Original Research Article

Expression of Ki-67 in premalignant and malignant lesion of cervix in tertiary care hospital in Uttar Pradesh

Pratima Singh*, Kanchan Garg, Ankita Singh, Shambhavi Tripathi

Department of Pathology, Hind Institute of Medical Sciences, Mau, Sitapur, Uttar Pradesh, India

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*Correspondence:
Dr. Kanchan Garg,
E-mail: kanchangarg68@gmail.com

ABSTRACT

Background: cancer is fast emerging as one of the most common causes of death related morbidities throughout the world. As far as India is concerned. Ki-67 is an ideal marker to assess the cellular proliferation. The study aimed to evaluate the proliferative activity by using Ki-67 in premalignant and malignant lesion of cervix. To study the various histomorphology features of cervical lesion and grading it into cervical intraepithelial neoplasia and malignant lesion. To access the expression of Ki 67 in different grade of lesions.
Methods: The study was carried in 60 cases of cervical biopsy that included 30 cases of CIN 1, CIN2 and 30 cases of CIN 3, SCC. Ki-67 staining was done on all cases which were diagnosed histologically as CIN or cervical carcinoma. The result was categorized in grading 1,2,3.
Results: Ki-67 expression was studied in all cases. There was an increase in the intensity of Ki-67 from CIN to carcinoma. The focal positivity in low grade lesion to diffuse positivity in higher grade lesion was seen in our study.
Conclusions: In this study, Ki-67 expression was helpful in differentiating dysplastic lesion from carcinoma and helps in confirming the histopathological diagnosis. Therefore Ki-67 marker could be used as biomarker in the evaluation of the proliferative activity and progressive potential of dysplastic and neoplastic changes.

Keywords: Cervical cancer, Ki-67, Squamous cell carcinoma

INTRODUCTION

Cervical cancer is the second most common cause of death in the western world, after cardiovascular disease.[1] Type of cancers that are associated with the highest rate of mortality. Cancer is the third most commonly diagnosed cancer and fourth leading cause of cancer death in females worldwide.[2] As per GLOBOCAL 2020 estimates made public in the year 2021,breast cancer and cervical cancer are the leading cause of cancer death in 110 and 36 countries.[3] The incidence of cervical cancer cases has increased from 529,800 in the year2008 to 604,127 in the year 2020 yet its proportion in total cancer cases has showed a decline from 9% to 6.5%.[2,4] As a result of concerted efforts to create awareness and mobilize screening. The natural history of cervical cancer represents a stepwise-progression from a histologically normal to frank invasive cancer.[5]

Cervical cancer is considered to be a multifactorial disease involving socioeconomics, cultural, immunological and epigenetic factors, as well as persistent human papilloma virus (HPV) infection presence of factors like smoking, high parity, inflammation and other inducing factors like heavy viral load and presence of specific viral variants (HPV type 16, 18, 31, 33, 45, 58 and others) result in progression to higher grades and cervical intraepithelial neoplastic

conditions which eventually progress to the invasive form of cervical cancer. Cervical cancer is a progressive disease which if identified at early stages of development could be prevented successfully from advancement and progression to more invasive stages. The concept of preinvasive disease is based on the fact that epithelial changes could be identified that had the appearance of invasive cancer but were confined to the epithelium and if not treated, dysplasia can progress to cervical cancer. Histological changes include cellular disorganization, nuclear abnormality and increased mitotic activity.

Ki-67, is a protein that is associated with all active phase (particularly interphase and mitosis) of cellular growth/ proliferation excepting the resting state. Ki-67 is a proliferative marker that is confined to the parabasal cell layer of normal stratified squamous epithelium. It has also been found to be associated with the clinical course of disease.[6,7] Thus it is considered as an ideal marker to assess the cellular proliferation of a given tissue at a given times. Ki-67 has also been found to be .4 associated with the clinical course of disease.[8,9] A number of previous studies have shown its role in screening, triaging of cervical cancer and precancerous lesion along with evaluating of treatment outcome and prognosis.[10-16]

METHODS

This study was conducted at, Hind institute of Medical Sciences Mau, Ataria Sitapur.60 patients of cervical cancer and cervical intraepithelial neoplasia were taken which were diagnosed by histopathological examination.

Cross sectional study conducted at Hind Institute of medical science, Mau, Ataria, Sitapur, from 5th December 2020 to 5th June 2022.

Inclusion criteria

Paraffin block preserved squamous intraepithelial specimen obtained from patients suspected of lesion of cervix and carcinoma cervix.

Exclusion criteria

Cervical biopsies reported as inflammatory condition. Samples showing features suggestive of lesion other than squamous intraepithelial lesion and squamous cell carcinoma of cervix.

All the specimens were formalin fixed and paraffine embedded. H&E staining was done and histopathological diagnosis was done. Immunohistochemical staining of Ki-67 was performed subsequently.

Immunohistochemical assessment

Immunohistochemical staining of Ki-67 was performed subsequently. The staining was performed according to the following protocol (Figure 1).
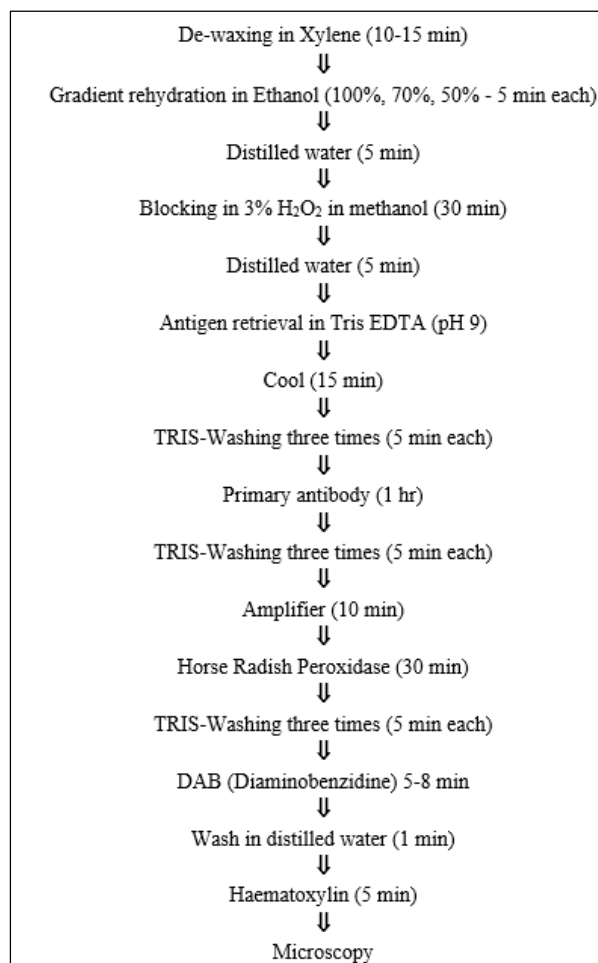

Figure 1: Protocol for immunohistochemistry.

IHC scoring

A semi-quantitative scoring method was used to quantify the level of Ki-67 expression as per following criteria.

Ethical approval

The study was carried out following the Helsinki Declaration for Research on Human Subjects.

Approval for the study was obtained from the Institutional Ethics Committee before the commencement of study. Informed consent was obtained from the patients.

Statistical analysis

The data was analyzed using IBM-Statistical Package for Social Sciences (SPSS) 21.0 version. Data has been represented as numbers and percentages for categorical data. Continuous data has been represented as mean±standard deviation. Kruskal-Wallis and Mann-Whitney U tests were used for analysis of data.

RESULTS

he Present Study was conducted to study the proliferative activity by using Ki -67 in premalignant and malignant lesion of cervix. For this purpose, a total of 60 laboratory preserved squamous intraepithelial specimen were accessed in department of pathology Hind Institute of Medical Sciences, Mau, Sitapur, Uttar Pradesh.

Table 1: Age profile of study population (n=60).

Age Group	No. of women	Percentage
30-39 years	9	15.0
40-49 years	33	55.0
50-59 years	13	21.7
>60 years	5	8.3
Mean age ±SD (Range) **Median (interquartile range)**	47.63±8.57 (30-73) 46 (42-51)	

Table 2: Presenting complains and relevant history.

Variable	No. of women	Percentage
Postmenopausal bleeding	22	36.7
White vaginal discharge	23	38.3
Abnormal uterine bleeding	33	55.0
Abdominal pain	30	50.0
H/O HPV infection	20	33.3
IUCD use	22	36.7

Table 3: Distribution of cases according to ki-67 expression status.

Ki-67 expression score	No. of patients	Percentage
Score 0	0	0
Score 1	26	43.3
Score 2	18	30.0
Score 3	16	26.7

Age of study population ranged from 30 to 73 years. Majority (n=33; 55.0%) .

Patient were aged 40-49 years followed by those aged 50-59 years (n=13;21.7%), 30-39 Years (n=9; 15%) and >60 years (n=5; 8.3%). Mean age of patients was 47.63±8.57 years. Median age of study population was 46 years with interquartile range falling in 42 to 51 years (Table 1).

Abnormal uterine bleeding (55%) was the most common presenting complaint followed by abdominal pain (50%), white vaginal discharge (38.3%) and postmenopausal bleeding (36.7%). There were 20 (33.3%) patients with a history of HPV infection while 22 (36.7%) revealed a history of IUCD use (Table 2).

Ki-67 expression was seen in all the cases. Maximum (n=26; 43.3%) cases had Ki-67 expression score 1 followed by score 2 (n=18; 30%) and score 3 (n=16; 26.7%) respectively (Table 3, Figure 2, 3, 4, 5).
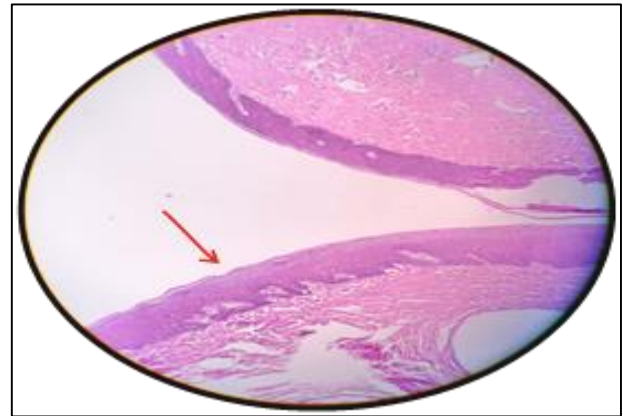

Figure 2: Photomicrograph (H&E) showing CIN1.

(Red arrow-Dysplastic cells are confined to the lower third of epithelium).

Table 4: Comparison of Ki-67 Expression between Malignant and Premalignant lesions.

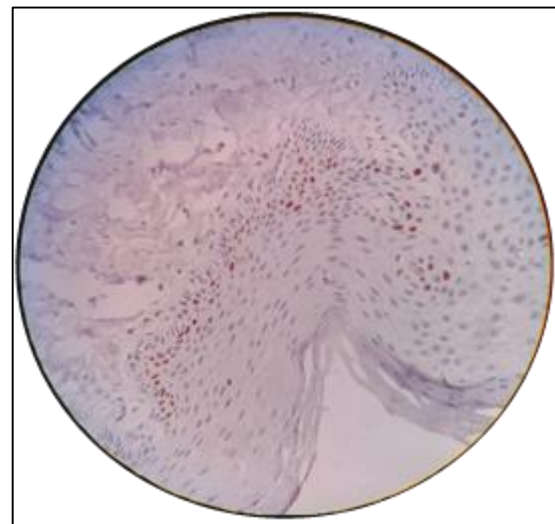| Lesion type	Score 1	Score 2	Score 3
Malignant (n=17)	0	3 (17.6%)	14 (82.4%)
Premalignant (n=43)	26 (60.5%)	15 (34.9%)	2 (4.7%)

Figure 3: Photomicrograph (IHC) showing Ki-67 grade 1 in CIN1.

In malignant group, majority (82.4%) had score 3 followed by score 2 (17.6%). None of the malignant cases had score 1. Compared to this, in the Premalignant group, majority (60.5%) had score 1 followed by score 2 (34.9%) and score 3 (4.7%) respectively. On comparing the data statistically, malignant group showed a significantly higher Ki-67 expression score as compared

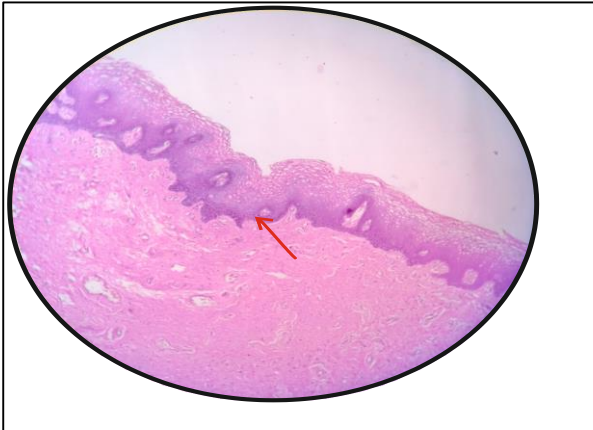to premalignant group (p<0.001) (Table 4; Figure 6, 7, 8, 9).

(Red arrow-dysplastic cells with loss of polarity of cells).

Figure 4: Photomicrograph (H&E) showing CIN2.

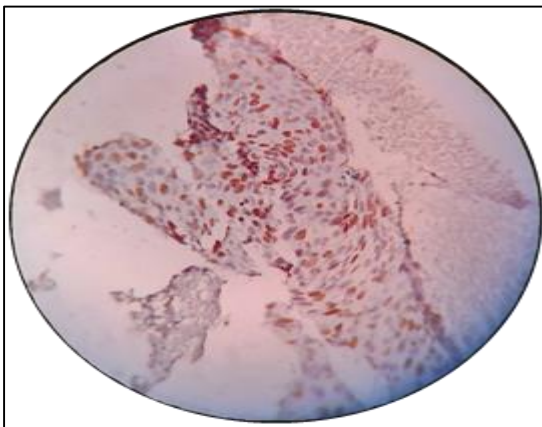(Red arrow-Dysplastic cells are confined to the lower two third of epithelium).

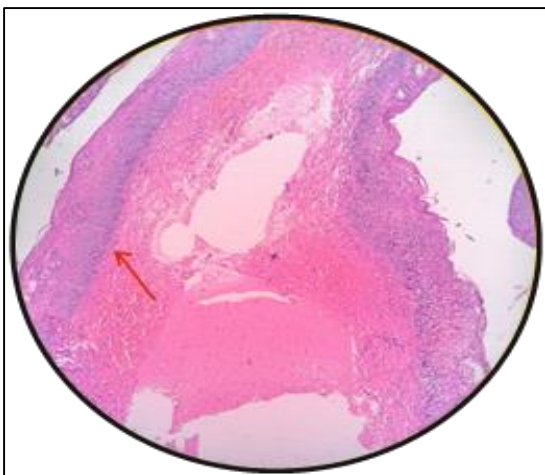**Figure 5: Photomicrograph (IHC) showing ki 67 grade 2 in CIN 2.**

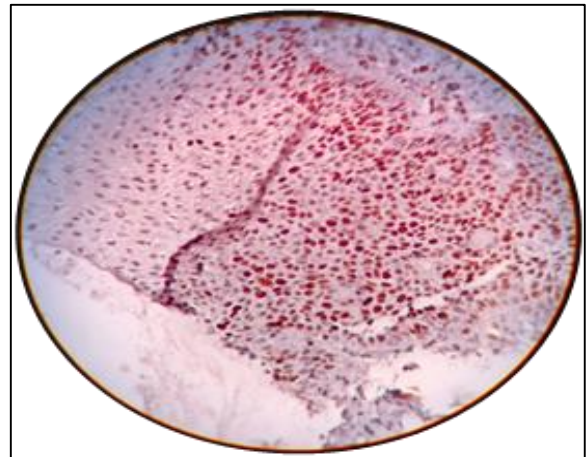**Figure 6: Photomicrograph (H&E) showing cin3**

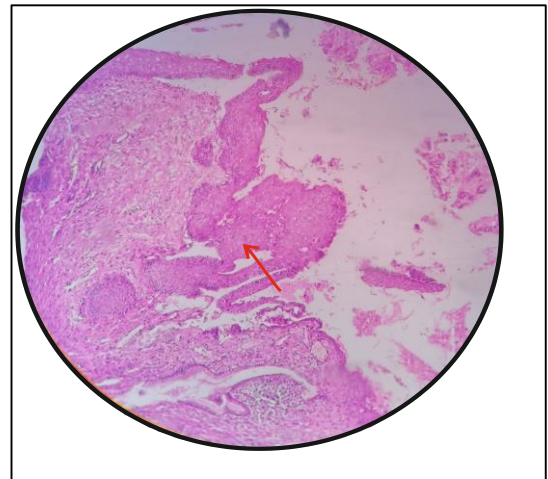**Figure 7: Photomicrograph (IHC) showing Ki-67 grade 3 in CIN3.**

Figure 8: Photomicrograph (H&E) showing SCC.

(Red arrow-Dysplastic cells are distributed in whole thickness of epithelium).
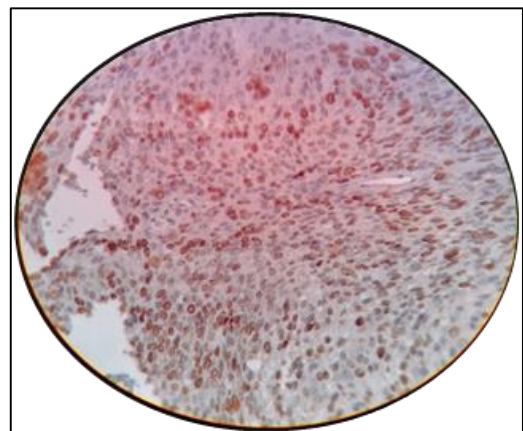

Figure 9: Photomicrograph (IHC) showing Ki-67 grade 3 in SCC.

DISCUSSION

Morphological characteristics at histopathological level are very important to establishment of a confirmed histopathological diagnosis. Immunohistochemistry is based on assessment of various biochemical changes taking place in the tissue and hence its role is not only limited to confirmation of a diagnosis but it also helps to provide a descriptive account of the various biochemical processes taking place in the tissue that are helpful in understanding the clinical course of disease, determination of treatment outcomes and prognosis too.[17-24]

With this background the present study was carried out to study the histomorphological features of cervical cancer and its grading into cervical intraepithelial neoplasia and malignant lesion and to correlate the IHC expression of Ki-67 in different grades of cervical lesions.

In the present study, age of specimen source ranged from 30 to 73 years with a mean age of 47.63±8.57 years and a median age of 46 years (interquartile range 42 to 51 years). The age profile of patients in the present study is similar to other study who reported it to be 47.74 years and 48.45 years respectively in CIN and carcinoma cases respectively (Combined average age 48.21years).[25] However, another author reported the mean age of patients to be 40.4 years which is slightly less than that in the present study.[26] In other studies, the mean age of patients has been reported to range from 37.40 years to 47 years.[13,27,28] A much younger age profile of the patients was reported by an author in 2019 who reported the median age of premalignant and malignant group patients in 25 to 28 years range.[29] In the present study, majority of patients (55%) were in age group 40-49 years. Another researcher also reported it to be the most common age group for both malignant and premalignant groups.[25] However, in 2019 a researcher found majority of premalignant as well as malignant patients to be below 30 years of age but this was the only study reporting such a young profile of affected women.[29] Otherwise the age profile of women in different studies shows a dominance of those aged 40 years or above as also seen in the present study.

The present study showed a dominance of illiterate women (63.3%) from lower socioeconomic class (85%), without habit of alcohol/smoking (81.7%), early marriage history (mean age at marriage 17.77 years; maximum age at marriage 22 years), early child bearing (mean age at first child 19.47±1.81; maximum age at first child 23 years) and premenopausal status.

In the present study IHC expression of Ki-67 was seen in all the cases, however, it was low (Score 1) in 26/60 (43.3%) and high (score 2 and 3) in 56.7% cases. Compared to the present study, another author who carried out his work found IHC expression in only 38% cases (that included only CIN III and malignant cases)

and in all of them this expression was of higher grades (Grades 2 and 3).[30] In 2016 the study conducted observed Ki-67 expression in only 93/180 (51.7%) of premalignant and malignant lesions.[31] However, unlike the present study, where Ki-67 expression was graded/scored, they noted it only in categorical terms using a subjective observer-based criteria. Other author in 2019 used a similar criterion as used by us and observed Ki-67 expression in 66.7% of cases.[32] Similar to the present study, in their study too, higher scores (2+ and 3+) were seen in almost half (48.1%) the cases. In the study that included only cases of low and high squamous epithelial lesions reported Ki-67 expression in all the cases but reported higher scores (2+/3+) in only 32.3% of cases.[33] In another study Ki-67 expression in all the premalignant as well as malignant cases was moderate to high expression was seen in 70% of cases. In other studies, too, majority of CIN and carcinoma cases have been shown to have a positive Ki-67 expression.[34,35] however, the proportion of higher grades of Ki-67 expression showed variation among different studies probably depending upon the grades of premalignant lesions and proportion of carcinoma cases in the study.

As is common knowledge, Ki-67 is a proliferative marker that is expressed throughout the cell cycle with the exception of G0 phase. It is quite effective at identifying cervical lesion with a high risk of developing into cancer. The findings of the present study were recorded and compare with the observation made by some previous study.

Limitations

The present study despite its limitations provided some useful information regarding the morphological features of cervical premalignant and malignant lesions and also provided that Ki-67 as a single marker could help to differentiate between different progressive stages of cervical cancer. Further studies on a larger sample size and their longitudinal follow-up to assess the role of Ki-67 in differentiating different grades of cervical malignancy and its prognosis are recommended.

CONCLUSION

The findings of the study showed that histomorphological features and Ki-67 expression are useful in diagnosing the nature and type of malignant and premalignant lesions of cervix. Ki-67 emerged as a useful independent marker to differentiate between malignant and premalignant lesions of cervix. It also helped to differentiate among different stages of premalignancy. Despite the limitation of a small sample size, the findings of the study were encouraging and highlighted the usefulness of Ki-67 to quantify the proliferative activity in cervical premalignant and malignant cases. Further longitudinal studies to highlight the prognostic value of Ki-67 in assessment of treatment response and survival are recommended.

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