

Immunohistochemical Expression of Bcl-2 in Oral Squamous Cell Carcinoma.

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Abstract: Currently, oral cancer is one of the most alarming health problems facing mankind. More than 90% of all oral cancers are oral squamous cell carcinoma (OSCC). Bcl-2 proteins are one of the most prominent anti-apoptotic proteins expressed in OSCC. They contribute to cancer development and mediate resistance to current anticancer treatments. Tissue homeostasis is violated in the process of tumorigenesis, by a subset of transformed cells which further gear them up with increased cell proliferation and decreased apoptosis. This study emphasizes the importance of Bcl-2 expression as a prognostic indicator in OSCC.

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I. Introduction

Currently, oral cancer is one of the most alarming health problems facing mankind in terms of morbidity and mortality. It is the sixth most common type of cancer worldwide. Oral cancer accounts for 4% of all malignancies in men and 2% in women.¹ More than 90% of all oral cancers are oral squamous cell carcinoma (OSCC),² which is often preceded by a premalignant lesion. According to the World Health Organization, OSCC is the eighth most common type of cancer worldwide, with geographical variations.³ The major etiological factors in the genesis of oral carcinoma constitute tobacco chewing/smoking, alcohol consumption, and human papilloma viruses.¹ A diet low in fresh fruits and vegetables has also recently been implicated in the etiopathogenesis of OSCC.² Overall incidence and mortality attributed to OSCC is increasing, with the current estimates of age-standardized incidence and mortality being 6.6/100,000 and 3.1/100,000 in men and 2.9/100,000 and 1.4/100,000 in women, respectively.⁴ Cancer is caused by the accumulation of genetic and epigenetic mutations in the genes that normally play a role in the regulation of cell proliferation, thus leading to uncontrolled cell growth. The genes involved in tumorigenesis include those whose products (1) directly regulate cell proliferation (either promoting or inhibiting), (2) control programmed cell death or apoptosis, and (3) are involved in the repair of damaged DNA. Depending on how they affect each process, these genes are named as tumor suppressor genes (growth inhibitory), proto-oncogenes (growth promoting), or anti-apoptotic genes (inhibits apoptosis).¹

B-cell lymphoma/leukemia-2 (Bcl-2) is an anti-apoptotic protein that interacts with and is regulated by p53. It is a part of the regulatory system that controls the cell cycle and the induction of apoptosis. Bcl-2 gene family and related proteins form the core of the apoptotic program and the major effector arm of the cell death program. Bcl-2 gene was first discovered in follicular non-Hodgkin's B-cell lymphoma. In this translocation, the Bcl-2 gene is moved from its normal chromosomal location at 18q21 in to proximity with powerful enhanced elements in the immunoglobulin heavy chain (IgH) locus at 14q32. This results in the deregulation of the translocated Bcl-2 gene and the overproduction of Bcl-2 mRNAs and their encoded proteins.⁵

II. Material And Methods

Study Design and Patient Selection

The current study was conducted on the archival formalin fixed, paraffin embedded tissues obtained from private college. The study included 12 cases of histologically diagnosed Oral squamous cell carcinoma. section of 3µ thickness were sectioned from each sample.

The work was approved by Institutional Review Board (IRB Ref. No. IGIDSIRB2016 NDP24PGMROP). and Institutional Ethical Committee, (IECRef. No. IGIDSIEC2016 NDP24PGMROP). Indira Gandhi institute of dental sciences, Sri Balaji vidyapeeth university.

INCLUSION CRITERIA:

Biopsied samples which were histopathologically diagnosed as OSCC were included in the study.

MATERIALS

PARAFFIN BLOCKS:

Paraffin embedded tissues of histologically confirmed Oral squamous cell carcinoma.

MARKERS FOR IMMUNOHISTOCHEMISTRY:

- Primary antibody
- 1. Bcl-2 [Rabbit monoclonal antibody] (PathnSitu Biotechnologies Private Limited.) stored at 4°C.
- Secondary kit (PolyExcel HRP/DAB Detection System) – Pathn Situ Biotechnologies Private Limited. stored at 4°C.

METHODOLOGY

Formalin fixed paraffin embedded tissues were sectioned at 3µm and mounted on charged slides. Incubated at 60°C – 70°C for 1 hour. Deparaffinized by xylene. Sections are hydrated through descending grades of alcohols. Antigen retrieval is done using multi epitope retrieval system (MERS) standard protocol. Endogenous peroxidase blocking was done by adding Poly Excel H₂O₂ on the section. Primary antibody was added on the sections and kept for 30 minutes in a moist chamber. Polyexcel HRP (secondary antibody) was added to the sections and incubated for 12 -15 minutes. DAB working solution was prepared (1 ml of DAB buffer + 1 drop DAB chromogen, mix well) in a dark chamber. Counterstained with Hematoxylin for 30 seconds. Sections are dried and mounted using DPX.

POSITIVE CONTROL:

Positive control sections were made from Lymphoma tissues for Bcl-2 which was treated in the same manner as the test groups.

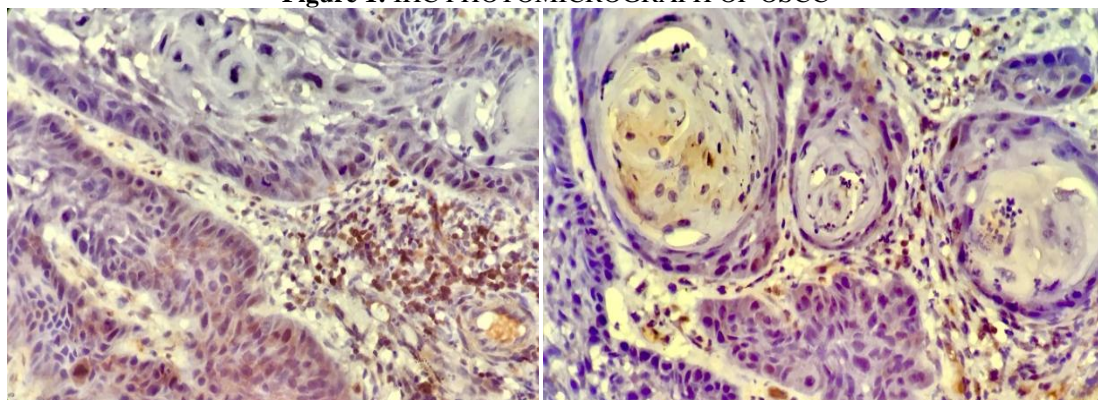
ANALYSIS OF IMMUNOREACTIVITY OF Bcl-2: (Table-1)

Ten random fields were selected under 100x magnification. Sudha et al⁶ criteria was followed; hundred cells were counted in ten fields and the percentage of positive cells were derived (Table-1). The scoring was done by two independent observers. Though there was positivity, if the percentage of positive cells scored is less than 10% it is considered under no stain category according to the criteria.

Table 1: Sudha et al⁶ scoring criteria

Staining intensity	Scoring
NO STAIN	<10% - (±)
MILD	10%-25% - (+)
MODERATE	25%-50% - (++)
INTENSE	>50% - (+++)

Figure 1: IHC PHOTOMICROGRAPH OF OSCC



Bcl-2 expression in OSCC

III. Result

- Table 2 , shows Out of 12 cases of OSCC; 8 cases showed mild expression and 4 cases showed moderate expression.

Table:2; Percentage of Bcl-2 IN OSCC, based on Sudha et al⁶

	Bcl-2		
	Mild	Moderate	Intense
Oral squamous cell carcinoma. (n=12)	8/66.6%	4/33.3%	NA

- Table 3, shows All cases of OSCC showed basal/para basal expression. 2 cases showed expression in the superficial layer and 4 cases showed expression in the tumor islands.

Table:3;Percentage of Bcl-2 topographic staining pattern in OSCC.

	OSCC(n=12)
Basal/para basal	12/100%
Superfial layer	2/16.6%
Tumor islands	4/33.3%

IV. Discussion

Bcl-2 expression appears to be altered to varying degrees in oral mucosal dysplasia. The recent retrospective cross sectional studies have suggested that the up regulation of Bcl-2 may be an early event in epithelial carcinogenesis.⁷

The carcinogenesis of OSCC is a multistage process involving the activation of oncogenes and the inactivation of tumor suppressor genes with an imbalance of cell death and growth. Although considerable interest has recently been focused on the identification of the regulator of apoptosis that may be a potential and key influence on the balance of cell death and cell growth in cancer.⁸ various studies have been conducted on the oral tissues to study the expression of Bcl2 in oral dysplastic lesions.

Coutinho-Camillo et al.³ characterized the expression of proteins that inhibit (Bcl-2, Bcl-x, Bcl-xL, Bcl-2-related protein A1, and BAG-1) or promote (Bak, Bax, Bim/Bod, Bim-Long, Bad, Bid, and PUMA) apoptosis and determine possible correlations between the expression of these proteins and the clinicopathological features of OSCC using immunohistochemistry. They suggested that the expression of apoptotic molecules might be used as a prognostic indicator for OSCC. in our study we noted increased expression of the Bcl-2 in OSCC, it denotes that Anti-apoptosis may take the major role in OSCC.

Popović et al.⁹ estimated the level of over expression of Bcl-2 proteins in OSCC using immunohistochemistry. The results showed a low percentage of positively stained Bcl-2 cells and concluded that the level of Bcl-2 expression could be a valuable predictor of tumor behavior and disease outcome. in our study we also concludes the same.

Juneja et al.⁸ did an immunohistochemical (IHC) study to evaluate and compare the expression of Bcl 2 protein in oral epithelial dysplasia and OSCC using a monoclonal antibody against anti-human Bcl-2 oncoprotein. For Bcl-2, 26.7% positivity was observed in oral epithelial dysplasia, and 30% in OSCC. They concluded that the altered expression of Bcl-2 may be an early molecular event, which leads to prolonged cell survival, an increased chance of accumulation of genetic alterations, and subsequent increase in malignant transformation potential.in our study we noticed 100% positivity in OSCC.

A study by Arul et al.¹⁰ evaluated correlated the expressions of anti-apoptotic marker Bcl-2 and proliferative marker MIB-1 in varying grades of OSCC using immunohistochemistry. Anti-apoptosis was found dominant in well-differentiated lesions than in moderately and poorly differentiated lesions. We also received the same results that well differentiated lesion expressed more compare to the moderate and poorly differentiated lesions.

Thomas and Sethupathy¹¹ investigated the expression of the apoptosis related proteins Bax, Bcl-2, and p53 in OSCC to explore the possible relationship among these apoptotic markers in oral carcinogenesis. They have observed the over expression of p53 and Bcl2 and the decreased expression of bax in patients with OSCC. They concluded that apoptotic mechanism can be accounted for oral carcinogenesis. We recorded the same results that increased expression of Bcl-2 in OSCC.

A study by Suri¹² evaluated the expression, as well as quantified and determined the intensity and the pattern of Bcl-2 in various histological grades of OSCC using immunohistochemistry. The number of cells expressing Bcl-2 increased from well-differentiated to poorly differentiated OSCCs, showing an inverse relationship with the degree of differentiation. We noted the same results.

V. Conclusion

Bcl-2 proteins are one of the most prominent anti-apoptotic proteins expressed in OSCC. Most of the studies suggest that the expression of Bcl-2 might be used as a prognostic indicator for OSCC. They contribute to cancer development and mediate resistance to current anticancer treatments. Several promising inhibitors of Bcl-2 proteins have been developed in recent years, and it is very important to utilize these compounds in the treatment of OSCC. Therefore, in future, Bcl-2 inhibitors should be included in personalized cancer treatments for those tumors that express addiction to Bcl-2 proteins.

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