Original Research Article

Clinicopathological study of premalignant and malignant lesions of cervix along with apoptotic index and Ki-67 expression

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ABSTRACT

Background: Cervical cancer is known to have a good response to radiotherapy. The response and prognosis are dependent on the level of apoptosis. Pap smear and histopathology are cost-effective methods in diagnosing premalignant and malignant lesions of cervix but not accurate in classifying and estimating the progression of the disease, especially in premalignant lesions. Therefore this study was undertaken to know the role of Ki-67 expression and apoptotic index in classifying accurately the premalignant lesions for better management.

Methods: The study included 540 cases diagnosed histologically as cervical intraepithelial neoplasia or carcinoma. The apoptotic index is calculated for all the 540 cases using light microscopy on Haematoxylin and Eosin stained sections. Ki-67 immunohistochemical staining was done for 100 cervical biopsies. Ki-67 expression was graded and the Ki-67 labelling index was calculated. Statistical evaluation was done using the unpaired t-test.

Results: The Apoptotic index increased with increasing grade of dysplasia. There is a significant difference in the mean apoptotic index between premalignant and malignant lesions of the cervix. The ki-67 index increased with increasing grade of dysplasia. There is a significant difference in the mean Ki-67 index between premalignant and malignant lesions of the cervix.

Conclusions: Apoptotic index and proliferative indices have been found useful in distinguishing between premalignant and malignant lesions of the cervix and gives an idea about the proliferative activity of the tumour for better management of the patient and to determine prognosis.

Keywords: Apoptotic index, Cervical intraepithelial neoplasia, Ki-67 labelling index.

INTRODUCTION

In India due to poor socioeconomic status, new cervical cancer cases are diagnosed annually and an increase in cervical cancer deaths.[1] Cervical screening programs by Pap smears, detect cervical precancerous lesions at an early stage. The gold standard for the diagnosis of cervical intraepithelial lesions is by histopathological examination. The diagnosis of cervical intraepithelial lesions is subjective and based on personal experience.

Pathologists show significant interobserver variability in classifying CIN and in distinguishing between reactive squamous proliferation and CIN grade.[2]

Proliferative markers and apoptotic indices have emerged as a diagnostic tool in premalignant and malignant lesions of uterine cervix.[2] Immunohistochemistry (IHC) or biomarker increases the accuracy and reproducibility of grading CIN and hence standardization of diagnosis.[3] A biomarker like Ki-67 is useful in identifying the various

grades of dysplasia and helps in disease progression.[4] There are many studies available in the English literature that have evaluated the apoptotic index in high-grade dysplasias and malignancy by light microscopy but there are very few studies available in India.

Therefore this study was undertaken to assess the importance of Ki-67 expression and apoptotic index in sub classifying accurately various grades of dysplasia and malignancy in lesions of the cervix.

Objectives

To study the distribution of various premalignant and malignant lesions of cervix in relevance with age and clinical presentation, to know the role of Apoptotic index in distinguishing various grades of cervical intraepithelial neoplasia and invasive cervical carcinoma and to know the Ki-67 expression as a proliferation marker in premalignant and malignant lesions of cervix.

METHODS

Study design

It is an observational study.

Study period and sample size

2 years from July 2017 to June 2019 at the department of pathology, Andhra Medical College, India. Sample size is taken to be 540. Inclusion criteria were all the specimens with a histopathological diagnosis of cervical intra-epithelial neoplasia and carcinoma cervix during the study period were included in the study. Exclusion criteria were inconclusive and inadequate cervical biopsies.

Methodology

A detailed clinical history was recorded as per the protocol. The tissues were subjected to routine paraffin-embedded processing and stained with haematoxylin & eosin.

The apoptotic index was measured in hematoxylin & eosin stained slides by calculating percent of apoptotic cells and apoptotic bodies from 1000 tumor cells at high magnification (400x).[5] Ki-67 Immunohistochemistry staining was done randomly on 100 cervical biopsies diagnosed histologically as CIN or cervical carcinoma. The Ki-67 stain was done using a poly excel HRP/DAB Detection system. Ki-67 expression and Ki-67 labeling index is calculated.

Grading of Ki-67 expression

Sections stained for Ki-67 proliferation (nuclear stain) were evaluated using scores from 1to3.[6] Score 1 was given for +++ high proliferation >50% positive cells,

score 2 was given for ++ moderate proliferation 30-50% positive cells and score 3 was given for + Low proliferation 10-30% positive cells.

Calculation of Ki-67 labelling index

MIB-1 labelling index (LI) was calculated by the number of positive cells per 100 cervical epithelial cells in different areas under 40X objective and mean calculated.[7]

LI = No. of cells showing positive staining x 100 ÷ Total no. of tumor cells counted

Statistical analysis

Data for apoptotic index and Ki-67 proliferative index was expressed as mean and an unpaired t-test to find the statistical difference of means between premalignant and malignant lesions.

RESULTS

540 cases of cervical lesions were studied during this 2 year study period. 494 were cervical punch biopsy specimens and 46 were hysterectomy specimens. In the present study number of premalignant lesions encountered are 282 cases which accounts for 52.23% and malignant lesions are 258cases which accounts for 47.77%. Among the premalignant group most common is CIN 1 which accounts for 192 cases (35.5%) followed by CIN 2:58 cases (10.5%) and CIN 3:32 cases (6.11%). Among the malignant group of lesions most common is non-keratinizing SCC which accounts for 212 cases (39.25%) followed by keratinizing SCC: 31 cases (5.92%), papillary SCC: 5 cases (0.92%), adenocarcinoma: 9 cases (1.66%) and adenosquamous: 1case (0.185%) (Table 1).

Table 1: Histological subtypes of premalignant and malignant cervical lesions.

Category (n=540)	N (%)
Squamous intraepithelial lesions (premalignant lesions)	282 (52.2)
CIN-1 (LSIL)	192 (35.5)
CIN-2 (HSIL)	58 (10.5)
CIN-3 (HSIL)	32 (6.11)
Malignant lesions	258 (47.77)
Squamous cell carcinoma (SCC)	248 (45.92)
Keratinizing SCC	31 (5.92)
Non keratinizing SCC	212 (39.25)
Papillary SCC	5 (0.92)
Adenocarcinoma	9 (1.66)
Adenocarcinoma NOS	5 (0.92)
Villoglandular adenocarcinoma	2 (0.37)
Endocervical adenocarcinoma	1 (0.185)
Serous carcinoma	1 (0.185)
Adenosquamous	1 (0.185)
Total	540 (100)

The premalignant lesions were found between age group ranging from 22 to 72 years. The mean age for premalignant lesions was found to be 42.85±10 years. Range (32.85 to 52.85) years. The malignant lesions were found between age group ranging 30 to 85 years. The mean age for malignant lesions was found to be 52.77±10 years. Range (42.77 to 62.77 years).

A most common symptom in cervical dysplasias is white discharge and heavy menstrual bleeding, whereas in carcinomas postmenopausal bleeding is a common symptom.

The apoptotic index was done for all 540 cases. There was an increase in mean Apoptotic index with increasing grade of dysplasia i.e. from CIN-1 (0.221±0.1115) to CIN-2(0.391±0.1490) to CIN-3 (0.688±0.1431) and the difference in mean values between CIN-1 and CIN-2, CIN-2 and CIN-3, CIN-1 and CIN-3 were found to be statistically significant, (p<0.001) (Table 2).

Table 2: Correlation of apoptotic index with grades of cervical dysplasias and carcinomas.

Category, (n=540)	N	Mean AI (%)±SD	Range	P value
Squamous intraepithelial neoplasia, (premalignant lesions)				
CIN-1	192	0.221±0.1115	0-0.4	<0.001
CIN-2	58	0.391±0.1490	0.1-0.7	
CIN-3	32	0.688±0.1431	0.4-0.9	
Carcinomas				
Keratinizing SCC	31	1.296±0.151	0.8-1.3	0.9
Nonkeratinizing SCC	212	1.304±0.4613	1.1-2.0	
Papillary SCC	5	0.7±0.10	0.6-0.8	
Adenocarcinoma	9	0.83±0.023	0.8-0.9	

An increase in apoptotic index (Figure 1) was observed from keratinizing squamous cell carcinoma (1.296±0.151) to Non-keratinizing squamous cell carcinoma (1.304±0.4613) but no statistical significance was observed on correlation. The mean Apoptotic index for adenocarcinoma is (0.83±0.023). One case of adenosquamous carcinoma shows Apoptotic index of 1.2. Overall mean Apoptotic index for premalignant lesions is 0.311±0.1966 and for malignant lesions is 1.28±0.4290. Statistical comparison between premalignant and malignant lesions was found to be highly significant (p<0.001) (Table 3).
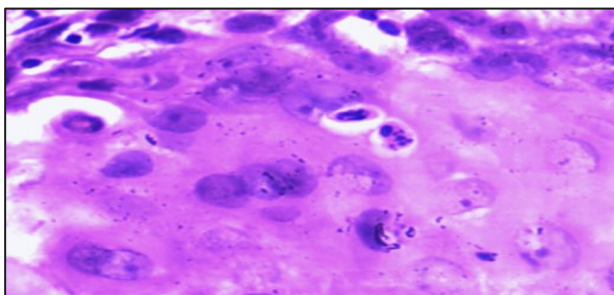
Figure 1: Photomicrograph showing apoptotic cell with deep eosinophilia of cytoplasm and pyknotic, round nucleus seen in keratinising squamous cell carcinoma.

In this study 100 cases of cervical dysplasias and carcinoma were subjected to immunohistochemical staining for Ki-67. It comprised of 45 cases of cervical dysplasia and 55 cases of carcinoma.

Table 3: Comparision of apoptotic index between premalignant and malignant lesions.

Type of lesions	Over all mean±SD of AI	P value
Premalignant lesions	0.311±0.1966	0.001
Carcinomas	1.28±0.4290.	

Out of 45 cases of cervical dysplasias 30 cases (66.67%) and 13 cases (33.33%) showed low and moderate proliferation and out of 55 cases of cervical carcinoma 35 cases (63.37%) showed moderate proliferation and 20 (36.33%) cases showed high proliferation (Table 4).

Table 4: Correlation of Ki-67 expression with grades of cervical dysplasias and carcinomas.

Category (n=100)	N	Low	Moderate	High
Premalignant lesions	45	30	15	-
CIN-1	15	15	-	-
CIN-2	15	15	-	-
CIN-3	15	-	15	-
Carcinomas	55	-	35	20
Keratinizing SCC	18	-	3	15
Nonkeratinizing SCC	30	-	28	2
Papillary SCC	2	-	2	-
Adenocarcinoma	4	-	2	2
Adenosquamous	1	-	-	1

There was an increase in mean Ki-67 labelling index with increasing grade of dysplasia, from CIN-1 (6.25±2.04) to CIN-2 (19.75±4.13) to CIN-3(34.81±3.27), and the p

value is found statistically significant among all the groups (p<0.0001).

The mean labelling index and standard deviation in keratinizing SCC (56.05±4.05), non-keratinizing SCC is (46.133±2.34), papillary SCC is (47.5±1), adeno-carcinoma is (50±1.58). One case of adenosquamous show labelling index of 51%. Correlation between keratinizing SCC and non-keratinizing SCC was found to be statistically significant (Table 5).

Table 5: Correlation of Ki-67 index with grades of cervical dysplasias and carcinoma.

Category (n=100)	N	Mean LI (%)±SD	Range	P value
Squamous intraepithelial neoplasia (Premalignant lesions)	**45**			
CIN-1	15	6.25±2.04	2-9	<0.001
CIN-2	15	19.75±4.13	13-27	
CIN-3	15	34.81±3.27	30-42	
Carcinomas	**55**			
Keratinizing SCC	18	56.05±4.05	47-61	<0.001
Nonkeratinizing SCC	30	46.133±2.34	42-52	
Papillary SCC	2	47.5±1	47-48	
Adenocarcinoma	4	50±1.58	48-52	
Adenosquamous	1	51	51	

The overall mean value of Ki-67 labelling index increased as the nature of the lesion progressed from dysplasia (20±12.03) to carcinoma (50±6.02). An unpaired t-test was done to find the statistical difference of means of Ki-67 labelling index which was found to be highly statistically significant (p=0.0001) (Table 6).

Table 6: Comparision of Ki-67 index between premalignant and malignant lesions.

Type of lesions	Mean value of Ki-67 Index	P value
Premalignant lesions	20±12.03	<0.0001
Carcinomas	50±6.02	

In this study 100 cases of cervical dysplasias and carcinoma were subjected to immunohistochemical staining for Ki-67. It comprised of 45 cases of cervical dysplasia and 55 cases of carcinoma.

Out of 45 cases of cervical dysplasias 30 cases (66.67%) showed low proliferation, 13 cases (33.33%) showed moderate proliferation and out of 55 cases of cervical carcinoma 35 cases (63.37%) show moderate proliferation and 20 cases (36.33%) cases show high proliferation (Table 4).

There was increase in mean Ki-67 labelling index with increasing grade of dysplasia, from CIN-1 (6.25±2.04) (Figure 2) to CIN-2 (19.75±4.13) (Figure 3) to CIN-3 (34.81±3.27) (Figure 4) and p value is found statistically significant among all the groups (p<0.0001).

The mean labelling index and standard deviation in keratinizing SCC (56.05±4.05), (Figure 5) non-keratinizing SCC is (46.133±2.34) (Figure 6), papillary SCC is (47.5±1), adenocarcinoma is (50±1.58) (Figure 7).

One case of adenosquamous show labelling index of 51%. Correlation between keratinizing SCC and non-keratinizing SCC was found to be statistically significant (Table 5).

The overall mean value of Ki-67 labelling index increased as the nature of the lesion progressed from dysplasia (20±12.03) to carcinoma (50±6.02). Unpaired test was done to find the statistical difference of means of Ki-67 labelling index which was found to be highly statistically significant (p=0.0001) (Table 6).
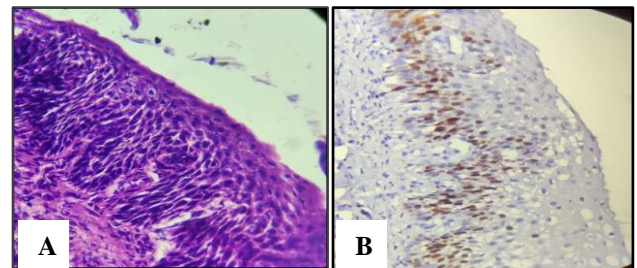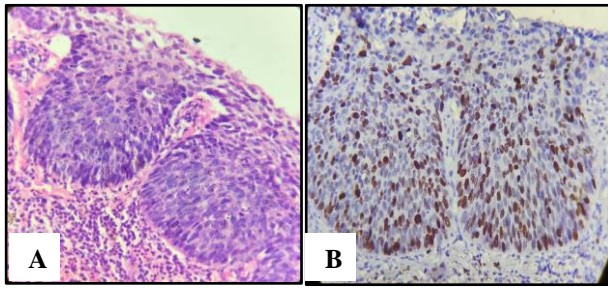

Figure 2: A) Mild dysplasia B) Mild dysplasia Ki-67 labelling index of 9% IHC.
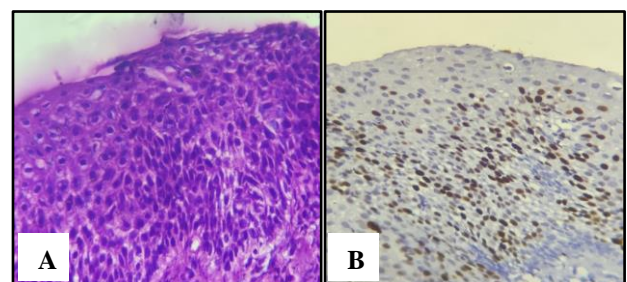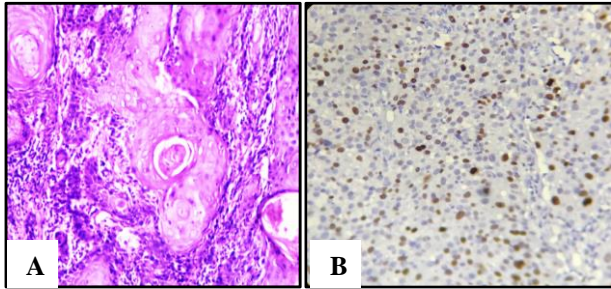

Figure 3: A) Moderate dysplasia B) Moderate dysplasia Ki-67 labelling index of 27% IHC.
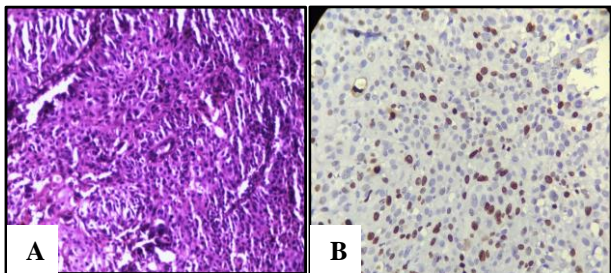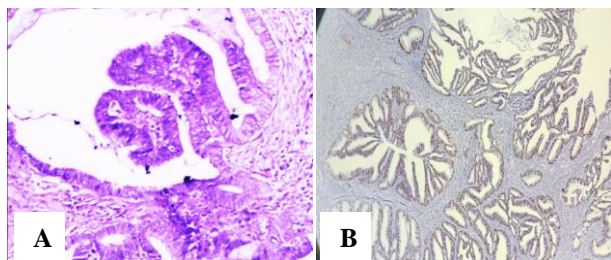
Figure 4: A) Severe dysplasia (B) severe dysplasia Ki-67 labelling index of 39% IHC.

Figure 5: A) Keratinizing squamous cell carcinoma (B) Keratinizing squamous cell carcinoma Ki-67 labelling index of 51% IHC.

Figure 6: (A) Non Keratinizing SCC (B) non Keratinizing SCC Ki-67 labelling index of 47% IHC.

Figure 7: A) Adenocarcinoma B) Adenocarcinoma Ki-67 labelling index of 52% IHC.

DISCUSSION

The biologic behaviour of the tumour depends not only on its proliferative activity index but also on the number of cells dying by apoptosis.[8] Almost all invasive cervical cancers are preceded by dysplasia and infection with high risk HPV.[9] Early identification of CIN-1 is the most suitable method to detect the risk of cervical cancer.[5]

In the present study, most common age group involved in premalignant lesions is 30-40 years which is correlated with studies done by Chauhan et al, Okwi et al.[9,10] Most common age group involved in carcinomas is 51-60 years which is correlated with Jain et al and Chauhan et al which implies that there is a long preinvasive stage.[11]

In spite of well described criteria high rates of interobserver variability occur in the histopathologic diagnosis of cervical neoplastic lesions.[12] Additional methods using biomarkers are essential to obtain more accurate results.

Ki-67 is a protein-related to cell proliferation and is expressed in cell nuclei throughout the entire cell cycle except G0. Ki-67 is present during all active phases of the cell cycle, but is absent from resting cells, which makes it an excellent marker for determining the so-called growth fraction of a given cell population.[13] The expression of Ki-67 starts from the beginning of the S phase, peaks at mitosis, and decreases during the G1 phase.[14] Under normal physiological conditions, its expression is limited in basal layer squamous epithelium of the uterine cervix.[15] Cell proliferation marker like Ki-67 is effective for confirmation of the diagnosis in equivocal cases and CIN grading. In the present study Ki-67 expression increased from CIN 1 to CIN 2 to CIN 3.

In a study done by Gupta et al out of 20 cases of dysplasias, 80% showed low-grade expression, 15% showed moderate grade expression and 5% showed high grade expression.[6] All cases of CIN-1 and CIN2 had a low grade expression. All cases of CIN-3 had moderate grade expression except 1 case which showed high-grade expression, almost similar to the findings of the present study in which all cases of CIN-1 and CIN-2 showed low Ki-67 expression and CIN 3 moderate Ki-67 expression.

In the study by Gupta et al, out of 26 cases of squamous cell carcinoma 73.1% showed moderate and 26.9% showed high proliferation which is almost similar to the present study in which out of 50 cases of invasive squamous cell carcinoma 66% showed moderate Ki-67 expression and 34% showed high Ki-67 expression. In the present study Ki-67 index increased with increasing grade of dysplasia.[6] In the present study, there was an increase in mean labelling index with increasing grade of dysplasia, from CIN-1 (6.25±2.04) to CIN-2 (19.75±4.13) to CIN-3 (34.81±3.27) and the p value was found to be statistically significant (p<0.0001) which is similar to Gogoi et al study, Chauhan et al and Gupta et al.[5,6,8]

In the present study the mean labelling index and standard deviation in keratinizing SCC (56.05±4.05), non-keratinizing SCC is (46.133±2.34). Correlation between keratinizing SCC and non-keratinizing SCC was found to be statistically significant, (p<0.05) which

correlated with Gupta et al and Chauhan et al.[6,8] In the study by Gogoi et al did not show any statistically significant difference.[5] In the present study there is a significant difference of mean Ki-67 labelling index between premalignant and malignant lesions of the cervix which is similar to studies done by Gogoi et al, Gupta et al and Chauhan R et al.[5,6,8]

Apoptosis is genetically regulated and it permits the elimination of damaged cells.[4] Relationship of Apoptosis with tumour growth and progression has been studied by several authors and apoptotic index is included among parameters used to measure tumour growth in various malignancies including cervical carcinoma. We observed that the Apoptotic index increased with increasing grade of dysplasia which is similar to the studies done by Gogoi et al, Mysorekar et al.[6,16]

In the present study, there is no statistical significance of mean apoptotic index within the carcinoma groups which is correlated with Gupta et al and Gogoi et al.[5,6] Statistical comparison between premalignant and malignant lesions was found to be highly significant (p<0.001) which was similar to studies done by Gogoi et al, Gupta et al, Bharadwaj et al, Dey et al and Mysorekar et al.[5,6,16-18]

CONCLUSION

Apoptotic index and proliferative indices have been found useful in distinguishing between premalignant and malignant lesions of cervix and gives an idea about proliferative activity of the tumour for better management of patients and to determine prognosis. In the present study the Apoptotic index increased with increasing grade of dysplasia. There is significant difference of mean apoptotic index between premalignant and malignant lesions of cervix. Ki-67 index increased with increasing grade of dysplasia. There is a significant difference of mean Ki-67 labelling index between premalignant and malignant lesions of cervix.

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