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CORRELATION BETWEEN MITOTIC AND KI-67 LABELLING INDEX IN PARAFFIN EMBEDDED CERVICAL CARCINOMA

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ABSTRACT

Introduction: In India cervical cancer is the second most frequent cancer among women. This study was carried out to study the role and correlation of ki-67 labelling index and mitotic index in the cervical carcinoma.
Methods: The present study was carried out on 40 cases of cervical carcinoma in 39 cervical biopsies and one hysterectomy specimens over a period of two years in the deparment of pathology F H medical college, Tundla, Firozabad, Uttar Pradesh, India. In all cases mitotic index and ki-67 labelling index were calculated.
Results: ki-67 labelling index and mitotic index were found to be correlated with each other and increase with the increasing grade of the cervical cancer.Conclusion: Both the proliferative indices, mitotic index and ki-67 labelling indices are useful in determining the grading of cervical cancer.

INTRODUCTION

Carcinoma of cervix is the second most common cancer among women in India. Cases from developing countries accounts for 80% of the total cervical carcinoma cases.[1] In 2004, 10,500 new cases of invasive cancer occurred and more than 50,000 cases of carcinoma in situ were detected.[2]

Ki-67 is a proliferation marker known as predictive factor for tumor development. It is expressed during all active phases of the cell cycle (G1, S, G2, M), except G0, thus being present only in dividing cells and absent in resting cells.[3,4]

Mitotic Index is the oldest way of assessing proliferation. Though many other ways of assessing proliferation have become available, the ease with which mitoses can be recognized with a well stained H and E slide has led to the increasing popularity of this way of counting of mitotic figures up to the present.[5]

There is a good correlation of growth fraction measured by Ki-67 with mitotic index and high histological grade of cervical carcinoma.[6]

The present study is conducted to evaluate the correlation of Ki-67 expression, and mitotic index, in tissue sections of cervical carcinomas of uterus. From the data acquired in this study it was possible to determine general regularities for low-grade and high grade

MATERIAL AND METHODS

The present study was conducted on 40 cases of uterine cervix consisting of 39 cervical biopsies & 1 hysterectomy specimen received in the Department of Pathology, F H Medical college, Tundla, Firozabad, Uttar Pradesh, India. It is a retrospective as well as prospective study conducted over a period of two years.

All histologically proven cases of cervical carcinoma were taken in the study. Tissue sections with inadequate study material and with extensive necrosis and haemorrhage were excluded from the study. The History was obtained from histopathological records as per proforma attached.

The tissue was fixed in formalin for 24 hours and after adequate fixation, it was processed and finally embedded in paraffin wax. Sections of 5µm (microns) were cut and stained with Haematoxylin and Eosin (H & E) stain, mounted to see under the microscope.

The cases were diagnosed as invasive squamous cell carcinomas and adenocarcinomas of cervix. The cases of SCC (squamous cell carcinomas) & adenocarcinoma were further categorized as grade I, II, III. The mitotic index was calculated as given below.

Immunostaining for ki-67

The sections from the tumor tissue were subjected to immunostaining for Ki-67 expression with monoclonal mouse anti Ki-67 antibody /clone MB67 code E762 procured from diagnostic biosystems.

1. Positive control section-Tonsils
2. Negative control section - By omission of primary antibody.

Mitotic index:-

Mitotic index (MI) was calculated in H and E stained sections using 40 X objective, evaluating 1000 tumor cells for the presence of mitoses. Mitoses can be recognised by the presence of hairy extensions when focusing up and down, while the nuclear envelope is absent and cytoplasm is basophilic rather than eosinophilic (Figure 1).[7]

The mitotic index was calculated by dividing the mitotic count determined in 10 consecutive HPF(high power field) by the total number of cells in these fields, and expressed as number of mitotic figures per 1000 cells.[7]

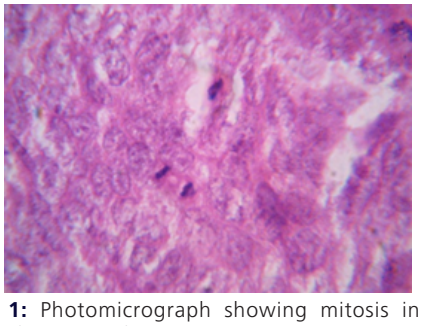

FIGURE 1: Photomicrograph showing mitosis in cervical carcinoma (H & E, 400X)

Ki-67 Score:-

This was evaluated by counting a minimum of 1000 tumor cells in consecutive fields (two to four) at high power (400 x) and expressed as a percentage of total cell count (labelling index, LI).[8] Ki-67 expression status was assessed according to the estimated proportion of nuclear staining of tumor cells that were positively stained.(Figure 2)

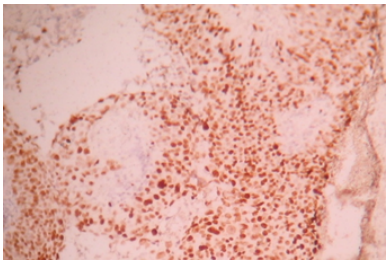

FIGURE 2: ki-67 expression in carcinoma cervix (400X).

Grading of Ki-67 expression was done by Costa S et al in their studies.[9]

ki-67 expression	% positivity of cells
Negativity	0%
Weak	<50%
Moderate	50-75%
Strong	>75%

RESULTS

The women in present study belonged to 20 years to more than 70 years of age. Maximum cases of cervical carcinoma were seen in 50-59 years of age group .Out of total 40 cases 38/40 (95%) are SCC cervix, while 2/40 (5%) are adenocarcinoma. TheSCC were further classified as keratinizing SCC 4/40 (10%), non keratinizing SCC 33/40(82.5%), poorly differentiated SCC 1/40(2.5%) and, adenocarcinoma 2/40.

In all the four cases of keratinizing SCC ki-67 positivity was <50%. In NKSCC 17/33(51.5%) cases had < 50% positivity, 15/33(45.5%) cases had 50-75% positivity, 1/33(3.0%) case had >75% positivity. Out of 40 cases 57.5% of cases had mitotic index <10, 40.0% of cases had mitotic index between 10- 20.Only one case was observed with mitotic index >20 .

The range of Ki-67 expression and mitotic index were more in grade II as compared to grade I. With grade III correlation is insignificant as there was only one case in grade III. (Table I) . The MI increased progressively with increasing grade of the carcinoma.

TABLE I Showing Correlation Of Ki-67 Labelling Index & Mitotic Index With Histological Grade

GRADE	NO OF CASES	Ki-67 LI	MITOTIC INDEX
GRADE I	4	13-37%	06- 09/1000 cells
GRADE II	33	12-92%	02-22/1000 cells
GRADE III	1	29%	10/1000 cells

p value between Grade I & II is very significant, but no correlation could be made with grade III due to small no of cases in grade III. (Table II)

TABLE II Showing Comparison Of Mean Ki-67 Labelling Index Between Various Grades Of Scc Cervix

GRADE	NO OF CASES	Mean Ki-67 LI ± standard deviation	p VALUE*
GRADE I	4	19.8 ± 11.5	p = 0.0077 between Grade I & II
GRADE II	33	47.9 ± 19.3	
GRADE III	1	29	

DISCUSSION

Cervical cancer develops from premalignant (CIN) to invasive stages, in a multistep process of carcinogenesis. During this process many molecular structures are denovo expressed while reference characteristics are lost. Immunohistochemical studies allow the identification of these molecular changes as tumor markers and contribute to improve our capacity in diagnosis and evaluation of prognosis.

Several candidate tumor markers for cervical neoplasia have been identified. Among these are the proliferation markers like Ki-67 and PCNA. The potential usefulness of determining the proliferation indices of tumors lies in the application of this knowledge in predicting the behavior and prognosis of individual tumors and in formulating treatment strategies based on thereon.

Mitotic index has also been used for many years as a measure of the proliferative character and so a measure of rate of growth of tumor. Tumors with a more rapid rate of growth will behave more aggressively than those growing less rapidly. In the present study Ki-67 expression was studied in various types of carcinoma cervix & correlation of immunostaining pattern was done with mitotic index, histological grade and with the various subtypes of carcinoma cervix.

In normal cervix the basal layers represent main proliferative pool with the basal providing a reserve. When CIN supervenes, this proliferative compartment expands commensurate with the grade of dysplasia. Increase in Ki-67 index had been demonstrated as the grade of CIN increases. Expression of Ki-67 in the cervical carcinoma was studied by various authors.

TABLE III showing ki-67 labelling index in various grades of scc cervix

Study	Ki-67 labelling index		
	Grade I (Well differentiated)	Grade II (mod. differentiated)	Grade III (poorly differentiated)
Wong FW et al 1990[10]	11.5-33 % (mean =22.2%)	20-55.3% (mean =33.9%)	15.6-69.1% (mean =34.5%)
Wong FW 1994[11]	14.8-30.5% (mean=22.72%)	15.6- 48.4% (mean=27.02%)	11.5-32.2% (mean=24.24%)
Present study 2007	13-37% (mean=19.8%)	12-92% (mean=47.9%)	29 % (mean=29%)

In the present study mitotic index in the grade I was from 13-37% (mean = 19.8%), grade II from 12-92% (mean = 47.9%), grade III was 29%. Ki-67 LI increased from grade I to grade II , while in grade III as there was only one case, no correlation could be made with grade III. Thus it is clear from present study that the proliferating index varies with the differentiation in a linear manner. It was similar to the study conducted by Wong et al10 in which all the lower grades cervical carcinoma had lower Ki-67 values than higher grade. It is different from the study of Wong FW[11] in which differentiation does not correlate with growth rate. (Table III)

TABLE IV Showing Mitotic Index In Various Grades Of Scc Cervix

Study	Mitotic index		
	Grade I (Well differentiated)	Grade II (mod. differentiated)	Grade III(poorly differentiated)
Wong FW et al 1990[10]	9-76/50 HPF (mean= 36.35)	25-125/50 HPF (mean = 70.25)	21-150/50 HPF (mean = 81.36)
Wong FW 1994[11]	45/50 HPF	74/50 HPF	60/50 HPF
Present study 2007	06- 09 /1000 cells (mean = 7.25)	02-22/1000 cells (mean = 10.49)	10/1000 cells

In the present study mitotic index in the grade I was from 6-9 /1000 cells (mean = 7.25), grade II from 2-22/1000 cells (mean = 10.47), grade III it was 11/1000. Similar to Ki-67 LI, MI increased with the grade in a identical manner. It was lesser in grade I as compared to grade II. It was in concordance with the study of Wong et al[10] in which similar findings were reported.

As both the mitotic and Ki-67 labelling indices correlated with the grade in a linear manner in the present study, this suggests that differentiation correlates with the grade in a linear manner or that this measure of growth rate might be important in predicting the prognosis in cervical cancer.

In present study in all the 40 cases correlation between Ki-67 labelling index and mitotic index was statistically very significant(p = 0.0001). In moderately differentiated carcinoma, both the indices showed a correlation with each other, which is also statistically very significant (p = 0.001) as both the indices were highest in moderately differentiated (grade II) carcinoma.

The prognostic implications of Ki-67 growth fraction have been described in various studies. In the present study Ki-67 measured growth fraction also varied widely from case to case in each histological grade. To support this, clinical data have shown that some well differentiated carcinoma may take a more rapid downhill course than others and some poorly differentiated carcinoma respond better to therapy than others. The prognostic implication of this Ki-67 finding with in a histological grade is well illustrated by Hall et al with respect to lymphomas. In their study

those cases with high Ki-67 showed better survival due to good response to therapy and those with a Ki-67 LI had poor survival due to increased chance of relapse. The Ki-67 LI can be used safely as a proliferation marker in cervical carcinomas, and changes in the Ki-67 LI during the early course of radiotherapy may predict the metastatic potential in cervical cancer. [12,13,14]

A correlation between Ki-67 growth fraction and survival has not been attempted in the present study because of insufficient follow up time.

There are advantages of this method over other methods of determining the proliferative index such as radioisotope labelling and immunoflourescent techniques. These include simplicity of technical application, retention of morphology to allow identification of nature of positive cells, and the absence of radiation hazards. The only disadvantage is laborious counting of large number of cells & the inter observer variability. However the application of automated image cytometry could overcome this in future.

CONCLUSION

Mean mitotic index and the Ki-67 index varied with the grade in a linear manner as higher levels of LI & MI had higher histological grade. Both indices increased from grade I to grade II which is statistically significant but the correlation between with grade III is insignificant as there was only one case in grade III.

In all the 40 cases irrespective of grade, correlation between Ki-67 index and mitotic index was statistically very significant ($p = 0.0001$) as higher level of LI was correlated with higher level of MI. In grade II SCC both the indices were highest and they correlated with each other in a linear manner which is also statistically very significant ($p = 0.001$).

Thus, it is concluded that both the mitotic & Ki-67 labelling indices are correlated with each other and both indices increase with increase in grade and proliferative activity of cervical lesion as determined by mitotic index and ki-67 antibody expression are reliable indicators of its malignant potential.

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