

IMMUNOHISTOCHEMICAL STUDY OF INVASIVE SQUAMOUS CELL CARCINOMA OF UTERINE CERVIX AND CERVICAL INTRAEPITHELIAL NEOPLASIA USING Bcl-2, p53 AND Ki-67

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ABSTRACT

BACKGROUND

p53 plays a key role in malignant transformation of cervical epithelium. Bcl-2 is a regulator of apoptosis and is expressed in a variety of cancers. Ki-67 is a proliferation marker.

MATERIALS AND METHODS

Retrospective study of 98 ISCC of uterine cervix and 45 CIN cases was undertaken. Archived tissue blocks were retrieved and subjected to Immunohistochemistry (IHC) for p53, Bcl-2 and Ki-67. Results were analysed for statistical significance.

RESULTS

26 cases belonged to stage IIIB & 18 to IIB i.e., higher stage of presentation was noted.

53% of CIN were HGCIN (CIN2+CIN3). Expression of Bcl-2 was higher in LGCIN (CIN1), as compared with HGCIN and the difference in immunoexpression was highly significant statistically. Immunoexpression of Bcl-2 was higher in CIN (44.44%) as compared to ISCC (19.38%) and the difference was significant statistically.

Ki-67 immunoexpression showed an increasing trend with the progression of the disease (ISCC>HGCIN>LGCIN).

There was significant difference in immunoexpression of Bcl-2 in p53 negative and p53 positive ISCC cases. Thus, this may suggest the p53 – Bcl-2 pathway of carcinogenesis in cervical cancer.

CONCLUSION

Bcl-2 can be used to distinguish between LGCIN & HGCIN because there is a significant difference of Bcl-2 expression in the two groups. Loss of Bcl-2 expression or absence of Bcl-2 expression could be used to predict invasion because there is a significant difference in Bcl-2 expression in CIN and ISCC.

KEYWORDS

Bcl-2, CIN, ISCC, Ki-67, p53.

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BACKGROUND

In developing countries like India, cervical cancer is the second most common cancer affecting women¹ while in the United States, cervical cancer is the third most common cancer in females.² Worldwide, cervical cancer is the third most common cancer among women and the second most frequent cause of cancer-related death, accounting for nearly 300,000 deaths annually. It is the leading cause of death from cancer among women in developing countries, where it causes about 190,000 deaths each year.³ The current estimates indicate approximately 132,000 new cases diagnosed and 74,000 deaths annually in India,

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accounting for nearly 1/3rd of the global cervical cancer deaths.⁴ Numerous biomarkers have been studied in CIN and carcinoma of uterine cervix. There are studies correlating markers like p53 and Bcl-2 with the prognosis of cervical lesion. Some other studies impart a diagnostic role to Ki-67 in cervical carcinoma. The present study was undertaken to study the immunoexpression of p53, Bcl-2, Ki-67 in CIN and ISCC of the uterine cervix.

Aims and Objectives

This study was undertaken to analyse Bcl-2, p53 and Ki-67 in ISCC and CIN and attempt correlation with clinical and radiological findings.

MATERIALS AND METHODS

This study included one hundred and forty-three cases of cervical lesions; out of which twenty-one cases were CIN1, five were of CIN2, nineteen cases were of CIN3/ C.I.S. and the remainder 98 cases were of ISCC. These cases were diagnosed and/or operated upon (biopsy and/ or hysterectomy) during the period from August 2012 to

August 2015. The tissues for histopathology (surgical specimens, biopsies) were received in the department of Pathology over a period of three years. We selected the representative slide and paraffin block for IHC staining. A technique of manual tissue array was employed for 11 cases subjected for IHC staining. For the remaining cases IHC was performed on entire tissue section. The antibodies to p53, Bcl-2 and Ki-67 were used. Appropriate positive controls were taken for the IHC stains as per literature. Breast carcinoma was taken as a positive control for p53; tonsil was taken as positive tissue control for Ki-67 and Bcl-2. Statistical analysis of various parameters was done using Primer of Biostatistics (7th edition) software.

For the staining of p53 and Bcl-2, biopsies were recorded as positive when >10% of tumor cells were stained. The percentage of positive tumor cells was graded according to the method by Crawford et al⁵ and Nair et al.⁶ When none or <10% of tumor cells were stained, it was regarded as negative; 1+, when 11–30% cells were stained; 2+, when 31–50% cells were stained; and 3+, when >51% cells were stained. The number of Ki-67–stained cells was obtained by counting 200 nuclei in 400 magnifications for each sample. After counting a total of 200 cells, the immune reactive score was expressed as a percentage of the total cell count or Ki-67 index. A cut-off level to define high and low proliferating tumours was 30%.

RESULTS

Out of 143 cases, squamous cell carcinoma cases were maximum (n=98), of which 44 cases were of keratinizing subtype (KSCC), 43 cases were of nonkeratinizing subtype (NKSCC), 9 cases were of Papillary Squamotransitional subtype (PST) and one each of micro invasive and small cell carcinoma subtypes.

The most common clinical feature in cases of CIN was Vaginal Discharge. The most common clinical feature in invasive squamous cell carcinoma was post-menopausal vaginal bleeding. Multiparity was common in most of the cases of CIN & ISCC. Most cases of ISCC presented at an advanced stage of the disease (IIIB & IIB). Most cases of CIN were High Grade CIN (CIN2+CIN3).

Expression of Bcl-2 was higher in Low grade CIN/LGCIN (CIN1), as compared with High Grade CIN/HGCIN (CIN 2+3) and the difference in immunoeexpression was highly significant statistically (X²=18.573, p value=0.000). Immunoeexpression of Bcl-2 was higher in CIN (44.44%) as compared to Squamous cell carcinoma (19.38%) and the difference was significant statistically (X²=8.539, p value=0.003).

Expression of p53 was higher in Low grade CIN/LGCIN (CIN1), as compared with High Grade CIN/HGCIN (CIN 2+3) although the difference in immunoeexpression was not significant statistically. Immunoeexpression of p53 was slightly higher in CIN (64.44%) than squamous cell carcinoma (58.16%), although the difference was not significant statistically (X²=0.27, p value=0.59). Ki-67 immuno-expression showed an increasing trend with the progression of the disease (ISCC>HGCIN>LGCIN).

There was significant difference in immunoeexpression of Bcl-2 in p53 negative and p53 positive ISCC cases (Bcl-2 immunoeexpression in p53 positive tumours was 26.3% and in p53 negative tumours was 9.76%, chi square= 4.184, p= 0.040). Thus this strongly confirms the p53-Bcl-2 pathway of carcinogenesis in cervical cancer.

Name of Study	% Positivity of p53 in		Trend from LGCIN to HGCIN
	LGCIN	HGCIN	
Shukla S et al ⁷	22.2%	60%	Increasing
Hunt CR et al ⁸	0%	70%	Increasing
Present Study	76.19%	54.16%	Decreasing

Table 1. Trend of Immunopositivity of p53 from LGCIN to HGCIN

DISCUSSION

Our observations are in accordance with Vassalo et al.⁹ Vassalo et al⁹ who concluded that the higher expression of p53 protein in early lesions supports the hypothesis of a partially protective role of the wild-type p53 in early stages of cervical lesions.

Immunohistochemistry for the analysis of p53 has been criticized, partly because of conflicting results in clinical studies. The method does not necessarily detect all mutations.¹⁰ This also includes the pitfalls of IHC- for instance DO 7 clone picks up both wild and mutant type p53. In the present study as well, DO7 monoclonal antibody was used, which can recognize both wild and mutant p53 protein. Heterogeneity of labelling may occur because not all tumor cells harbour mutant p53 gene and not all p53 mutations result in accumulation of p53 protein. For instance, Nylander¹¹ et al showed 50% tumours which were p53 negative had p53 mutations. They concluded that the absence of p53 expression does not necessarily indicate that p53 mutation is not present.

Name of Study	% of Immuno-Positivity		Trend
	LGCIN	HGCIN	
Looi M L et al ¹²	0%	33%	Increasing
Shukla S et al ⁷	88.89%	80%	Decreasing
Kamaraddi S et al ¹³	55.56%	66.67%	Increasing
Dimitrakakis C et al ¹⁴	45%	39.75%	Decreasing
Present Study	66.67%	25%	Decreasing

Table 2. Trend of BCL-2 Expression from LGCIN to HGCIN in Various Studies

We found that among the CIN, immunoeexpression of Bcl-2 was higher in low grade CIN (CIN1) as compared to high grade CIN (CIN2 & 3) and this difference was significant statistically (X²=18.573, p=0.000). This is in accordance with Shukla S, et al⁷ and Dimitrakakis C et al¹⁴ but in discordance with Looi ML et al¹² and Kamaraddi S et

al.¹³ Dimitrakakis C et al¹⁴ however found that it increased from CIN1 to CIN2 but decreased suddenly in CIN3 to a low value.

Name of Study	% in LGCIN	% in HGCIN	Trend
Agoff SN et al ¹⁵	72.36	92.22	Increasing
Looi ML et al ¹²	25	33	Increasing
Raju Kalyani et al ¹⁶	100	94.5	Decreasing
Zhong P et al ¹⁷	77.71	97.34	Increasing
Present Study	90.48	100	Increasing

Table 3. Trend of Immunopositivity of Ki-67 from LGCIN to HGCIN

Our observations were in accordance with most other studies. The difference in immunoeexpression of Ki-67 in LGCIN and HGCIN is not significant statistically ($X^2=0.675$, p value=0.411).



Figure 1. Gross Photograph of a Fresh (Unfixed) Surgical Specimen of a case of Squamous Cell Carcinoma Cervix Showing a Classical Cauliflower-Like Growth

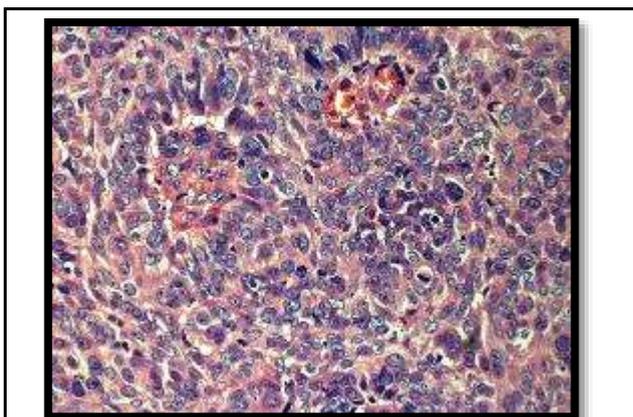


Figure 2. Photomicrograph of a case of LCNKSCC (H&E, X400)

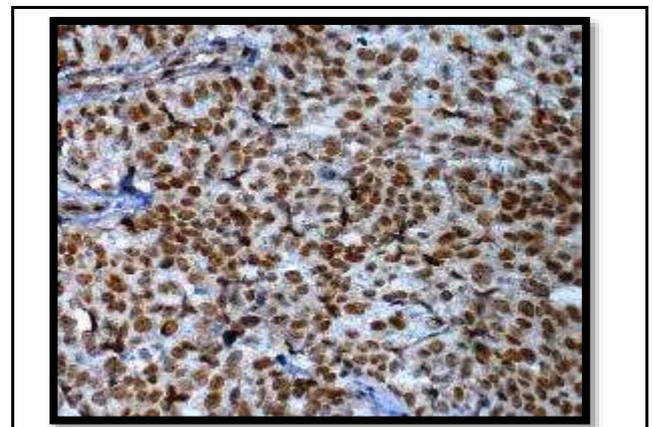


Figure 3. Photomicrograph showing Immunoeexpression of p53 (around 80% positivity) in a case of LCNKSCC(X400)

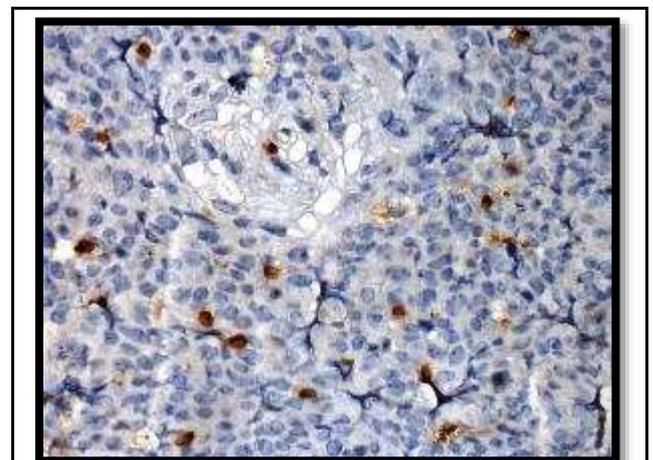


Figure 4. Photomicrograph showing Immunoeexpression of BCL-2 in a case of LCNKSCC showing Complete Lack of Immunoeexpression in the Tumour Cells. The Few Positive Cells seen are Intra-Tumoural Lymphocytes (X400)



Figure 5. Ki-67 Immunopositivity (Almost 100%) in a case of LCNKSCC (X100)

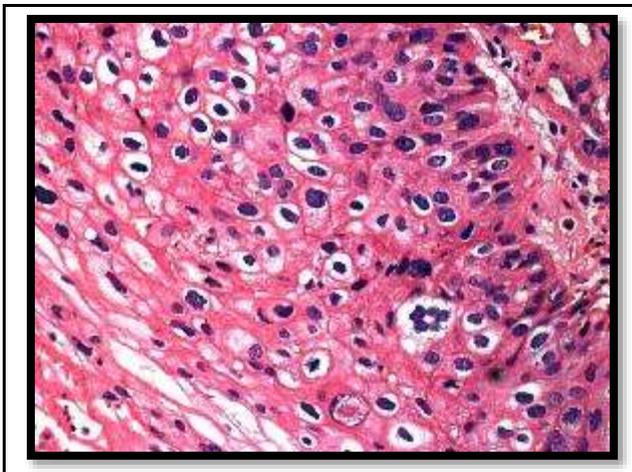


Figure 6. Photomicrograph of a case of LCKSCC (H&E, X 400)

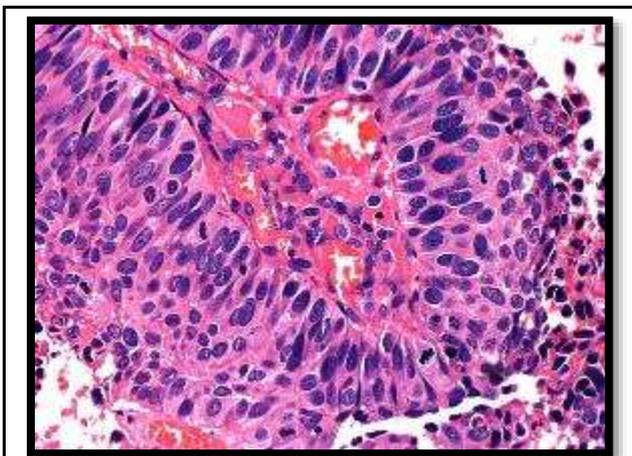


Figure 7. Photomicrograph of a case of Invasive Squamotransitional Cell Carcinoma Cervix (H&E, 400)

CONCLUSION

- 1) Bcl-2 can be used to distinguish between LGCIN & HGCIN because there is a significant difference of Bcl-2 expression in the two groups.
- 2) Loss of Bcl-2 expression or absence of Bcl-2 expression could be used to predict invasion because there is a significant difference in Bcl-2 expression in CIN and ISCC.
- 3) p53 cannot be used for prognostication of squamous cell carcinoma of cervix because there is no significant correlation between p53 expression & stage of ISCC.
- 4) The possible use of Ki-67 for diagnosis of CIN needs to be investigated using studies which compare Ki-67 immunorexpression in non-neoplastic lesions like cervicitis with that in CIN.
- 5) The expression of p53, Bcl-2 and Ki-67 may be used to differentiate between CIN2 & CIN3 because markers are expressed in all the layers in CIN3 while expression is confined to lower layers in CIN1 & 2. Since the treatment of CIN2 & CIN3 is similar at present, this

finding does not have a clinical significance but may be used for research purposes.

- 6) The Bcl-2 expression is significantly more in the group of p53 positive ISCC. This indicates the presence of a separate pathway of carcinogenesis involving Bcl-2 and p53. This finding may become significant when targeted therapies for this pathway of carcinogenesis becomes available.

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