

Role of KI-67 and P16 as Markers of Prognostic Indices in Premalignant and Malignant Lesions of Cervix

Dina Mary M¹, Shantaraman K²

ABSTRACT

Introduction: Since cervical cancer is highly attributed to the effects of HPV, an infectious agent, it can either be prevented or treated at a pre-invasive stage there by reducing morbidity and mortality; hence the need for additional biomarkers and parameters of cell proliferation and cell death as important diagnostic and prognostic tools. This study was done to evaluate the proliferative indices in cervical pre cancerous and cancerous lesions using Ki-67 and p16 as bio markers.

Material and Methods: A total of 50 cases including CIN-1, CIN-2, CIN-3 and malignancy of the cervix were evaluated. Strong nuclear positivity for Ki-67 was considered positive. The scoring was graded 1,2,3 for 10-30%, 30-50% and >50%. Staining intensity for p16 (nuclear or/and cytoplasmic staining) was done and the results were categorized as Grade 1,2,3 for 1-10%, 10-50% and >50% positive cells.

Results: Ki-67 and p16 expression were seen in CIN-I,II,III and squamous cell carcinomas of the cervix. Ki-67 expression was in increasing grades from CIN to carcinoma. p16 had a better expression in high grade intra epithelial lesions (60-80%) and malignancies (100%) of the cervix compared to low grade dysplasias (21%).

Conclusion: Thus Ki-67 and p16 expression in pre malignant and malignant lesions of the cervix can be used in conjunction with the histo morphological features to study their proliferative potential.

Keywords: Ki-67, p16, CIN, Carcinoma Cervix.

INTRODUCTION

Carcinoma cervix is a major burden on the population and the government especially in developing countries like India, although its incidence has been controlled in developed nations. This decrease may be attributed to the efficacy of cervical cytology screening programmes which are done in a categorical manner.

Carcinoma cervix stands next only to breast cancers that affect the female population being responsible for about 5% of deaths due to malignancies worldwide.¹ The estimated incidence of cervical cancer is 470,000 and remains as a leading cause of morbidity and mortality worldwide.² Approximately 230,000 women die each year from cervical cancer; over 190,000 of these women are from developing countries. The contribution is roughly around 25% by the Indian women to the total occurrence in the world.³

Biopsy cervix is definitive in establishing a diagnosis in cervical lesions. However, it has its own limitations such as the study of morphological parameters alone with no information as to the fate of the lesion (progression/regression). Further interobserver variability needs special mention.^{4,5} Hence to overcome these draw backs and

to increase the accuracy in deciding the magnitude of progression, bio markers come into role. The carcinoma cervix has histopathologically, well characterized precursor lesions (CIN), which slowly progresses to the well differentiated tumour. The vast majority of HPV infections (up to 90%) regress spontaneously, without treatment, after a few months. If the viral infection persists, however, the risk of developing a precancerous lesion increases as well as the risk of developing an invasive carcinoma.⁶ This transformation of cervical epithelial cells from CIN to carcinoma takes 10 – 15 yrs.⁷ During this transformation period, many important markers of tumor progression, are expressed.

Carcinoma cervix, thus because of its long period of transformation, provides a opportunity to study about the expression of biomarkers of tumor progression. Because of the above advantages, present study is designed to evaluate the mitotic count and the expression of immunomarkers, Ki-67 and p16 in different grades of cervical precancerous and cancerous lesions to understand their role in cervical carcinogenesis.

Study was done to evaluate the proliferative indices in cervical pre-cancerous and cancerous lesions – Ki-67 and p16 in IHC stained sections and to correlate the proliferative indices with varying grades of pre malignant lesions and various histological sub types of cervical carcinoma.

MATERIAL AND METHODS

The study was undertaken in the Department of Pathology at Tirunelveli Medical College Hospital. A total of 50 cases including cervical intra epithelial neoplasia and carcinoma were included in the study. Ethical clearance and informed consent was taken before the start of study.

Inclusion criteria: Cervical biopsies diagnosed cervical intra epithelial neoplasia (I,II,III) and carcinoma cervix, Hysterectomy specimens diagnosed cervical intra epithelial neoplasia (I,II,III) and carcinoma cervix in the age group of 20 to 75 yrs.

Exclusion criteria: Cervical biopsies reported as inflammatory conditions, Cervical biopsies diagnosed mesenchymal lesion, Hysterectomy specimens for causes

¹Assistant Professor, ²Professor, Department of Pathology, Tirunelveli Medical College Hospital, Tamilnadu, India

Corresponding author: Dr. Shantaraman K, Professor, Department of Pathology, Tirunelveli Medical College, Tamilnadu, India

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other than carcinoma cervix. Immunohistochemistry stains done for Ki-67, p16.

STATISTICAL ANALYSIS

Descriptive statistics like mean and percentages were used to interpret the data.

RESULTS

The table shows an association between the expression of Ki-67 and p16 in cervical lesions (both benign and malignant) since 28 cases out of the 50 cases showed positivity for both the markers. Only 8 turned out to be negative for both. P value is <.005 and hence statistically significant (table 1,2). P16 showed only 21% positivity in CIN-I lesions whereas in high grade dysplasias the positivity was above 60%. This may point towards the association of HPV with high grade dysplastic lesions and malignancies of the cervix. However large cell non keratinizing carcinomas showed only 70% positivity which emphasizes the need to study if HPV is associated in these malignancies. Ki-67 showed around 60% positivity even in CIN-I lesions though the grade was low. This rate keeps increasing then onwards (table-3).

DISCUSSION

Ki-67 as well known is a proliferative marker that is expressed in all stages of the cell cycle except G0 phase. It is highly accurate in demonstrating the cervical lesions with high potential of turning malignant. Thus this marker is widely used to prognosticate dysplastic lesions of the cervix. P16 is a tumor suppressor protein that is encoded by the CDKN2A gene. Its major role in cell cycle is down regulation of cell cycle by inhibiting the progression of cell cycle from G1 to S phase. It has its effect expressed through the HPV genome. It has wide application in malignancies like squamous cell carcinoma and melanoma. With the studies available,⁸⁻¹⁰ the expression of Ki-67 and p16 were studied in both dysplastic and malignant lesions of the cervix to understand the prognosis of the same which cannot be done through histopathological examination though wide arrays of potential biomarkers have been evaluated for the diagnostic usefulness of cervical cancer and its precursors. These studies were mainly done to minimize the surgical intervention and to bring down the costs of repeated screening programmes.

In the study by Srivastava S. et al. (2010),¹¹ the expression of MIB-I increased from normal cervical epithelia to varying severity of CINs to carcinoma. MIB-I positivity was seen in 14 of 15 cases of CIN I, 15 of 15 cases of CIN II, 3 of 3 cases of CIN III and 15 of 15 cases of carcinoma cervix. In this study also, 9 of 14 CIN-I, 5 of 8 CIN-II, 6 of 6 CIN-III cases, 20 of 21 carcinoma cases showed Ki-67 expression. The % expression also showed a constant increase from 64% in CIN-I to 100% in malignant cases. However, one case of CIN-I showed grade III positivity which may indicate the lesion would progress vigorously. In a study by Klaes et al. (2001),¹² positive expression of p16 was found in all CIN I lesions (n = 47), all CIN II lesions (n = 32), all CIN III lesions (n = 60) and 56 of 58 invasive Squamous cell carcinoma

Diagnosis	No. of cases
CIN-I	14
CIN-II	8
CIN-III	6
Carcinoma	22

Table-1: Distribution of cases

P16			P value
	Positive	Negative	
MiB	Positive	28	0.013
	Negative	3	

Table-2: Correlation between Ki-67 and p16 expression in study cases

Lesions	Total	MIB		P16	
		Positive	%	Positive	%
CIN-I	14	9	64.2	3	21.42
CIN-II	8	5	62	5	62
CIN-III	6	6	100	5	83
LCK	8	8	100	8	100
LCNK	13	12	92.3	9	69.2
Adeno	1	0	0	0	0

Table-3: Comparison of Ki-67 and p16 positivity in study cases

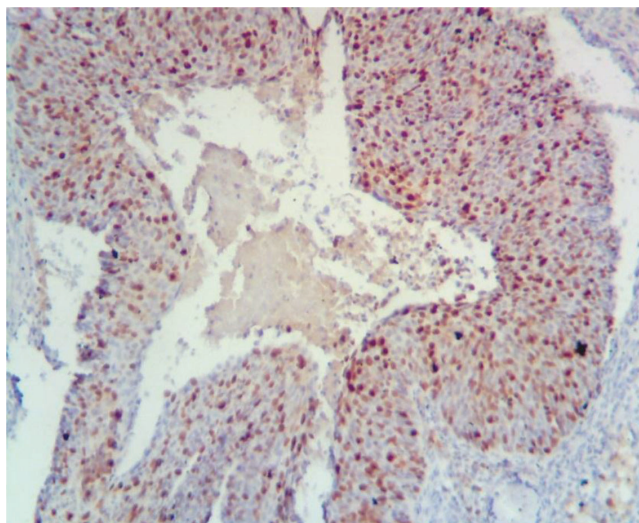


Figure-1: Photomicrograph showing 3+ nuclear positivity of Ki-67 in a case of large cell non keratinizing carcinoma

(SCC). However this expression was found to be minimal in inflammatory and low grade dysplasias.

In the present study, p16 expression was very much less (4/14, i.e.21%) in CIN-1 compared to the above study. The expression in CIN II and CIN-III was also less (62% and 83%). However the expression in malignant lesions was similar to the above study.

In the same study by Srivastava S. et al. (2010), correlation between Ki-67 and p16 expression with an increasing p16 expression with consistently increasing MIB-1 LI in the groups of increasing severity. In this study also, the correlation between the two markers was seen and it was also statistically significant.¹¹

In Gupta et al.¹³ study, which comprised of 20 cases of

dysplasia and 30 cases of carcinomas revealed that the mean value of LI (Labeling Index) was found to increase as the nature of the lesion changed from dysplasia (16.94) to carcinoma (50.754) with the difference being found to be extremely statistically significant (p value <0.0001).

CONCLUSION

To conclude, Ki-67 and p16 expression in pre malignant and malignant lesions of the Cervix can be used in conjunction with the histo morphological features to study their proliferative potential and thereby their progression which would have a major impact on the treatment.

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