STUDY OF IMMUNOHISTOCHEMICAL EXPRESSION OF Ki-67 IN SQUAMOUS CELL CARCINOMA OF CERVIX

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ABSTRACT

BACKGROUND

Cervical cancer is the second most common cancer cause of death among women in developing countries. Recently parameters of cell proliferation have emerged as important tools in the evaluation of dysplastic and neoplastic lesions of the cervix.

The aim of this study was to study the immunohistochemical expression of Ki-67 in squamous cell carcinoma of the cervix.

MATERIALS AND METHODS

63 cases of histologically proven squamous cell carcinoma (SCC) of the cervix were included in the study. Ki-67 immunostaining was done and labelling index was calculated. ANOVA test was applied to see correlation of Ki-67 expression with different grades of squamous cell carcinoma.

RESULTS

The Ki-67 labelling index was maximum in poorly differentiated SCC and minimum in well-differentiated SCC and p value amongst these groups was found to be statistically significant.

CONCLUSION

Ki-67 labelling index has a positive association with histological grading in squamous cell carcinoma of cervix.

KEYWORDS

Ki-67, Squamous Cell Carcinoma of Cervix.

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BACKGROUND

Cervical cancer is the most common cancer cause of death among women in developing countries.¹ It can involve the uterine corpus by direct extension or through parametrium by lymphatic invasion to the uterine wall.² Mortality due to cervical cancer is also an indicator of health inequities, as 86% of all deaths due to cervical cancer are in developing, low and middle income countries.³ Every year in India, 122,844 women are diagnosed with cervical cancer and 67,477 die from the disease. India has a population of 432.2 million women aged 15 years and older who are at risk of developing cancer. It is the second most common cancer in women aged 15–44 years.⁴ India also has the highest age standardised incidence of cervical cancer in South Asia at 22, compared to 19.2 in Bangladesh, 13 in Sri Lanka, and 2.8 in Iran.⁴

The Ki-67 protein is a cellular marker for proliferation which can be detected within the cell nucleus. In all active phases of the cell cycle Ki-67 is present i.e. G1, S, G2, and M, but absent in resting cells (G0).⁵ To determine the growth fraction of a given cell population Ki-67 is an excellent

marker.⁶ Ki-67 is of great importance in the tumour grading, proliferative degrees and prognosis.⁷ Ki-67 expression is normally confined to the parabasal and basal layers in the normal cervical epithelium.⁸ Some studies have showed that Ki-67 protein could be a biomarker in the evaluation of the proliferative activity and progressive potential of normal, dysplastic and neoplastic changes.⁹ An Asian study observed Ki-67 may serve as a helpful adjunct to routinely-stained histological sections in differentiating SCC.¹⁰

The present study aims at assessing the Ki-67 expression in Squamous Cell Carcinoma (SCC) of the cervix and correlating its expression with the histological grades of SCC.

MATERIALS AND METHODS

A total of 63 histologically proven cases of cervical squamous cell carcinoma received in the Department of Pathology, Sri Ram Murti Smarak Institute of Medical Sciences, Bareilly, from January 2015 to December 2015 were included in the study. The study was approved by the institute's ethical committee.

Immunostaining for Ki-67 (BioGenex) was carried out. All slides were deparaffinised in xylene, rehydrated in graded alcohols, washed in PBS buffer, boiled in EDTA solution (pH 6.0, 100°C, 2.5 minutes) for antigen retrieval and cooled down. Following endogenous peroxide and protein blocking step, the slides were incubated with primary antibodies. After brief washes, incubation in a cocktail of biotinylated rabbit anti-mouse IgG/IgM for 30 min. was performed.¹¹ The sections were then washed and incubated with streptavidin-biotinylated horseradish peroxidase complex for 30 min., reacted with 3-amino-9-ethylcarbazole and hydrogen

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peroxide. To visualise the end product haematoxylin was used as a counter stain.

Ki-67 labelling index was calculated by the number of positive cells per 200 cervical epithelial cells under X400 magnification. Positive nuclei were expressed as the percentage of total nuclei counted.¹¹

Modified Broder's grading system was used for grading squamous cell carcinomas. Carcinomas were divided into three grades: well-differentiated squamous cell carcinoma (WDSCC), moderately differentiated squamous cell carcinoma (MDSCC) and poorly differentiated squamous cell carcinoma (PDSCC). WDSCC showed abundant intercellular bridging, cytoplasmic keratinisation and keratin pearls, nuclear pleomorphism was minimal and mitotic figures were <2/hpf. In MDSCC individual cell keratinisation, moderate nuclear pleomorphism and mitotic figures up to 4/hpf were present. In PDSCC immature tumour cells with scant cytoplasm, marked nuclear pleomorphism, and mitotic figures, >4/hpf were present.¹²

All the data was entered in database file and analysis was done using SPSS 20 version. ANOVA test was applied to see correlation of Ki-67 expression with different grades of SCC. P value less than 0.05 was considered as significant.

RESULTS

The mean Ki-67 Index % observed was 54.0 ± 2.64 for those diagnosed as well-differentiated squamous cell carcinoma (SCC-WD), 63.0 ± 4.17 for patients diagnosed as moderately differentiated squamous cell carcinoma (SCC-MD), and 85.5 ± 9.3 for patients diagnosed for poorly differentiated squamous cell carcinoma (SCC-PD), and the difference in the Ki-67 Index among different forms of squamous cell carcinoma was highly statistically significant $P < 0.001$ (Table 1).

Increase in Ki-67 expression was seen with the increasing grade of SCC. All 5 patients of SCC-PD were highly proliferative with an expression of Ki-67 in 70% or more of the cells. Among the SCC- MD lesions, 34/49 (69.4%) expressed Ki-67 in 30 to <70% of the cells and 9 out of 49 (18.4%) cases showed Ki-67 expression in 70% and more than of the cells. In SCC-WD lesions, mostly 5 patients out of 9 (55.5%) had expression of Ki-67 in 30 to <70% of the cells. (Table 2).

Diagnosis (SCC) (N=63)	KI-67 Index % (Mean $\pm$ SD)	P value
SCC-WD (n=9)	54.0 ± 2.64	<0.001
SCC-MD (n=49)	63.0 ± 4.17	
SCC-PD (n=5)	85.5 ± 9.3	

Table 1. Expression of mean Ki-67 Index (%) in Squamous Cell Carcinoma

KI-67 Index (%)	Diagnosis		
	SCC-WD (n=9)	SCC-MD (n=49)	SCC-PD (n=5)
>0 to <10%	0 (0.0%)	0 (0.0%)	0 (0.0%)
10 to <30%	4 (44.4%)	6 (12.24%)	0 (0.0%)
30 to <70%	5 (55.5%)	34 (69.38%)	0 (0.0%)
70 to 100%	0 (0.0%)	9 (18.36%)	5 (100%)

Table 2. Ki-67 expression in Squamous Cell Carcinoma

Figure 1. Photomicrograph of SCC-WD Showing Ki-67 Positivity (IHC Ki-67, 40X)

Figure 2. Photomicrograph of SCC-MD Showing Ki-67 Positivity (IHC Ki-67, 40X)

Figure 3. Photomicrograph of SCC-PD Showing Ki-67 Positivity (IHC Ki-67, 40X)

DISCUSSION

In the present study, total number of cases was 63. Among them, most (77.7%) were diagnosed as Moderately Differentiated Squamous Cell Carcinoma (SCC-MD) followed by 14.2% cases of Well-Differentiated Squamous Cell

Carcinoma (SCC-WD) and 7.9% cases of Poorly Differentiated Squamous Cell Carcinoma (SCC-PD) respectively. Similar results were found by Hemlata Panwar et al¹³ where out of the 78 cases of biopsy-proven invasive carcinoma, 54 cases had moderately differentiated squamous cell carcinoma, followed by 10 cases of well-differentiated SCC and 5 cases of poorly differentiated SCC.

Our cases showed an increase in the Ki-67 expression in relation to increase in the grade of the lesion. The mean Ki-67 Index % observed was 54.0 ± 2.64 for patients diagnosed as Well Differentiated Squamous Cell Carcinoma (SCC-WD), 63.0 ± 4.17 for patients diagnosed as Moderately Differentiated Squamous Cell Carcinoma (SCC-MD), and 85.5 ± 9.3 for patients diagnosed as Poorly Differentiated Squamous Cell Carcinoma (SCC-PD), and the difference was highly significant statistically ($P < 0.001$). Similar result was found by Gupta K et al¹⁴ in their study which reported the mean LI in WD SCC was 55.333 ± 7.789 ; in MDSCC was 43.976 ± 3.152 and in PDSCC was 80 ± 5 . The study conducted by Pahuja et al¹⁵ showed an increase in LI from WDSCC to PDSCC with maximum mean value in PDSCC similar to the present study. The study conducted by Nam et al¹⁶ also showed an increase in LI from WD SCC to PD SCC.

Mehrotra A et al¹¹ reported Ki-67 labelling index (LI) increased from dysplasia to carcinoma group. Statistical analysis showed that Ki-67 LI was significantly higher in diseased group as compared to normal group ($P < 0.001$). Carreras R et al¹⁷ showed increase in the Ki-67 expression in relation to increases in the severity of the lesions which is similar to our study.

In SCC-WD lesions, 5 patients out of 9 (55.5%) had expression of Ki-67 in 30 to <70% of the cells, 4/9 (44.4%) had expression of Ki-67 in 10 to <30%. None of the cells showed expression of 0 to <10% and in between 70 to 100%. Among the SCC- MD lesions, 34/49 (69.4%) showed expression of Ki-67 in 30 to <70% of the cells, 9 out of 49 (18.4%) cases showed Ki-67 expression in 70% and more than of the cells and 6 out of 49 (12.24%) cases showed Ki-67 expression in 10 to <30%. None of the cases showed Ki-67 expression in 0 to <10%. All 5 patients of SCC-PD were highly proliferative with an expression of Ki-67 in 70% or more of the cells. None of the cases showed Ki-67 expression in 0 to <10%, 10 to <30% and 30 to <70% category (Table 2). Reuschenbach M et al¹⁸ found the proportion of lesions with Ki-67 expression in 70% or more of the cells was somewhat higher in the carcinomas (21/46, 45.7%) than in the CIN3/carcinoma in situ (CIS) group of cases.

CONCLUSION

We conclude that MIB-1 staining shows an increasing trend in the Ki-67 labelling index with increasing grades of SCC.

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