Ki-67 Expression and Apoptotic Index in Premalignant and Malignant Lesions of Uterine Cervix

Pranjal Kumar Gogoi[1], Mondita Borgohain[2], Ramesh Sonowal[3]

ABSTRACT

Introduction: Cervical cancer in India ranks as the 2nd most frequent cancer among women. Proliferative and apoptotic indices have emerged as important diagnostic and prognostic tools. The study aimed to evaluate the role of Ki-67 expression and apoptotic index (AI) as a diagnostic tool in premalignant and malignant lesions of uterine cervix and to determine the potential of Ki-67 expression and apoptotic index in reliably distinguishing cervical intraepithelial neoplasia (CIN) from invasive carcinoma cervix.

Material and methods: Ki-67 immunohistochemical (IHC) staining was done on 57 cervical biopsies, diagnosed histologically as CIN or cervical carcinoma. Ki-67 is expressed as Ki-67 labelling index (LI), which is calculated as the number of Ki-67 positive cells per 100 cervical epithelial cells. AI was measured in haematoxylin and eosin stained slides by calculating percentage of apoptotic cells and apoptotic bodies from atleast 1000 tumor cells in each case at high magnification (400X). Data were analysed using MS Excel 2010.

Results: There was an increase in both Ki–67 labelling index and apoptotic index with increasing grades of dysplasia. Statistical comparison of Ki–67 labelling index and apoptotic index between CIN and carcinoma; and also between different grades of CIN and was found to be highly significant (*P* value <0.05).

Conclusion: Both Ki-67 labelling index and apoptotic index has potential to reliably distinguish different grades of CIN; and can also distinguish CIN from invasive carcinoma cervix, thus both indices can be used as a diagnostic tool in premalignant and malignant lesions of uterine cervix.

Keywords: Apoptotic index, Cervical carcinoma, Cervical intraepithelial neoplasia, immunohistochemistry, Ki-67, MIB-1

INTRODUCTION

Cervical carcinoma is the fourth most common cancer in women worldwide.[1] The incidence of cervical cancer has diminished mainly in developed countries due to successful cytological screening programmes. Today more than 80% of woman dying from cervical cancer live in developing countries[2]

Most developing countries like India have national guidelines for cervical cancer screening but such screening programmes have failed mainly due to limited resources and large population. As a result, very often diagnosis of cervical cancer is based on opportunistic screening or after the onset of symptoms.[3]

Cervical cancer is a preventable disease because invasive cervical carcinoma always arises from a benign premalignant condition termed as cervical intraepithelial neoplasia (CIN) which is a completely curable condition. CIN may regress to normal or progress to invasive cancer if left untreated. Most cervical carcinomas are of squamous cell type.

Although the Papanicolaou (Pap) smear screening programmes and histologic interpretation of biopsy specimen by the pathologists has appreciably decreased the number of deaths due to cervical cancers, but the degree of this reduction and the cost-effectiveness of current screening programs remain the subject of debate. The false-negative rate for Pap tests in the presence of invasive cancer is up to 50%, so a negative Pap test should never be relied on in a symptomatic patient.[4] Therefore, an objective biomarker like Ki-67 will be helpful in the identification of truly dysplastic and cancerous cells and/or predict disease progression.

Histopathological evaluation is known as ‘‘Gold standard’’ for the diagnosis of CIN lesions. Histopathological diagnosis that directs treatment is also affected by high rates of disconcordance among pathologists. Pathologists show significant interobserver variability in classifying CIN and in distinguishing between reactive squamous proliferations and CIN grade.[5]

Ki-67 is an antigen present in proliferating cells. MIB-1 is a monoclonal antibody which recognize Ki-67 antigen in the G1, S, G2 and M phase of cell cycle, but it is absent in the G0 phase.[6] Hence, this antibody can be used as a marker of proliferation in neoplastic lesions and can be of diagnostic and prognostic value.[7] VonHoven in 1996 suggested it as a sensitive biological indicator of progression in CIN lesions[8]

Apoptosis is genetically regulated cell death which permit elimination of damaged cells.[9] Apoptosis can be identified morphologically by both light and electron microscopy. During the past few years mechanistic studies on apoptosis have been done, revealing its basic molecular mechanisms. Investigators working on neoplastic cells have discovered new analytical tools to study the role of apoptosis in cancer. Apoptosis and its relationship with growth and progression of tumor have been studied in numerous papers and “apoptotic index” is included among the parameters used to measure tumor growth in various types of neoplasms including cervical cancer. “Apoptotic index” is measured in haematoxylin and eosin stained slides by calculating percentage of apoptotic cells and apoptotic bodies from atleast 1000 tumor cells at high magnification (400X)[10]

Therefore this study was undertaken to assess the importance of Ki-67 expression and apoptotic index in accurate diagnosis and classification of cervical neoplastic lesion, so that it can be used as an indispensable part of histological examination and

[1]Demonstrator, [2]Professor, Department of Pathology, [3]Associate Professor, Department of Obstetrics and Gynaecology, Assam Medical College and Hospital, Dibrugarh, India

Corresponding author: Dr. Pranjal kumar Gogoi, Department of Pathology, Assam Medical College and Hospital, Dibrugarh, PIN-786002, Assam, India.

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eliminate the false positive and false negative diagnosis due to interobserver variability.

MATERIAL AND METHODS

This study was conducted in Dept. Of Pathology, Assam Medical College and Hospital, Dibrugarh. All cervical specimens received during one year period (June 2014-May 2015) were to be studied. We received total 57 cervical specimens during study period.

Criteria for Selection of Cases

Inclusion Criteria: Cervical biopsy specimens received at the Department of Pathology A.M.C.H during the study period, diagnosed histologically as CIN or invasive cervical carcinoma.

Exclusion Criteria: Inconclusive and inadequate cervical biopsies.

The type of study was hospital based cross-sectional study. In all the cases relevant history was taken, clinical examination was done, and important investigations were noted. Prior consent was taken from patient. Ethical approval was taken from college ethical committee.

Specimen from study included colposcopic punch biopsy and hysterectomy specimens. Specimen were processed in 10% neutral buffered formalin followed by sectioning and paraffin embedding. One section was stained with hematoxylin-eosin (H & E) staining for observing histological typing and apoptotic index. Rest of the sections were kept for Ki-67 immunostaining. Samples were divided into seven groups-C.I.N-1, C.I.N-2, C.I.N-3, W.D.S.C.C, M.D.S.C.C, P.D.S.C.C AND OTHER TYPES. Ki-67 immunostaining was done according to manufacturer's protocol and Ki-67 labelling index was assessed.

Reporting of Ki-67 for cervical tissue[11]

The sections stained for Ki-67 were observed under light microscope with a magnification of 400X, immunopositivity was considered when there was strong brown to black color nuclear staining. The slides were first assessed for staining in lower third (basal and parabasal layer) of cervical epithelium, which is a normal finding and act as an internal positive control. Ki-67 staining is considered positive when staining occur in the upper two-third of the epithelium.

Grading of Ki-67 Expression[12]: The sections stained for Ki-67 proliferation (revealed as nuclear staining) was graded as:

- High Grade : >50% positive cells
- Moderate Grade : 30%-50% positive cells
- Low Grade : 10-30% positive cells.

Calculation of Ki-67 Labelling Index[11]: Ki-67 labelling index (LI) was calculated by the number of cells showing positive staining per 100 cervical epithelial cells in separate representative areas of tumour and the mean was calculated. Ki-67 labelling index was calculated as follows:

$$\text{Labelling index} = \frac{\text{No. of cells showing positive staining}}{\text{Total No of cells}} \times 100$$

Apoptotic Index (AI):[13] The H and E sections were examined using a'40x'objective. From each section four areas devoid of any preservation or fixation artefact were selected. AI was measured in haematoxylin and eosin stained slides by calculating percentage of apoptotic cells and apoptotic bodies from atleast 1000 tumor cells in each case at high magnification (400X).

STATISTICAL ANALYSIS

Samples were categorised into premalignant and malignant groups. Premalignant lesions are again subdivided into C.I.N-1, C.I.N-2 and C.I.N-3. Malignant lesions were subdivided into W.D.S.C.C, M.D.S.C.C, P.D.S.C.C AND OTHER TYPES. Mean and standard deviation of Ki-67 labelling index and apoptotic index were calculated for all the subgroups. MS Excel 2010 was used for data analysis. Statistical significance among premalignant and malignant groups and also between their subgroups were assessed using Student t-test with $P<0.05$ being significant.

RESULTS

57 cases of cervical lesions were studied during this study period. 21 were operatively resected specimens and 36 were colposcopically obtained punch biopsy specimens. Samples consisted of 19 CIN cases (8 cases of CIN-1, 6 cases of CIN-2 and 5 cases of CIN-3), and 38 carcinoma cases (14 cases of W.D.S.C.C, 16 cases of M.D.S.C.C, 5 cases of P.D.S.C.C, 1 case of Adenocarcinoma, 2 cases of adenosquamous carcinoma).

Most common chief complaint was abnormal vaginal bleeding/ post-menopausal bleeding (84.21%) followed by post coital bleeding (40.35%), vaginal discharge (63.16%), pelvic pain (19.30%) and pain during coitus (14.04%).

Out of total 19 cases of CIN, age ranged from 30–80 years with a mean age of 47.74 years. Highest incidence was noted in the age group of 40-49 years followed by age group of 50-59 years. The youngest patient had age of 32 years, and the oldest patient was 80 years old.

Out of total 38 cases of carcinomas, age ranged from 30–80 years with a mean age of 48.45 years. Highest incidence was noted in the age group of 40–49 years followed by age group of 50–59 years. The youngest patient had age of 34 years, and the oldest patient was 71 years old.

In our study most CIN cases (73.68%) had low grade expression of Ki-67 and most carcinoma cases (76.32%) had high grade expression of Ki-67.

Mean and standard deviation of Ki–67 labelling index for CIN–1, CIN–2, CIN–3 were 11.38 ± 1.85, 17.83 ± 5.91 and 38 ± 8.25 respectively. The mean LI increased with increasing grades of CIN, statistical comparison between groups showed highly significant difference among different grades of CIN ($P<0.0001$). Mean and standard deviation of Ki–67 labelling index for WDSCC, MDSCC, PDSCC and other cancers were 60.21 ± 11.99, 57.94 ± 14.68, 72.60 ± 5.03 and 66.33 ± 11.59 respectively. Comparison of Ki–67 LI between different groups of carcinoma did not show statistically significant difference ($p>0.05$). Statistical comparison of Ki-67 LI between CIN-2 and CIN-3, CIN-1 and CIN-3; and between CIN-1, CIN-2, CIN-3 and carcinomas showed significant difference ($P<0.05$), though comparison between CIN-1 and CIN-2 LI values did not show significant difference ($P<0.05$) (Figure 1-5).

Overall Mean and standard deviation of Ki-67 LI for CIN and carcinoma groups was 20.74 ± 12.66 and 61.37 ± 13.13 respectively, statistical comparison between CIN and carcinoma was found to be highly significant ($P<0.0001$).

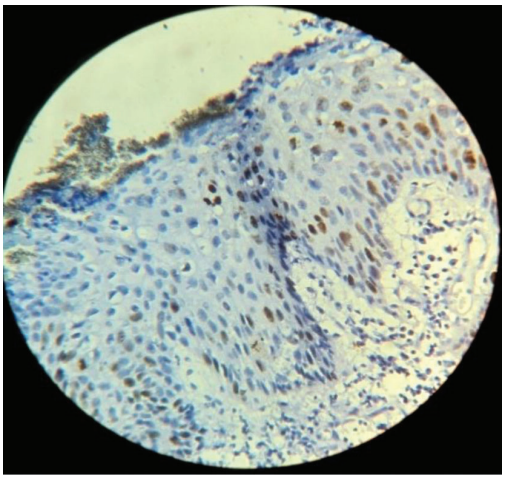

Figure-1: CIN–1 (Ki–67, X400)

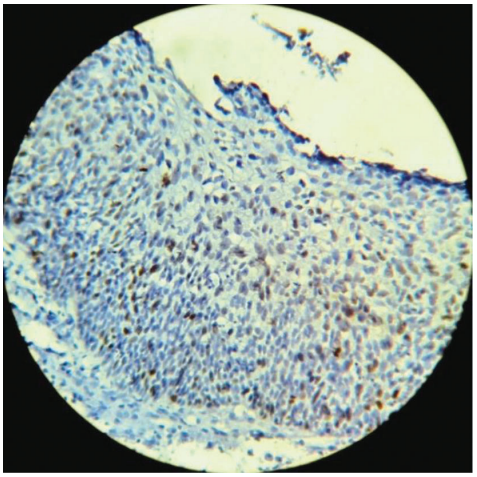

Figure-2: CIN–2 (Ki–67/X400)

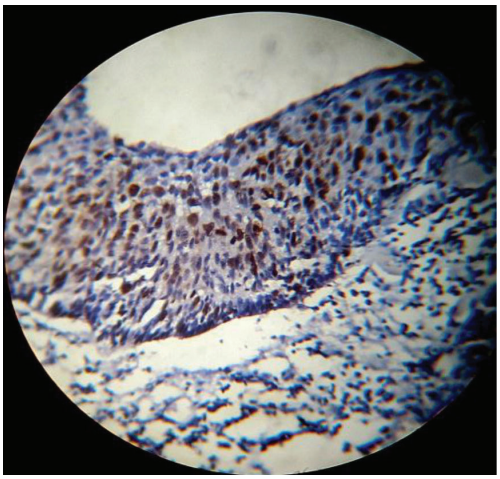

Figure-3: CIN–3 (Ki–67/X400)

Mean and standard deviation of apoptotic index for CIN–1, CIN–2, CIN–3 were 0.44 ± 0.21, 1 ± 0.38 and 2.44 ± 0.48 respectively. The mean AI increased with increasing grades of CIN, statistical comparison between groups showed highly significant (P<0.0001) difference among different grades of CIN. Mean apoptotic index of WDSCC, MDSCC, PDSCC and other cancers were 3.71 ± 0.89, 3.62 ± 0.86, 3.94 ± 0.34 and 4.7 ± 0.50 respectively. Comparison of apoptotic index between different groups of carcinoma did not show statistically

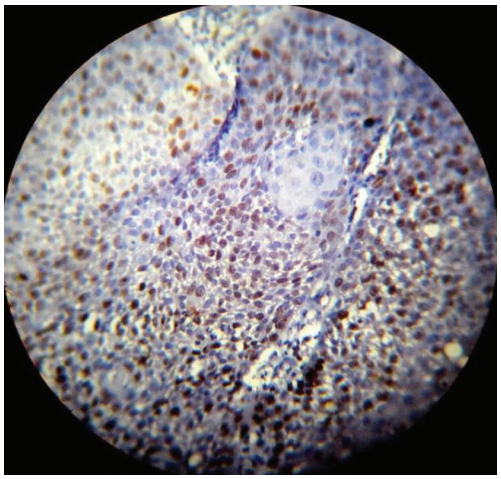

Figure-4: SCC (Ki–67/X400)

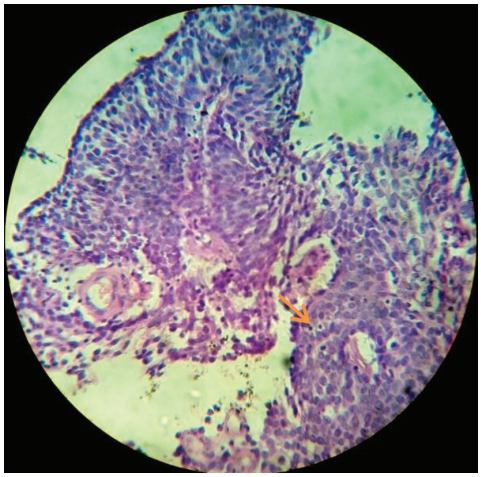

Figure-5: SCC showing apoptotic cells

significant difference (P>0.05). Statistical comparison of Ki-67 LI between CIN-1 and CIN-2; CIN-2 and CIN-3 and also between CIN-3 and carcinomas showed significant difference (P<0.05).

Overall Mean and standard deviation of apoptotic index for CIN and carcinoma were 1.14 ± 0.90 and 3.78 ± 0.83 respectively, statistical comparison between CIN and carcinoma was found to be highly significant (P<0.0001).

DISCUSSION

Almost all of the invasive cervical cancers are preceded by CIN[14] and persistent infections with high risk HPV lead to progression of CIN to invasive cancer, but only detection of HPV is not sufficient to assess the risk of cervical cancer since it is a common finding in reproductive age group females and progression to cervical cancer will occur in just a minor proportion of infected women. Therefore early identification of infected patients who may progress to cancer is needed, so detection of CIN–1 is the most suitable method to detect risk of cervical cancer.

In Pap smear test early recognition of CIN–1 is difficult because often precancerous lesions and carcinomas are missed in Pap smear due to either sampling problems or to diagnostic problems due to inter and intra observer variation in reporting.

Inspite of well described criteria, high rates of interobserver variability occur in histopathologic diagnosis of cervical

neoplastic lesions.[15] Additional methods using biomarkers are essential to attain more accurate results. Cell proliferation marker like Ki-67 is effective for confirmation of the diagnosis in equivocal cases and CIN grading.[14,16]

Apoptosis is accepted as a physiological process for controlling cell death and its disturbance is thought to cause carcinogenesis. Many studies are available that have assessed apoptosis by light microscopy in different types of human tumours, but few such studies are available from India, which have established the diagnostic significance of apoptosis in neoplastic lesions of uterine cervix.

The findings of the present study were recorded and compared with the observations made by some previous workers.

Cell proliferation marker like Ki-67 is strongly associated with tumor cell proliferation and growth and significantly higher in malignant tissue than in normal tissue. In a study done by Gupta K et al out of 20 cases of dysplasias, 16 (80%) showed low grade expression, 3 (15%) showed moderate grade expression and 1 (5%) high grade expression. All cases of CIN-1 and CIN-2 had low grade expression. All cases of CIN-3 had moderate grade expression (3cases) except 1 case which showed high grade expression.[17]

In a study done by Zhong P. et al most cases of CIN-1 (75%) showed low grade expression, CIN-2 cases showed both moderate grade and high grade expression and most cases of CIN-3 showed high grade expression.[18] The increasing Ki-67 grades with increasing severity of dysplasia from CIN-1 to CIN-3 in the above mentioned studies done by Gupta K. et al and Zhong P. et al is almost similar to findings of our study.[17,18]

Gupta K et al studied 26 cases of SCC, of which 19 (73.1%) showed moderate and 7 (26.9%) showed high proliferation. Out of 6 cases of WDSCC, 4 cases showed high grade expression and remaining 2 cases showed moderate grade expression. All 17 cases of MDSCC showed moderate grade expression. All 3 cases of PDSCC showed high grade expression.[17]

In a study done by Zhong P. et al, out of 22 cases of SCC, 19 cases showed high grade expression and only 3 showed moderate grade expression.[18] High grade Ki-67 expression by majority of cervical carcinomas found in above mentioned studies done by Gupta K. et al and Zhong P. et al is almost similar to findings of our study.[17,18]

In a study done by Gupta K. et al, mean LI increased with increasing degree of dysplasia, from CIN-I (5.54 ± 2.185); to CIN-II (18.9 ± 2.491); to CIN-III (42.5 ± 7.937), and p value amongst these groups was found to be statistically significant. The mean LI found in WD SCC was 55.333 ± 7.789; MD SCC was 43.976 ± 3.152 and in PD SCC was 80 ± 5. The mean value of LI increased as the lesion progressed from dysplasia (16.94 ± 14.871) to SCC (50.754 ± 12.625) and the alteration was statistically significant (P <0.001).[17]

In a study done by Mehrotra A, Goel MM, the LI increased with increasing severity of intraepithelial neoplasia to the carcinoma group. Mean labeling index of SCC (31.40±15.68) and non-SCC group (37.69± 11.54) was higher than that of CIN group (8.23± 6.17) and normal epithelium (1.44 ±1.06).In case of dysplasia CIN-III cases present maximum labeling index as compared to other CIN lesions. Statistical analysis showed that Ki-67 LI was significantly higher in diseased group as compared to normal group (P<0.0001 for all the groups).[11]

Isacson et al investigated proliferation and apoptosis in cervical neoplasia cases consisting of 19 LSIL, 11 HSIL and 8 SCC, Ki-67 index increased progressively from LSIL (20±1.9) to HSIL (60.4±2.2) to SCC (69.1±2.8) and the difference was found to be statistically significant (p value <0.05).[19] 24 patients diagnosed with SIL were studied by Simionescu C et al, Ki67 immunoexpression increased with severity of lesions. The Ki-67 proliferation index varied widely (3–22%) in LSIL lesion, in HSIL lesions with atypical mitosis the proliferation index had values more than 42%.[20]

In a study done by Tan GC et al, thirteen of the 25 (52%) premalignant (CIN 3) cases were positive for Ki-67 protein. In contrast, 34 of the 36 (94.4%) malignant (SCC) cases were positive. The average percentages of Ki-67 expression were 12% and 64.9% for CIN 3 and SCC respectively. The difference of Ki-67 protein expression between CIN 3 and SCC was statistically significant (p value <0.0001).[21]

In all the above mentioned studies there is a uniform interpretation that Ki-67 LI increases with increasing grades of CIN and also increases in progression from CIN to carcinoma which is statistically significant (p<0.05), the findings are similar to our study.

In a study done by Bhardwaj S and Wani FA, the mean AI and standard deviation of premalignant group of lesions were: mild dysplasia- 0.62 ± 0.6, moderate dysplasia- 1 ± 0.44 and severe dysplasia- 1 ± 0.6; and for malignant group of tumours, i.e., keratinizing squamous cell carcinoma- 3.38 ± 1.15, non-keratinizing squamous cell carcinoma- 2.92 ±1.41. Statistically significant difference with a p value of <0.0001 was noted between premalignant and malignant group of lesions.[22]

In a study done by Dey P.et al, there was an increase in mean apoptotic index with greater degree of dysplasia i.e. from CIN-1 (0.89±0.35); to CIN-2 (1.59±0.69); to CIN-3 (1.60±0.76); to carcinoma (3.36±1.59). Statistical comparison between CIN group and carcinoma showed a highly significant difference with a p value of <0.0001.[23] In a study done by Nam et al., the apoptotic index (AI) significantly increased (P < 0.001) as the grade of cervical neoplasia increased: 0.06 ± 0.02% (mean ± SD) in normal cervical epithelium, 0.17 ± 0.05% in LSIL, 1.44 ± 0.93% in HSIL, and 3.54 ± 0.79% in squamous cell carcinomas, respectively[24]

Shoji et al. investigated 46 cases of CIN-1 and CIN-2, 75 cases of CIN-3, 16 cases of MIC, and 44 cases of SCC, apoptotic index (AI) increased as the grade of cervical neoplasia increased: mean and standard deviation were 0.26 ±0 56 in CIN1/ CIN2, 0.82±1.43 in CIN3, 1.81 ±1.58 in MIC and 4.65±2.95 in squamous cell carcinomas respectively, the difference being statistically significant between all groups.[25] In a study done by Mysorekar VV et al, there was an increase in mean apoptotic index with greater degree of dysplasia i.e. from CIN-1 (2.13±1.06); to CIN-2 (2.60±1.61); to CIN-3 (3.15±1.62); to invasive SCC (4.49±2.23). The AI values were found to be significant (P<0.01) among all groups.[13]

All above mentioned studies have almost comparable AI values as in present study and there is a progressive increase of AI with progression of cervical neoplasia from CIN to invasive carcinoma which is statistically significant (P<0.05), the findings are similar to our study.

CONCLUSION

We conclude that both biomarkers have potential to reliably distinguish cervical intraepithelial neoplasia from invasive cervical neoplasia and can be accepted as a diagnostic tool in premalignant and malignant lesions of uterine cervix. These will help to plan the management in cases of cervical lesions and to determine the prognosis. Moreover these techniques are simple and inexpensive in comparison to HPVDNA test and can be easily done in formalin fixed paraffin embedded tissues in a clinical laboratory.

However, a much larger study needs to be done over a longer period of time with interlaboratory standardization to truly determine the value of the biomarkers as a diagnostic and prognostic tool in cervical neoplastic lesions.

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