Evaluation of BCL-2 Oncoprotein in Oral Epithelial DysplasIa & Quamous Cell Carcinoma : An immunohistochemical study

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Introduction

Oral epithelial dysplasia is the histologic marker of premalignancy and as such predictive of an increased rate of development of squamous cell carcinoma. Squamous cell carcinoma constituted about 90% of oral malignancy. Despite advances in the detection and management of cancer survival rate has remain poor [3]. Oral cancer may develop as a two stage process first stage as premalignant lesion and second as development of cancer with in this lesion. Epithelial dysplasia is the most important indicator of malignant potential. However subjective variation has restricted the role of epithelial dysplasia as a predictor for malignant transformation[4,14].

More recent advances in oncogenic events deal with increase cell growth, proliferation & also tumour suppressor gene which regulate cell growth and proliferation in a negative fashion. E.g.: p53 gene. Fewer studies have addressed the role of apoptosis in the natural history of oral cancer. A group of genes have been identified which regulate apoptosis. The first of this group the bcl-2 was identified in molecular analysis of the t (14:18) chromosome translocation in follicular B-cell lymphoma. The gene is located at chromosome 18q21. The bcl-2 is unique among protooncogene, being localized to mitochondria and extending cell survival by blocking programmed cell death[5]. It is topographically restricted to cells in proliferating zones and cells with long life span & it is downregulated in terminally differentiated cells. Enhanced expression of bcl-2 oncoprotein in human neoplasm suggests a role for this oncoprotein in the pathogenesis of neoplasia. However studies involving expression of bcl-2 in oral cancer and precancer is lacking and the possible role of bcl-2 in oral tumours remains unknown. Therefore the present study was carried out to evaluate bcl-2 oncoprotein expression in mild, moderate, & severe epithelial dysplasia & squamous cell carcinoma and to determine its role in early stages of oral carcinogenesis.

Aims & Objectives

It was carried forth with the following aims and objectives

1. To grade the degree of dysplasia. 2. To evaluate bcl-2 oncoprotein in oral dysplasia and carcinoma 3. To correlate its expression with the degree of dysplasia and carcinoma 4. To elucidate the role of bcl-2 in early stages of carcinogenesis. 5. To evaluate prognostic significance of bcl-2 in oral precancerous and cancer lesions.

Materials & Methods

This study was carried out in the Department of Oral Pathology & Microbiology, GDC&H, in Mumbai. The expression of bcl-2 oncoprotein was studied in tissue of 80 patients, 60 premalignant lesions & 20 squamous cell carcinoma, 60 cases of epithelial dysplasia were subdivided into mild, moderate & severe dysplasia graded according to the extent of nuclear atypia & number of epithelial cell layer involved and the opinion of three experienced inter observers from the department were taken into consideration.

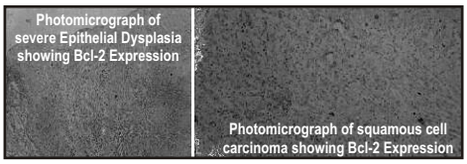
A detailed case history, blood evaluation was done for each patient prior to biopsy procedure. The tissues were stained with H&E stain. The immunohistochemical staining was carried out using bcl-2 Monoclonal Mouse Antihuman Antibody Clone124 (DAKO). The immunohistochemical detection kit used in this study was DAKO LSABR2 System Peroxidase and immunostaining was performed as per the instructions recommended by the manufacturer. The substrate chromogen (DAB) forms the brown coloured end product at the site of target antigen. The slides were then assessed for positivity.

Interpretation of Staining

Presence of brown coloured end product at the site of target antigen was indicative of positive reactivity.

Counting of cells

The cells immunoreactive for bcl-2 were scored in 4 randomly field of a cm^2 area under 400X using a grid. The count of positive cells was described as X cells/ cm^2



Discussion

The balanced between cell proliferation and cell death is essential for the maintenance of normal tissue and organs. This maintenance of balance seems to be disturbed in tumour cells. The tumour may be caused by either an increase in cell proliferation or a decrease in cell death or both.[9] To date most of our knowledge considering oncogenic events has concentrated on mechanism of increased cell growth and proliferation.

Bcl-2 initiates a new gene family involved in the regulation of cell death and survival.20 it may propogation and accumulation of contributing genetic alteration by inhibiting apoptosis[5]. The bcl-2 protooncogene on chromosome 18q21 is composed of 2 exons which encodes 2 closely related proteins bcl-2 *alpha* & bcl-2*beta* (26&21 Kd) which differ only at carboxyl terminals.

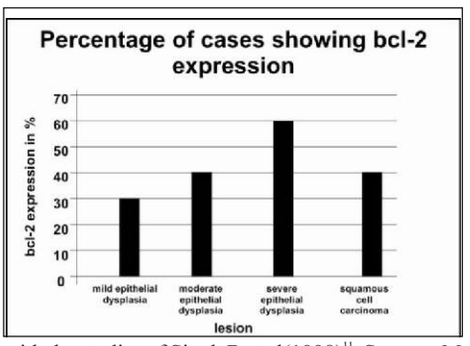
The t(14:18) translocation brings bcl-2 in juxtaposition with the immunoglobulin heavy chain regulatory region causing over expression of the protein. It appears to block a final common pathway in apoptosis possibly by preventing damaged DNA acting as a signal for activation of Programmed Cell Death (PCD) or by blocking the actions of the PCD gene product themselves. It therefore prevents premature death of mitotic cells and extents survival during which other mutation could occur. Bcl-2 also plays a role in the differentiation of epithelial cells. It has been suggested that the expression bcl-2 is downregulated in terminally differentiated cells.[2,6]

In the present study the expression of bcl-2 has been studied in different degree epithelial dysplasia and squamous cell carcinoma. A direct relationship between degree of epithelial dysplasia and the likelihood of lesion progressing has been well established. The rate of malignant transformation is higher in severe dysplasia as compared to mild and moderate dysplasia.

The present study indicated a higher incidence of bcl-2 expression on severe dysplasia than in mild / moderate or squamous cell carcinoma.

Out of 60 cases of epithelial dysplasia 26 cases (43.3%)showed positive for bcl-2. In 20 cases of mild epithelial dysplasia 6 cases (30%) showed bcl-2 expression. In moderate epithelial dysplasia of 20 cases, 8 (40%) showed bcl-2 expressionwhile in 20 cases of severe epithelial dysplasia 12 cases (60%) showed bcl-2 expression. In squamous cell carcinoma group of 20 cases 8 (40%) showed bcl-2 expression.

The results in our studies were in accordance



with the studies of Singh B et al(1998)[11], Seagusa M et al(1995)[10],Sinicrop et al (1995)[12], McAlinden et al (2000)[8], Sulkoskam et al (2001).[13]

The possible explanation of increased expression of bcl-2 in severe dysplasia can be given as due to the process of cell division in the basal layer, all of the more superficial cells move towards the surface and undergo morphological & chemical changes (differentiation process). In the epithelium showing epithelial dysplasia there is a loss of this normal maturation sequence in the epithelium, so that all of the cell layers have a similar morphology, and there is no change or differentiation in the cells as they approach the surface. This lack of differentiation affects the entire thickness of the epithelium.# our data thus raise the possibility that bcl-2 over expression in severe epithelial dysplasia closely related to loss of cell differentiation. There is a variation in result in squamous cell carcinoma group of which may be attributed to the smaller sample size of our study.

But there is a statistical significant difference in immunoreactivity between severe epithelial dysplasia and squamous cell carcinoma, wherein squamous cell carcinoma showed low incidence of bcl-2 expression. This finding can be co-related with the fact that bcl-2 expression down regulates in terminally differentiated cells, as bcl-2 is a part of differentiation process serving to facilitate the apoptotic response,

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