



STUDY OF EXPRESSION OF BCL-2 MARKER IN PREMALIGNANT LESIONS OF CERVICAL CANCER

Pathology

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ABSTRACT

Background: Cervical cancer is one of the common cancers in women of developing countries. Persistent human papilloma virus (HPV) is probably the best predictor of cervical carcinogenesis, but HPV DNA test being costlier expression of certain markers of cell proliferation and apoptosis have been studied. The present study was conducted to evaluate the expression of bcl-2 in premalignant lesions of uterine cervix.

Methods: Specimens of cervical biopsy retrieved from incident cases for routine histopathological evaluation from the Department of Obstetrics and Gynaecology, Gauhati Medical College and Hospital, Guwahati, India formed the source of data.

Results: A total of 40 cases were studied in the present study comprising of 23 cases of Cervical intraepithelial neoplasia (CIN) 1, 9 cases of CIN 2, 6 cases of CIN 3, and 2 cases of Carcinoma in situ (CIS). It was observed that there was increase in bcl-2 positivity with increasing grade of CIN (CIN 1=69.57%, CIN 2=77.78%, CIN 3=83.33%, CIS=100%).

Conclusions: Bcl-2 being an intracellular membrane protein prevents apoptotic cell death. Hence, bcl-2 can be used as a biomarker to know the severity of the premalignant lesions. Thus, it may also help in identifying the high risk premalignant lesions which needs further surveillance and treatment.

KEYWORDS

Cervical cancer, Premalignant, Bcl-2.

INTRODUCTION:

Human cancers arise from a sequence of multiple genetic and epigenetic alterations which involve the growth regulatory genes. Evasion of apoptosis is one of the fundamental changes in the cell physiology required for malignant transformation. Apoptosis, a programmed cell death, is regulated by a balance between the proapoptotic and anti-apoptotic genes. It requires activation of anti-apoptotic genes, mainly the bcl2 gene, and inactivation of proapoptotic genes, mainly the p53 gene, for cell survival and proliferation to occur which results in neoplasia.⁽¹⁾

Cervical cancer is the fourth most common cancer and fourth most common cause of cancer death in women worldwide. In developing countries like India it is the second most common cancer in women.⁽²⁾ Human papilloma virus (HPV) infection is the most common cause of cervical cancer which accounts for approximately 80%.⁽³⁾ Cervical cancer shows bimodal distribution of age, 2 peaks are shown at 35-39 years and 60-64 years.⁽⁴⁾

HPV DNA testing is probably the best predictor of cervical carcinogenesis.⁽⁵⁾ But HPV testing being costlier, modulation of apoptosis and apoptotic regulatory proteins by HPV oncoproteins has been implicated in cervical carcinogenesis.⁽⁶⁾ Bcl-2 is an important regulator of apoptosis. The regulation of apoptosis by bcl-2 is implemented through the mitochondrial pathway.⁽⁷⁾ P53 induces cell death can be blocked by increased expression of bcl-2, thereby promoting cell survival. An inverse relation between p53 and bcl2 expression has been observed in various human cancers, on the contrary, in carcinoma of the uterine cervix, expression of both has been observed, the significance of which is not known.⁽⁸⁾

The present study was done to evaluate the expression of bcl-2 in premalignant lesions of uterine cervix by immunohistochemistry. The bcl-2 expression will be correlated with the histological grade of the lesions.

MATERIALS AND METHODS:

Study sample: Specimens of cervical biopsy retrieved from incident cases for routine histopathological evaluation from the Department of Obstetrics and Gynaecology, Gauhati Medical College and Hospital, Guwahati, India from March 2019 to February 2020 formed the source of data for the study.

Study design: Cross sectional study.

Sample size: 40.

Study period: Archival cervical biopsy specimens over 1 year were studied.

Study method: The study comprised of 40 cases of premalignant lesions of cervix. The detailed clinical history including age, clinical signs and symptoms, and results of relevant investigations done were collected from the patients' case files.

Paraffin blocks of these patients were retrieved and 3-5 μ m thick sections were taken on poly-L-lysine coated slides. These slides were stained with Hematoxylin and Eosin and Immunohistochemical stain for bcl-2 protein were done.

The technique for IHC included antigen retrieval in citrate buffer in a microwave oven, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse monoclonal antibody against bcl-2 protein. Positive and negative controls were run with each batch of slides. The H and E stained slides were studied for the histopathological changes and the categorization of the cervical lesion. The immunostained slides were examined for cytoplasmic staining of bcl-2.

Bcl-2 immunostaining gave brown cytoplasmic reactivity. A case was taken as positive if more than 10% cells showed cytoplasmic reactivity.

Statistical analysis: Descriptive statistics of bcl-2 analysed and explained in percentage.

RESULTS:

In this study total 40 cases were studied comprising of 23 cases of Cervical intraepithelial neoplasia (CIN) 1, 9 cases of CIN 2, 6 cases of CIN 3, and 2 cases of Carcinoma in situ (CIS).

Age range of the patients were 28 years to 73 years with mean age being 49.55 $\pm$ 11.32 years. In the present study, it was observed that white discharge was the most common presenting symptom [Table 1].

The immunostaining of bcl-2 was cytoplasmic, which was of two types, diffuse type and localized to the basal layer. Total 30 out of 40 CIN cases (75%) showed positive bcl-2 expression. Bcl-2 positivity was shown by 16 out of 23 CIN 1 cases (69.57%), 7 out of 9 CIN 2 cases (77.78%), 5 out of 6 CIN 3 cases (83.33%) and 2 out of 2 CIS cases (100%). In the present study, it was observed that bcl-2 positivity was increased with increasing grade of CIN [Table 2] {Figure 1 to 3}.

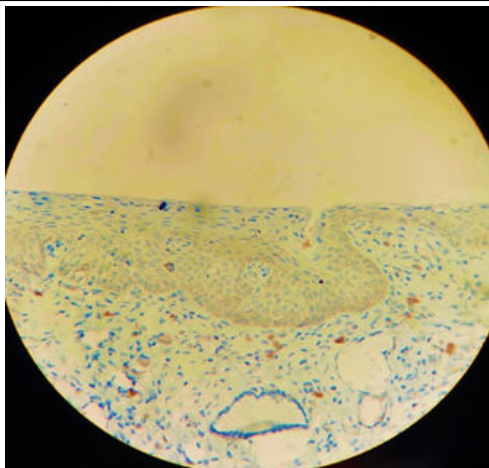
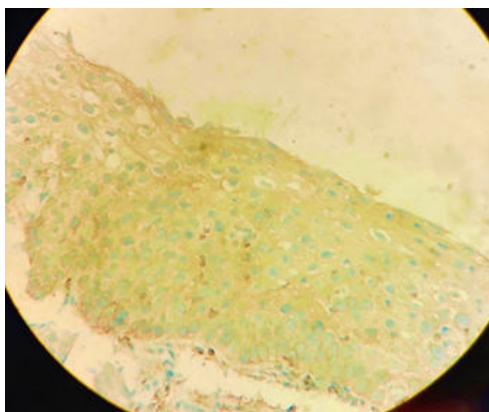
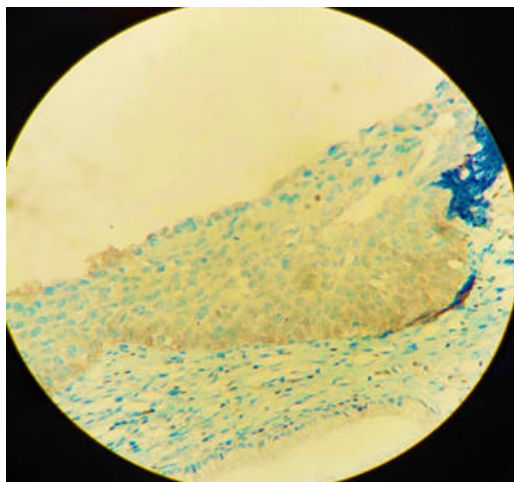
Table 1: Clinical presentation of the patients:

Case	Post coital bleeding	White discharge per vaginum	Bleeding per vaginum	Prolapsed uterus	Total no. of cases

CIN 1	0	10	1	12	23
CIN 2	2	4	3	0	9
CIN 3	1	1	4	0	6
CIS	1	0	1	0	2

Table 2: Bcl-2 positivity in premalignant lesion of cervix:

Type of lesions	Total	No. of bcl-2 positive cases	Percentage of bcl-2 positive cases
CIN	40	30	75%
CIN 1	23	16	69.57%
CIN 2	9	7	77.78%
CIN 3	6	5	83.33%
CIS	2	2	100%

**Figure 1: Cytoplasmic positivity of Bcl-2 in CIN 1 (IHC, 10x view).****Figure 2: Diffuse cytoplasmic positivity of Bcl-2 in CIN 3 (IHC, 40x view).****Figure 3: Diffuse cytoplasmic positivity of Bcl-2 in CIS (IHC, 40x view).****DISCUSSION:**

Worldwide carcinoma cervix is both fourth most common cause of cancer and fourth most common cause of cancer death in females.⁽²⁾ Persistent HPV infection has been implicated in approximately 80-90% of carcinoma cervix.⁽⁵⁾ Numerous biomarkers of cell proliferation and apoptosis have been studied in relation to carcinogenesis of cervical cancer. Bcl-2 is an intracellular membrane protein which inhibits apoptotic cell death. Bcl-2 overexpression is present in both malignant and premalignant lesions of cervix. Limited numbers of studies are available on bcl-2 expression.

This study was taken up to evaluate the bcl-2 expression on premalignant lesions of uterine cervix by using immunohistochemistry.

In the present study, bcl-2 positivity was found to increase with increasing grade of CIN (CIN 1 = 69.57%, CIN 2 = 77.78%, CIN 3 = 83.33%, CIS = 100%). This was similar to the findings of studies done by Saegusa M et al⁽⁹⁾, Pillai MR et al⁽¹⁰⁾, Dimitrakakis C et al⁽¹¹⁾, Grace VB et al⁽⁸⁾, Singh M et al⁽¹²⁾, Kamaraddi S et al⁽¹³⁾ who also found the same. Conversely, the study done by Shukla S et al⁽¹⁴⁾ had found reduced bcl-2 expression with increasing grade of CIN, however it was not found to be significant.

CONCLUSION:

Bcl-2 is an intracellular membrane protein which prevents apoptotic cell death that can be used as a biomarker to know the severity of the premalignant lesions. Thus, determining bcl-2 expression help in identifying the high risk premalignant lesions which need further surveillance and treatment.

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