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Evaluation of BCL-2 Marker in Identifying High Risk Premalignant Lesions of Cervix

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ABSTRACT:
Introduction: In developing countries like India, cervical cancer is the most common cancer in females. Cervical cancer is the second most common cancer after breast cancer in females in developed countries. Squamous cell carcinoma(SCC) is more common than adenocarcinoma and other types. Human papilloma virus(HPV) is causative organism for cervical carcinoma. Bcl-2 is an intracellular membrane protein which inhibits apoptotic cell death. Bcl-2 can indicate the initiation of carcinogenic process of HPV infection. Over expression of bcl-2 is seen in premalignant and malignant lesions of cervix and thus can be useful in identifying high risk premalignant lesions(CIN) of cervix.
Aim: To evaluate the role of bcl-2 marker to identify women who have high risk premalignant lesions of cervix and improve surveillance in those women.
Materials and methods: The study was performed on cervical biopsy specimen received in department of Pathology, SBKS MI & RC, Waghodia, Gujarat during May 2022 to August 2023(16 months). The study was performed on 38 cervical biopsy specimens received in department of Pathology. All cases of cervical lesions with age 26-65 years were included. Total 38 cases of CIN (cervical intraepithelial neoplasia) and carcinoma cervix were evaluated for expression of bcl-2 by immunohistochemistry.
Results: The study included total 38 cases. Out of which, 26 cases were of CIN and 12 cases were of carcinoma cervix. The mean age of patients diagnosed with carcinoma cervix was higher as compared to CIN. Higher number of malignant lesions (75%) stained positive for bcl-2 in comparison with premalignant lesions (46.15%). As the grade of CIN lesions increases, bcl-2 positivity increases.
Conclusion: Bcl-2 is an intracellular membrane protein which inhibits apoptotic cell death. It helps to identify high risk premalignant cervical lesions, as bcl-2 positivity increases as the grade of CIN increases and carcinoma cervix shows more bcl-2 positivity in comparison with CIN lesions which can improve further surveillance.

KEYWORDS: Cervical intraepithelial neoplasia, bcl-2 marker, carcinoma cervix, immunohistochemistry.

INTRODUCTION

In developing countries like India, cervical cancer is the most common cancer in females. Cervical cancer is the second most common cancer after breast cancer in females in developed countries [1]. Squamous cell carcinoma(SCC) is more common than adenocarcinoma and other types. Human papilloma virus(HPV) is causative organism for cervical carcinoma. Cervical cancer is most common in woman >30 years old, therefore co-testing using the combination of pap cytology plus HPV DNA testing is preferred cervical screening method.

More than 200 types of HPV have been identified on deoxyribonucleic acid (DNA) sequence analysis. About 80-90% of cervical carcinomas contain DNA sequences of specific HPV subtypes, especially those of HPV 16 and 18[2-4]. Carcinogenicity of HPV for cancer cervix is partly related to expression of two early genes, E6 and E7 oncogenes. E6 and E7

oncogenes interfere with the functioning of suppressor gene p53 and pRB respectively which are apoptotic proteins.

Bcl-2 is an intracellular membrane protein which inhibits apoptotic cell death. Bcl-2 over expression can block p53 mediated G1 arrest [5]. Thus, Bcl-2 can indicate the initiation of carcinogenic process of HPV infection. Over expression of bcl-2 is seen in premalignant and malignant lesions of cervix and it is suggested that bcl-2 expression is a relatively early event in cervical tumor genesis. Bcl-2 positivity also confer a better 5-year survival rate and prognosis [6]. Bcl-2 positivity increases as the grade of CIN lesions increases and carcinoma cervix shows more bcl-2 positivity in comparison with CIN lesions [7]. It is found that, bcl-2 positivity strongly associated with development of invasive cervical cancer. The pattern may be useful in identifying woman at high risk for invasive carcinoma and thus the requirement of immediate treatment [8].

The present study was done to evaluate the expression of bcl-2 in premalignant and malignant lesions of cervix by immunohistochemistry. The bcl-2 expression were correlated with the stage of cervical cancer lesion.

II. MATERIALS AND METHODS

The study was performed on cervical biopsy specimen received in department of Pathology, SBKS MI & RC, Waghodia, Gujarat during May 2022 to August 2023(16 months). The study was retrospective and observational in nature. The study was performed on 38 cervical biopsy specimens received in department of Pathology. All cases of cervical lesions with age 26-65 years were included. The exclusion criteria were inadequate biopsy sample, ulcerated or necrotic tissue and biopsies of secondary carcinomas of cervix.

Total 38 cases of cervical intraepithelial neoplasia(CIN) and carcinoma cervix were evaluated for expression of bcl-2 by immunohistochemistry(IHC). The specimen received were processed in fully automated tissue processor by passing through various processes of alcohol, xylene and wax. After tissue processing, paraffin embedded tissue blocks were prepared. From these blocks 3-5 micron thick sections were cut and stained with routine Haematoxylin and Eosin(H&E) staining method [9-10]. The slides were examined microscopically to confirm the diagnosis of cervical lesion. The H&E stained slides were studied and categorization of cervical lesion was done.

Bcl-2 marker was investigated by immunohistochemistry, using Envision flex IHC kit purchased from DAKO. Appropriate controls were run in every case [11]. Basic steps in IHC were fixation, antigen retrieval (to increase the availability of proteins for detection), blocking (to minimize pesky background signals) and antibody labeling and visualization. The immunostained slides were examined for cytoplasmic staining of bcl-2. In each case, the proportion of positive staining cells (expressed as percentage) and intensity of staining (expressed as -: negative/no staining, +: weak staining, ++: moderate staining and +++: intense staining) were evaluated. A case was taken as positive if more than 10% cells showed cytoplasmic reactivity [12].

All the clinical findings, pap smear reports, histopathological biopsy reports and immunohistochemical analysis were correlated to improve diagnostic efficacy of cervical lesions. All the observations were carried out by two observers in order to eliminate inter-observer bias, Results obtained of bcl-2 staining were analyzed.

III. RESULTS

The study included total 38 cases. Out of 38 cases, there were 11 cases of CIN I, 8 cases of CIN II, 7 cases were CIN III, 9 cases were squamous cell carcinoma(SCC) of cervix and 3 cases were adenocarcinoma of cervix.

Table 1: Age-wise case distribution

Age	CIN (n=26)	Carcinoma cervix (n=12)
26-35	8	1
36-45	10	2
46-55	5	4
56-65	3	5
Mean ± SD	39.75±8.5	50.12±7.25
p-value	0.031	

Table 2: Parity-wise case distribution

Parity	CIN (n=26)	Carcinoma cervix (n=12)
0-2	12	1
3-4	9	3
5-6	4	6
≥7	1	2
Mean ±SD	2.95±1.25	4.58±1.60
p-value	0.005	

Table 3: Clinical symptoms of patients

Case	Pelvic/Lower back pain	Discharge per vaginum	Bleeding per vaginum	Post-coital bleeding	Total no. of cases
CIN I	4	6	1	0	11
CIN II	3	4	1	0	8
CIN III	2	2	2	1	7
SCC	1	1	4	3	9
Adenocarcinoma	0	0	2	1	3

The patients included in this study were between 26 to 65 years of age. Table 1 indicates that the mean age of patients diagnosed with carcinoma cervix was higher (50.12) as compared to CIN(39.75) and this difference was statistically significant (p-value=0.031). Table 2 indicates that patients with CIN had lower parity while patients with carcinoma cervix had higher parity, which was statistically significant (p-value=0.005). Table 3 indicates that common symptoms in CIN patients were pelvic or lower back pain and discharge per vaginum and in carcinoma cervix were bleeding per vaginum and post-coital bleeding.

Table 4: Positivity of Bcl-2 in CIN and Carcinoma cervix

Lesions	Bcl-2 Positive	Bcl-2 Negative	Total
CIN I	4 (36.36%)	7	11
CIN II	4 (50%)	4	8
CIN III	4 (57.14%)	3	7

SCC	7 (77.78%)	2	9
Adenocarcinoma	2 (66.67%)	1	3

Table 5: Comparison of Bcl-2 immunostaining in CIN and Carcinoma cervix

Immunohistochemistry	**CIN (n=26)**	**Carcinoma cervix (n=12)**
Bcl-2 Positive	12/26 (46.15%)	9/12 (75%)
Bcl-2 Negative	14/26 (53.85%)	3/12 (25%)
p-value	0.043	

The immunostaining of bcl-2 was cytoplasmic. Table 4 indicates that, as the grade of CIN lesions increases, bcl-2 positivity increases (CIN I-36.36%, CIN II-50%, CIN III-57.14%). Table 5 indicates that higher number of malignant lesions(75%) stained positive for bcl-2 in comparison with premalignant lesions(46.15%), which was statistically significant (p-value=0.043).

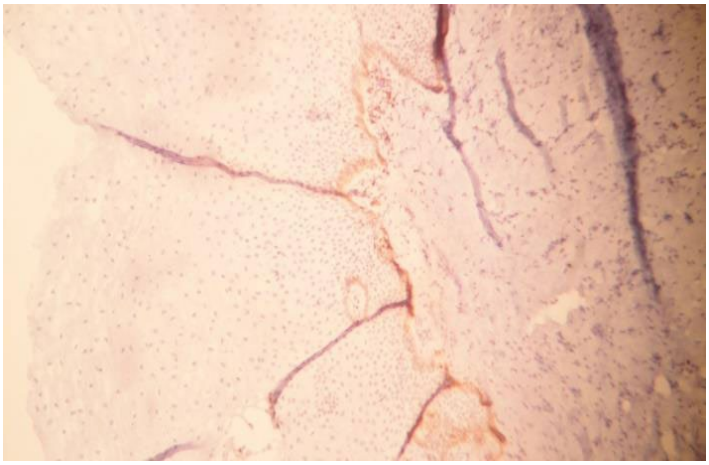

Image 1: Bcl-2 positivity in CIN II Lesions (Low power)

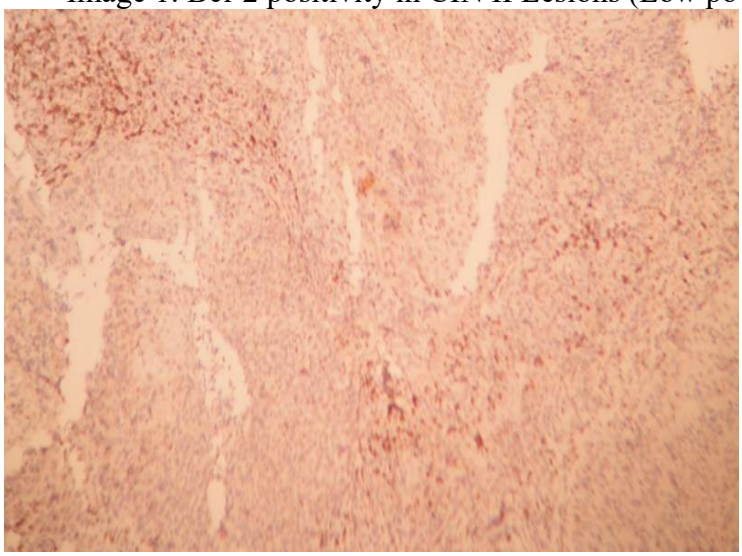

Image 2: Bcl-2 positivity in CIN III Lesions (High power)

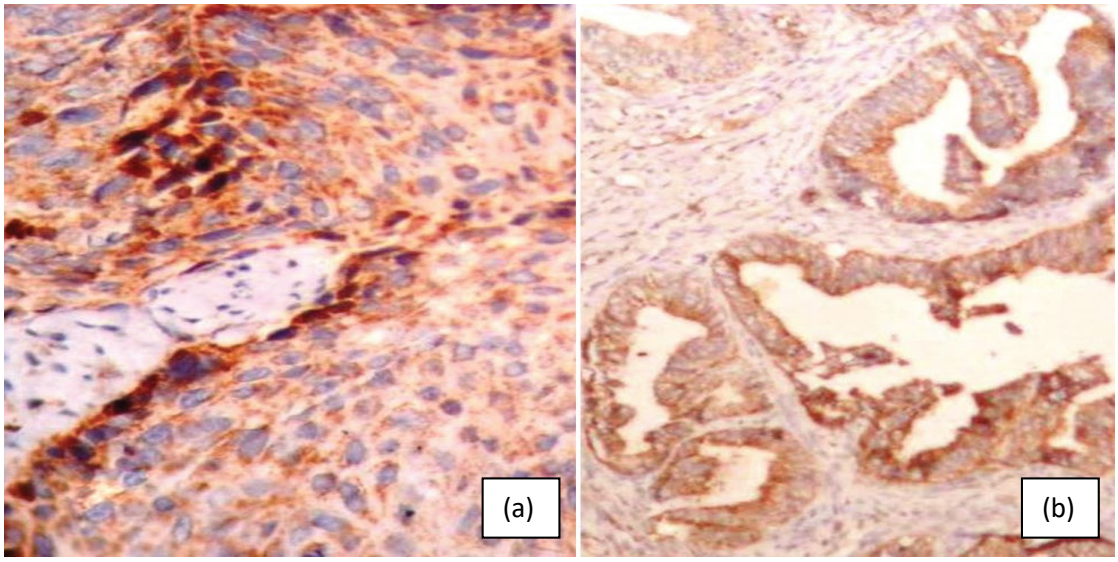

Image 3: (a) Bcl-2 positivity in squamous cell carcinoma of cervix (high power), (b) Bcl-2 positivity in adenocarcinoma of cervix (high power)

IV. DISCUSSION

Carcinoma cervix is the most common cancer among females in India [1]. Persistent HPV infection causes 80-90% of cervical cancers [2]. Biomarkers of cell proliferation and apoptosis had been studied which indicate process of carcinogenesis and process of HPV infection. Bcl-2 is a biomarker an intracellular membrane protein which inhibits apoptotic cell death. Over expression of bcl-2 is seen in pre-malignant and malignant lesions of cervical cancer.

This study was done to evaluate bcl-2 marker to identify high risk premalignant lesions of cervix. Total 38 cases of CIN and carcinoma cervix was included. In this study, the mean age of patients diagnosed with carcinoma cervix was higher compared to CIN and the patients with carcinoma cervix had higher parity compared to CIN patients. The patients with carcinoma cervix expressed more bcl-2 positivity as compared to CIN patients and Bcl-2 positivity increased as the grade of CIN increased.

The studies by Kurvinen K et al, Pillai MR et al, Brychtova S et al have also shown increasing expression of bcl-2 as the grade of CIN increasing [5,8,13]. The study by Shukla et al has shown decreased expression of bcl-2 with increasing grade of CIN [12].

V. CONCLUSION

Bcl-2 is an intracellular membrane protein which inhibits apoptotic cell death, by which severity of cervical premalignant lesion(CIN) can be known. It helps to identify high risk premalignant cervical lesions as bcl-2 positivity increases as the grade of CIN increases and carcinoma cervix shows more bcl-2 positivity in comparison with CIN lesions which can improve further surveillance.

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