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Original Research Article

A CROSS-SECTIONAL STUDY ON THE CORRELATION OF KI-67 EXPRESSION WITH CLINICAL AND PROGNOSTIC PARAMETERS IN SQUAMOUS CELL CARCINOMA OF THE CERVIX

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ABSTRACT

The study aimed to evaluate the expression of the proliferation marker Ki-67 in squamous cell carcinoma of the cervix and its correlation with age, parity, hormonal status, Broder's grading, and clinical staging. The cross-sectional study included 65 biopsy/hysterectomy-proven cases of squamous cell carcinoma. The mean age of the patients was 53 years, and all cases showed Ki-67 expression (100% positivity). The study found a statistically significant correlation between the Ki-67 score and age (p=0.046) as well as Broder's tumor grading (p=0.00). However, there was no association of the Ki-67 score with parity, hormonal status, and FIGO clinical staging. The findings suggest that Ki-67 expression may serve as an adverse prognostic marker in cervical invasive squamous cell carcinoma.

Keywords: Squamous cell carcinoma, cervical carcinoma, Ki-67, Broder's grading, Clinical stage.

INTRODUCTION

Cancer of the cervix has been the most important cancer among women in the past two decades.[1] As of March 2016, Indian Cancer Registries indicate that cervical cancer contributes to approximately 6–29% of all cancer in females. More than 85% of patients were from the age group 40 years and above.[2] The common histological type found in the ectocervix is squamous cell carcinoma (SCC) and that in the endocervix is adenocarcinoma.[3]

The proliferation of tumor cells is an important characteristic behaviour and therefore cell biological markers reflecting the proliferation rate of tumors have been investigated in various malignancies. However, for proliferation markers like BM28 and Ki67, no relation with survival has been observed.[4]

Ki-67 is a nuclear protein that is expressed by multiplying cells in all the phases of cell cycle (G1, S, G2, M) except G0. It can predict the possibility of development of a tumor and so is called a proliferation marker. The level of Ki-67 expression is used to determine the cell proliferation status.[5,6] It is normally expressed in basal and parabasal layers of normal cervical epithelium. However, when it shows positivity in the rest of the layers it implies dysplasia and carcinoma.[7] The increase in the number of positive cells has a significant positive correlation with the ascending grade of CIN.[8] It can therefore be a predictor of the malignant potential and prognosis of lesions.[9]

Aim and Objectives

1. To study expression patterns of Ki67 in squamous cell carcinoma.
2. To identify the association of Ki67 with the age, menopausal status, parity of patient, histopathological grade, and stage of SCC.

MATERIAL AND METHODS

This cross -sectional two-year study was conducted in the tertiary teaching institute of central India from January 2018 to December 2019.

Inclusion Criteria

All histopathological proven cases of squamous cell carcinoma of the cervix with age more than 18 years, whose biopsies and/or hysterectomy specimens were received for histologic study.

Exclusion Criteria

Very small biopsies with inadequate tissue for further processing to apply immunohistochemistry

Methods of Evaluation

1. Clinical details pertaining to age, parity, clinical signs & symptoms were noted.
2. Histological study of all cervix cancer cases was done.
3. Tumor grading was done on hematoxylin and eosin-stained slides using modified Broder's grading system.[10]
4. Staging of tumor was done based on International Federation of Gynecology and Obstetrics (FIGO) staging system.[11]
5. Immunohistochemical study of Ki67 was done in all cases of SCC.

IHC staining decision criteria

The slides were examined for the presence of staining as well as for the percentage and intensity of stained cells. The pathological diagnosis was considered definite if Ki67 were located in the nucleus of tumor cells indicated by tan or brown particles. At high magnification 10 different views were selected; for each view, 100 tumor cells were counted. Accounting to the percentage of positive cells Ki67 expression was considered positive or negative.

Scoring of Ki67 staining was done as per the standard guidelines and expression patterns of various IHC markers.[12]

IHC staining decision criteria

Data analysis: Data collected was entered in Microsoft Excel and presented with the help of descriptive statistics using SPSS software version 27. For qualitative variables, data was analysed by frequency and percentage and association was measured by chi-square test.

Table 1: Ki67 scoring system[12]

Staining	Score
< 10% Positive staining	0
10-30% Positive staining	1
30-50% positive staining	2
>50% positive staining	3

RESULTS

During the study period, a total of 65 SCC cases were diagnosed and enrolled in the index study. The mean age of the patient was 53 years with an age range of 30 years to 82 years. Amongst 65 cases, 72.3% of women were postmenopausal. The 40% of total women had parity ≤2, 56.9 % of women had parity between 3 to 5 and 3.1% of women had parity more than 5. The commonest presentation was postmenopausal bleeding (47.7%) followed by intermenstrual bleeding (30.8%). In the present study, moderately differentiated SCC was the commonest histological grade and constitutes 69% of the total cases.

In the present study, all cases of SCC were Ki-67 positive. Ki-67 score 3+ was the most common and observed in 50.8% (33 cases) of SCC cases. Ki-67 score 2+ and 1+ was observed in 30.8% and 18.5% of cases respectively. We also correlated the Ki-67 scoring with age, which showed a statistically significant rise in scores with increasing age. However, parity and menopausal status were not associated with the Ki-67 score. [Table 2]

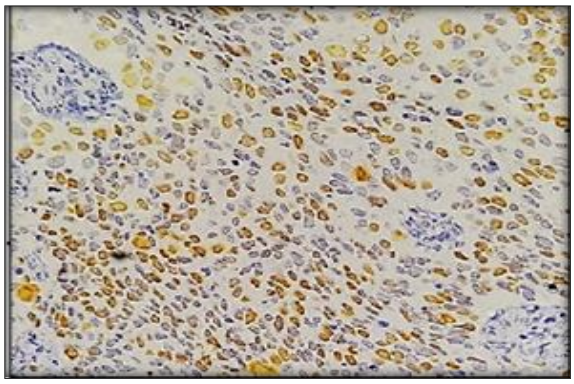

Figure 1: Photomicrograph showing Ki-67 positivity in moderately differentiated SCC (x400)

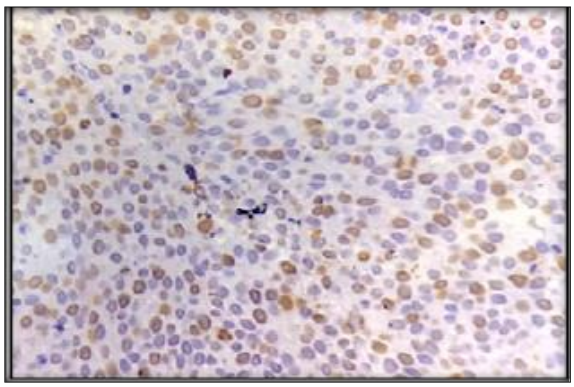

Figure 2: Photomicrograph showing Ki-67 positivity in poorly differentiated SCC (x400)

The association between the Ki-67 score and TNM staging of SCC of the cervix was not statistically significant. However, the associaation between Broder's grading and Ki-67 scoring was statistically significant. (Table 3) The Ki-67 score was high with moderately differentiated and poorly differentiated SCC. [Figure 1 & 2]

Table 2: Correlation of Ki-67 score with Age, parity and menopausal state

Characteristics	Total cases n=65	Ki-67 score (%)			P value
		1+ n=12	2+ n=20	3+ n=33	
Age (Years)					
<30	1	0	0	1(100)	0.046*
31-40	14	5 (35.7)	3 (21.4)	6 (42.9)	
41-50	17	2 (11.8)	7 (41.2)	8 (47.1)	
51-60	16	5 (31.2)	1 (6.2)	10 (62.5)	
>60	17	0	9 (52.9)	8 (47.1)	
Parity					
0-2	26	8 (30.8)	5 (19.2)	13 (50)	0.077
3-5	37	3 (8.1)	14 (37.8)	20 (54.1)	
>5	2	1 (50)	1 (50)	0	
Menopausal status					
Pre-menopausal	47	6 (12.8)	17 (36.2)	24 (51.1)	0.100
Postmenopausal	18	6 (33.3)	3 (16.7)	9 (50)	

Table 3: Correlation of Ki-67 score with tumor grading and staging

Characteristics	Total cases n=65	Ki-67 score (%)			P value
		1+	2+	3+	
Stage					
I	6	2 (33.3)	1 (16.7)	3 (50)	0.512
II	24	6 (25)	8 (33.3)	10 (41.7)	
III	32	4 (12.5)	9 (28.1)	19 (59.4)	
IV	3	0	2 (66.7)	1 (33.3)	
Broder's grading					
Well differentiated	15	8 (53.3)	5 (33.3)	2 (13.3)	0.000*
Moderately differentiated	45	4 (8.9)	14 (31.1)	27 (60)	
Poorly differentiated	5	0	1 (20)	4 (80)	

DISCUSSION:

The present study was conducted to evaluate the expression of Ki-67 in a total of 65 SCC cases.

The mean age in the current study was 53 years which was similar to other studies conducted for Ki-67 expression in premalignant and malignant lesions of cervix.[12,13]

In the current study, Ki-67 expression was observed in all cases (65%). This finding was in line with the study from Nigeria by Ike AO *et al*.[14], and the study by Elsokary AN *et al*.[15] who observed 100% positivity in 35 cases and 30 cases of SCC and its variants respectively. The concordant results were also observed in studies from South India and North India.[12,13,16] However, Tan GC *et al*.[17] and Odujoko OO *et al*.[18] observed Ki-67 positivity in 64.9% and 59.7% of cases respectively. This variation could be because of differences in the demographic profile of the study population, antigen retrieval method, and different antibodies used.

Many studies evaluated the role of Ki-67 to differentiate between normal epithelium and intraepithelial lesions (dysplasia/SIL) and also to differentiate high-grade squamous intraepithelial lesions (HSIL) and invasive SCC. They found Ki-67 expression was greater in dysplastic epithelium than the normal and metaplastic epithelium and the staining was stronger in high-grade intraepithelial lesions (HSIL) than the low-grade (LSIL) and concluded it as good tool to reduce the inter-and intra-observation variability in assessment of dysplasia.[14,19,20] Similarly, Singh P *et al*.[16], Tan GC *et al*.[17] and Raju K *et al*.[21] found Ki-67 as helpful adjunct tool in differentiating HSIL from SCC in difficult situations. However, the current study not included the dysplastic/intraepithelial lesions to study this aspect of Ki-67.

The present study explored the correlation between the Ki-67 scoring and age, parity, and hormonal status in cases of invasive SCC and did not find any correlation of Ki-67 score with parity and menopausal status. However, in current study we observed a significant increase in Ki-67 score with age. None of the study with the best of our knowledge correlated the Ki-67 expression with age. The current study has also correlated Ki-67 expression with the prognostic parameters like clinical staging and Border's tumor grading. Consistent with the findings of Raju K *et al*.[21] the study found no association between Ki-67 expression and clinical staging. However, Priyanka RD *et al*,[13] and Elsokary A *et al*. [15] found a statistically significant correlation of higher Ki-67 score with advanced FIGO stage. Priyanka RD *et al*. [13] observed 3+ Ki-67 score in 25 %, 64% and 81% of total cases of Stage I, II and III respectively.

Similarly, the current study has found a maximum number of cases of moderately differentiated SCC and established a significant correlation of tumor grading and Ki-67 expression, which is consistent with Elsokary A *et al*.'s[15] findings. The present study found that the Ki-67 score was 3+ in 80% of poorly differentiated SCC and 60% of moderately differentiated SCC, and 13.3% of well-differentiated SCC. These findings suggest that Ki-67 expression can be a valuable tool in assessing the prognosis of invasive SCC.

CONCLUSION

The present study evaluated the Ki67 expression in 65 cases of invasive SCC of cervix and found 100% positivity. The study found a significant correlation between Ki67 expression and age. The current study also found a statistically significant association of Ki-67 expression with the histological grade of the invasive cervical carcinoma. In conclusion, Ki67 expressions were directly associated with the severity of cervical lesions. Therefore, Ki-67 may be used as adverse prognostic marker in invasive cervical carcinoma.

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