 percent of the patients while expression was mild and moderate in 40.74 percent and 7.41 percent of the patients respectively. None of the patients showed strong expression of P63. Out of 12 cases of CIN I, P63 expression was absent in 8 patients (66.67 percent) while expression was mild and moderate in 3 patients (25 percent) and 1 patient (8.33 percent) respectively. Out of 9 cases of CIN II, P63 expression was absent in 2 patients (22.22 percent) while expression was mild and moderate in 4 patients (44.44 percent) and 3 patients (33.33 percent) respectively. Out of 11 cases of CIN III, P63 expression was absent in 1 patient (9.09 percent) while expression were mild, moderate and strong expression was seen in 1 patient (9.09 percent), 5 patients (45.45percent) and 4 patients (36.36 percent) respectively. Significant results were obtained while comparing the expression of P63 among different grades of CIN. Out of 6 patients of CGH, mild expression was present in 2 patients (33.33 percent) while it was absent in the remaining 4 patients (66.67 percent) [Table/Fig- 3].

Comparison of expression of ki67 among different pathologies

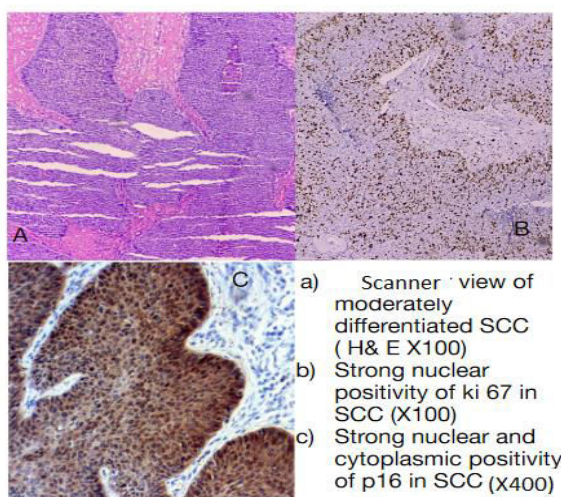
While analysing expression of Ki67 in SCC patients, it was seen that mild and moderate expression was present in 12.5 percent and 50 percent of the patients respectively while strong expression was seen in 37.5 percent of the patients

Expression	Carcinoma		CIS		CGH		Squamous metaplasia	
	Number of patient	Percentage of patients	Number of patients	Percentage of patients	Number of patients	Percentage of patients	Number of patients	Percentage of patients
Absent	0	0	14	43.75	4	66.67	42	51.85
Mild	1	12.5	7	21.88	2	33.33	11	40.74
Moderate	4	50	9	28.12	0	0	1	7.41
Strong	3	37.5	2	6.25	0	0	0	0
Total	8	100	32	100	6	100	54	100
Chi-square value	48.32							
p- value	0.00001 (Significant)							

[Table/Fig- 4] Comparison of expression of ki67 among different pathologies

Ki67 expression - Out of 54 patients of squamous metaplasia, expression of Ki67 was absent in 51.85 percent of the patients while expression was mild and moderate in 40.74 percent and 7.41 percent of the patients respectively. None of the patients showed strong expression of Ki67. Out of 12 cases of CIN I, Ki67 expression was absent in 9 patients (75 percent) while expression was mild and moderate in 2 patients (16.67 percent) and 1 patient (8.33 percent) respectively. Out of 9 cases of CIN II, Ki67 expression was absent in 3 patients (33.33 percent) while expression was mild and moderate in 4 patients (44.44 percent) and 2 patients (22.22 percent) respectively. Out of 11 cases of CIN III, Ki67 expression was absent in 2 patients (18.18 percent) while expression were mild, moderate and strong expression was seen in 1 patient (9.09 percent), 6 patients (54.55 percent) and 2 patients (18.18 percent) respectively. Significant results were obtained while comparing the expression of Ki67 among different grades of CIN. Out of 6 patients of CGH, mild expression was present in 2 patients (33.33 percent) while it was absent in the remaining 4 patients (66.67 percent). [Table/Fig- 4]

[Table/Fig- 5] a) Low power view of moderately differentiated SCC (H& E X100) b) Strong nuclear positivity of ki 67 in SCC (X100) c) Strong nuclear and cytoplasmic positivity of p16 in SCC (X100)



DISCUSSION

Cervical cancer is the one of the most common cause of cancer in women in the age group 15-45 years, main etiology being young age of onset of sexual intercourse and HPV infection. Early detection of viral infection helps in prophylaxis and prompt treatment.[4]

P16 expression in this study was found to be 27.8% (15 patients out of 54) within squamous metaplasia with a gradual increase from CIN I, II and III (25%, 55.56% and 81.82% respectively) and maximum in squamous cell carcinoma (100%) indicating a possibility of HPV infection. Also there was a positive correlation between expression intensity of p16 and the degree of severity of cervical lesions. Similar results were shown by Wang et al (2004), who in their study showed p16 overexpression in CIN (75%) and SCC (75%) than in normal or inflammatory conditions of the cervix (12.5%). [6] Other studies conducted by Murphy et al, (2005), Benevolo et al, (2006), Queiroz et al, (2006), Aslani et al, (2013), also reported P16 positivity in 80% to 100% cases of invasive carcinoma, 45% to 100% in CIN II/3, and 0% to 15% in non-dysplastic lesions. The variation of expression rates may partly depend on the sample processing, IHC technique and the criteria defining positive expression. [7,8, 9, 10]

While comparing the overall expression of Ki67 among patients with SCC, CIN, CGH and squamous metaplasia, significant results were obtained with a p value of 0.00001 and an overall positivity of 48.15%, 33.33%, 56.25% and 100% in squamous metaplasia, CGH, CIN and SCC respectively. An increasing staining intensity of Ki67 with increasing severity of cervical lesions was observed. The results were in concordance with the results obtained by Hebbar et al (2017), Nam et al (2008), Kim et al (2011) who reported a positive correlation between the degree of dysplasia and Ki67 expression, which is why Ki67 has been increasingly studied for the evaluation of low-grade lesions of the cervix. It has high specificity and reproducibility. Diffuse positive staining was found to be consistent with CIN, but Ki-67 staining could not differentiate between dysplasia and immature squamous metaplasia. Immunopositivity for Ki67 increased linearly as the CIN grade increased. [11,12,13]

While comparing the overall expression of P63 among the patients of SCC, CIN, CGH and squamous metaplasia, significant results were obtained with a p value of 0.00006 and an overall positivity of 48.15%, 33.33%, 65.62% and 100% in squamous metaplasia, CGH, CIN and SCC respectively. An increasing staining intensity of Ki67 with increasing severity of cervical lesions was observed. The results were in concordance with the results obtained by Selvi et al (2014) and Vosmik et al (2013). [14,15]

CONCLUSION

Ki-67, P63 and P16 expression in reactive, pre malignant and malignant lesions of the cervix can be used in conjunction with the histo morphological features to study their progression risk, proliferative potential and HPV 16 infection. Positive expression indicates an increased risk of progression to high-grade lesions. It is likely that in the coming years IHC, along with molecular investigations, will play an important role in the field of diagnostic uterine cervical pathology.

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