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Original Research Paper

Ki-67 Expression in Squamous Intraepithelial lesions and Carcinoma cervix by Immunohistochemistry

Medical Science

KEYWORDS : Ki-67 Antigen, Immunohistochemistry, Squamous intraepithelial lesions, CA cervix.

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ABSTRACT

***Aim**: Carcinoma of the cervix is one of the most common malignancies in women. Uncontrolled cell proliferation and malignant transformation are the basic elements in the development of cancer. The Ki-67 antigen detects cells in all active phases of the cell cycle and has been used as an indicator of Squamous intraepithelial lesions(SIL) The main aim is to study the proliferative activity by using the Ki-67 proliferative marker in Squamous intraepithelial lesions and carcinoma of the uterine cervix by immunohistochemistry. **Materials and methods:** A total of 50 cervical specimens were collected of which 15 cases were diagnosed as Squamous cell carcinoma of Cervix (CA cervix), 15 cases were diagnosed as High Grade Squamous Intraepithelial Lesion (HSIL), 15 cases as Low Grade Squamous Intraepithelial Lesion (LSIL) and 5 cases of normal cervix. Hematoxylin-Eosin slides of all the tissues were evaluated. The formalin fixed, paraffin-embedded blocks were sliced in 3 – 4 μm thickness for IHC. The Avidin Biotin Complex (ABC) detection system was used. Immunoreactivity was regarded as positive when dark brown, homogenous or punctate staining was localized and limited exclusively in the nucleus of squamous cells. **Results** : The intensity of immunostaining in LSIL, HSIL and CA cervix were evaluated. Ki-67 scoring was graded as 0,1+,2+ &3+. Statistical analysis was performed using 't' test. Statistically significant difference was found between LSIL &HSIL, LSIL and CA cervix ('t' test, p value 0.0001). **Conclusion:** We conclude that Ki – 67 is an important proliferative marker in precancerous and cancerous lesions of cervix. A higher Ki67 expression in increasing grades of CIN(cervical intraepithelial neoplasia) and SCC (squamous cell carcinoma) and its positive correlation emphasizes its role as a strong prognostic factor.*

Introduction

Carcinoma of the cervix is one of the most common malignancies in women. It is estimated to be the second most common cause of cancer in women worldwide. Uncontrolled cell proliferation and malignant transformation are the basic elements in the development of cancer including cervical cancer and its precursors. Better understanding of cell proliferation activity allows a more rational therapeutic approach. Loss of genomic integrity is a defining feature of many human malignancies, including preinvasive / squamous intraepithelial lesions (SIL) / cervical intraepithelial neoplasia (CIN) and invasive cervical squamous cancer lesions.

Antigen Ki-67 also known as Ki-67 is a nuclear protein that in humans is encoded by the MKI 67 gene and is necessary for cellular proliferation. The Ki-67 protein is a cellular marker for proliferation. Furthermore it is associated with ribosomal RNA transcription. Inactivation of antigen Ki-67 leads to inhibition of ribosomal RNA synthesis.

The fraction of Ki-67-positive tumor cells (the Ki-67 labelling index) is often correlated with the clinical course of cancer. The best-studied examples in this context are carcinomas of cervix, the prostate, brain, breast and nephroblastoma .

The Ki-67 antigen detects cells in all active phases of the cell cycle and has been used as an indicator of SIL. Expressed normally in the parabasal cells of mature squamous epithelium, qualitative evaluation of Ki-67 cells involving the upper two-thirds of the epithelium has been reported to have improved specificity in detecting SIL.

The present study was undertaken to study the expression of Ki-67 positive cells in Squamous Intraepithelial Lesions and Carcinoma of the uterine cervix.

Materials and Methods

II a. Experimental Design

This study was conducted at Meenakshi Medical College Hospital and Research Institute, Enathur, Kancheepuram, Tamil Nadu, India. Fifty cases were selected from August 2012 until July 2014, out of which 25 specimens were obtained from dilatattion and curettage, 15 specimens were obtained from total abdominal hysterectomy and 10 specimens were obtained from colposcopy guided cervical biopsy. These specimens were obtained from both out patients and in patients of Meenakshi Medical College Hospital.

Informed consent was obtained from each patient before surgery for the use of cervical tissues for the present study. Each cervical biopsy specimen was examined by a pathologist for histological examination. Histological features of all cases were studied with hematoxylin and eosin.

Inclusion criteria:

All female cases irrespective of age and other physical conditions were selected for study over a period of two years from August 2012 to July 2014 and the paraffin blocks of patients with squamous intra epithelial lesions of cervix and Carcinoma Cervix were subjected to Immunohistochemical study.

Exclusion criteria:

Samples were excluded if histopathological examination showed features suggestive of lesions other than Squamous intraepithelial lesions and Squamous cell carcinoma of cervix.

Immunohistochemical study (IHC) :

Hematoxylin-Eosin slides of all the tissues were evaluated, and for each case, the best paraffin blocks with highest tumor content were chosen in order to prevent artifact staining. These formalin fixed, paraffin-embedded blocks were

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sliced in 3 – 4 μm thickness for IHC. The Avidin Biotin Complex (ABC) detection system was used on specimens of formalin- fixed, paraffin embedded tissue sections.

Scoring system Immunoreactivity was regarded as positive when dark brown, homogenous or punctate staining was localized and limited exclusively in the nucleus of squamous cells. The intensity of immunostaining was evaluated by repeated staining of the same specimens[1].

Grading
(0) :No immunostaining
(1+) :Weak positive immunostaining (positive cells less than 10 %)
(2+):Positive immunostaining (positive cells 10 % to 50%)
(3+):strong positive (positive cells.>50%)

The extent was semi quantitatively estimated with a range of 0% to 100%. Percentages were estimated by counting at least 50 cells and then establishing the ratio of immunoreactive cells to total number of cells multiplied by 100; percentages were rounded to the nearest 10%. When less than 10% of cells were positive a score of 1 was used, 10 % to 50% cell positivity was scored as 2 , more than 50% positive cells was scored as 3 , no immunostaining was labelled as 0[1].

III. Statistical Analysis
Statistical analysis was carried out using SPSS version 19.0 (IBM SPSS, US). Quantitative data were expressed using range, mean, SD and median whereas qualitative data were expressed as frequency and percentage. P value was assumed to be statistically significant at 0.0001.

IV. Ethical Concern
Ethical clearance was obtained from the Ethical committee meeting conducted at Meenakshi Medical College and Research Institute, Kanchipuram, Tamil Nadu, India.

V. Results and Observation
A total of 50 cervical specimens were collected of which 15 cases were diagnosed as Squamous cell carcinoma of Cervix, 15 cases were diagnosed as High Grade Squamous Intraepithelial Lesion (HSIL), 15 cases as Low Grade Squamous Intraepithelial Lesion (LSIL), and 5 cases of normal cervix

Incidence of various types of lesions of cervix

S. No	Types of Lesion	No. of cases (50)	Percentage
1	CA Cervix	15	30%
2	HSIL	15	30%
3	LSIL	15	30%
4	Normal	5	10%

The age incidence in squamous intra epithelial lesion ranged from 23 - 53 years, with the mean age of 39 years. The age incidence for carcinoma cervix ranged from 34-59 years with the mean age of 49 years. SIL and carcinoma of cervix were more common in multiparous women than in nulliparous.

In our study most of the patients presented with abnormal vaginal bleeding(65%) followed by dysmenorrhea(35%) and white discharge. Significant proportion of patients remained asymptomatic (20%). Rare symptoms included urinary disturbances and vaginal mass.

Immunoreactive Ki-67 positive cells showed dark brown, homogenous or punctate staining, limited exclusively to the nucleus. In LSIL three patients (20%) had negative proliferative index and 15 patients showed weak positive (80%). Fig[1a & 1b].

Ki 67 positive proliferative index was found in all the15 patients of HSIL with a score of 2 in 9 cases (60%) and score of 3 in 6 cases (40%). Fig [2a & 2b].

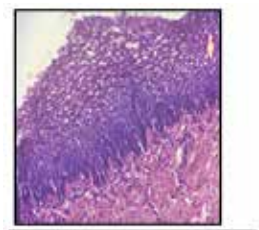


Fig. 1a. Photo micrograph of LSIL (H & E, magnification 400 X)

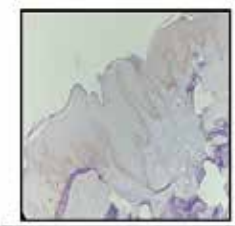


Fig. 1b. Photo micrograph of Ki – 67 expression in LSIL (IHC, 100 X)

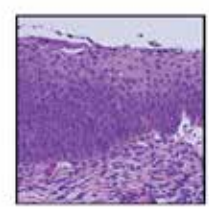


Fig. 2a. Photo micrograph of HSIL (H & E, magnification 100 X)

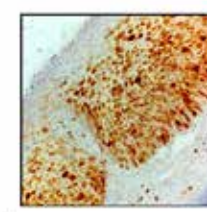


Fig. 2b. Photo micrograph of Ki – 67 expression in HSIL (IHC, 400 x)

Table.1. Incidence of Ki – 67 Expression in LSIL& HSIL

	Ki – 67 score	No. of cases	Percentage
LSIL (15)	0	3	20%
	1	12	80%
HSIL (15)	2	9	60%
	3	6	40%

Ki 67 positive proliferative index was seen in all the 15 cervical carcinoma cases,with a score of 2 in 2 patients (13%) and a score of 3 in 13 patients(87%). [Fig.3a, 3b & 3c].


Fig. 3a. Gross photograph of Squamous cell carcinoma of cervix

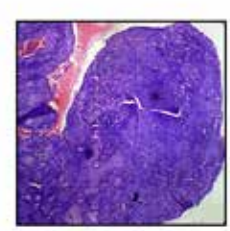


Fig. 3b. Photo micrograph of CA cervix (H & E, magnification 100 X)

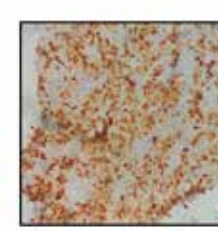


Fig. 3c. Photo micrograph of Ki – 67 expression in CA cervix (IHC, magnification 100 X)

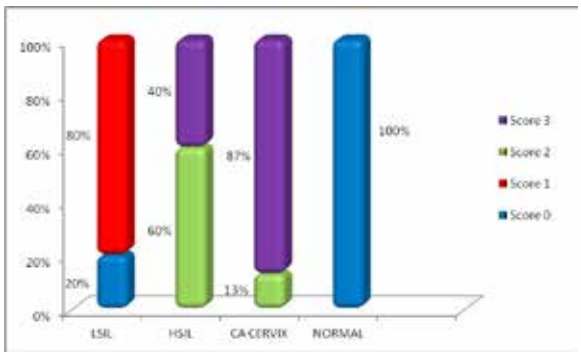
Table.2. Incidence of Ki – 67 Expression in CA Cervix

S. No	Ki – 67 score	No. of cases (15)	Percentage
1	2	2	13%
2	3	13	87%

Ki – 67 score is zero in all normal cervix biopsy specimens.

Table. 3. Ki -67 Scoring in various types of lesions

S. No	Ki – 67 Score	LSIL (15)	HSIL (15)	CA Cervix (15)	Normal **(5)**
1	0	3 (20%)	0	0	5 (100%)
2	1	12 (80%)	0	0	0
3	2	0	9 (60%)	2 (13%)	0
4	3	0	6 (40%)	13 (87%)	0



Statistically significant difference was found between LSIL &HSIL, LSIL and CA cervix ('t' test, p value 0.0001).

We also investigated the distribution of Ki – 67 positive nuclei in the cervical epithelium .In LSIL 12 cases(80%) showed positive in the lower third of the cervical epithelium and 3cases(20%) showed no expression .In HSIL Ki – 67 positive cells are distributed in the lower and Middle third of the cervical epithelial layer in 10 cases(67%) and 5 cases(33%) .showed positivity in Lower, Middle and Upper thirds of cervical epithelium.

In carcinoma cervix Ki – 67 positive cells are distributed in all the Lower, Middle and Upper thirds of cervical epithelium in all 15 cases of carcinoma cervix (100%).

Table 4.Distribution of Ki -67 positive cells in the cervical epithelium

S. No	Cervical Layer	LSIL (15)	HSIL (15)	CA Cervix (15)
1	No expression	3	0	0
2	Lower third	12	0	0
3	Lower & Middle third	0	10	0
4	Lower, Middle & upper third	0	5	15

Discussion

The demographic profile of squamous cell carcinoma of the uterine cervix which is the second most common cancer worldwide and which ranks first in India has been well established by several studies.[2-4].

Squamous Intraepithelial Lesions of cervix are commonly seen in women of child bearing age, mostly in the fourth decade. In our study the average age incidence of the patients with Squamous intraepithelial lesions and carcinoma cervix were 39 years .and 49 years respectively. This correlates well with studies done by Satija A et al[6] and Francheschi S et al[2]. According to these studies, the peak age for SIL incidence in India is 35 – 45 years and cervical cancer incidence in India is 45-54 years, which is similar to the rest of South Asia (WHO Information Centre on HPV and Cervical Cancer) [3,6].

Most of the patients in our study belonged to a lower socioeconomic status with high parity similar to that observed by previous Indian studies.[2,3,5,7].

It is widely accepted that multiparity is associated with increased risk for Cervical Carcinoma and Squamous Intraepithelial Lesions. According to Eluf Neto et al[5], multiparity is believed to be a risk factor for Carcinoma Cervix and SIL, especially among human papilloma virus (HPV)-positive women.

In our study, Cervical carcinoma and SIL were common in multiparous and Para II women, compared to nulliparous and primiparous women. 66% of SIL cases and 73% of Cervical Carcinoma cases were multiparous and Para II. This is in concordance with other studies in literature such as Parazzini et al[8] Munoz et al [9] and Brinton et al [10].

The analysis of various symptoms of CA Cervix and SIL shows that abnormal vaginal bleeding was predominant in 65% of patients followed by dysmenorrhea in 35% and white discharge in 30% of cases. Nearly 20% of patients remained asymptomatic. Rare symptoms included urinary symptoms and mass in vagina. These results are comparable with studies done by Canavan & Sultana et al, [11,12].

Cell proliferation can be followed up by the expression of some cell proteins such as PCNA(proliferating cell nuclear antigen) and Ki-67 antigen. Ki-67 is a nuclear antigen expressed during G1, S, M and G2 periods of cell cycle. The expression level of Ki-67 indicated the status of cell proliferation. Studies have shown that Ki-67 protein could be a biomarker in the evaluation of the proliferative activity and progressive potential of normal, dysplastic and neoplastic changes.

Many studies have shown a significant difference in the expression of Ki67 in normal cervix, squamous intraepithelial lesions and invasive Squamous cell carcinomas of the cervix.

Our study showed that Ki67 expression ranged from negative in normal epithelium to score of 1 to 3 in SIL to score of 2 and 3 in SCC which is in accordance with previous studies[13,14]In our study, Ki -67 score was one (+) in 80% of cases in LSIL, 60 % cases of HSIL and 13% cases of CA Cervix expressed Ki 67 score of two (++). Whereas 87% cases of CA Cervix and 40% cases of HSIL had Ki – 67 score of three (+++).

Sagol O et al [14] showed a significant difference in expression of Ki67 between squamous cell carcinoma of cervix and SIL groups and between low and high grade SIL groups which is concurrent with our study.

Milana Panjkovic et al[1] showed that all cases of CIN1 (LSIL) had negative proliferative index. All CIN II and CIN III (HSIL) cases showed positive Ki – 67 proliferative index. There was significant relation between the proliferative Ki-67 activity and CIN / SIL grade. Statistically significant difference was found between CIN1 (LSIL) and CIN2 (HSIL) group while no significant difference was found between CIN2 and CIN3 (Two subgroups of HSIL).

There was also a progressive increase in the Ki67 index from the adjoining CIN areas to Squamous carcinoma of cervix which highlights the higher proliferation which

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drives the clonal expansion in carcinogenesis.

Several studies have shown significant difference in the Ki67 expression in varying grades of SIL and CA cervix with difference in thickness of epithelium affected. [15,16,17.]

Around 67% cases of HSIL showed Ki- 67 positive cells in lower and middle third and 33% cases of HSIL showed Ki – 67 positive cells throughout the cervical epithelial layer.

All the cases of Carcinoma cervix showed Ki -67 positive cells throughout all the thirds of cervical epithelial layer. These results correlate well with the study done by Isacson et al[13]]and Milana et al[1]

According to Isacson et al study, the Ki67 expression was observed in parabasal, lower third, lower third with middle third and full thickness in normal, CIN I, CIN II and CIN III lesions respectively[13]

Sahebali et al[18] performed Ki-67 immunostaining in cervical cytology samples. There were a significantly higher number of immunopositive cells in high-grade squamous intraepithelial lesions as well as in HPV16 positive samples. The infection of cervical cells by HPV manifests itself by changes in the function or expression of the host genes, and the detection of these alterations can play a role in diagnosis.

These changes cause dysregulation of the cell cycle, manifested by abnormal proteins such as Ki-67. The detection of abnormal expression can identify clinically important cases of HPV infection with risk of progression towards dysplasia and carcinoma[19].

Ki-67 proliferative marker is very important for predicting the progression of LSIL and HSIL lesions but according to recent studies it is not enough. Kruse et al[19]. showed that other markers are also important such as Rb-positive nuclei in the deeper half of the epithelium and the proportions of CK13 and CK14-positive cells Expression of p16 is also an important prognostic factor, which is connected with the expression of Rb-E2F complex and influence of E7 oncoprotein on it.

Conclusion

From our study ,we conclude that Ki – 67 is an important proliferative marker in precancerous and cancerous lesions of cervix .There is a significant positive relation between the Ki – 67 score, Ki – 67 proliferative index, distribution of Ki 67 positive cells and increasing grade of SIL. A higher Ki67 expression in increasing grades of CIN and SCC and its positive correlation emphasizes its role as a strong prognostic factor.

The limitations of our study using immunohistochemical analysis of Ki - 67 alone should be acknowledged. For completion, it may require further validation using other methods including Bcl-2 and p53, molecular and cytogenetic methods for understanding the underlying molecular mechanisms that determine Ki -67 overexpression and other pathways in development of precancerous and cancerous lesions of cervix.

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