

## Original Article

# Clinicopathological significance of PTEN and bcl2 expressions in oral squamous cell carcinoma

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**Abstract:** A high frequency of mutations at the PTEN locus has been noticed in carcinoma of oral. However, the role of PTEN alternations and its association with outcome variables in the genesis of oral carcinoma is not understood fully. The purpose of our study was to examine the impact of PTEN and Bcl2 in the genesis of Squamous cell carcinoma of oral. Total numbers of 60 histopathologically confirmed cases of Squamous Cell Carcinoma and 15 cases of inflammatory lesion of oral specimens were studied. We assessed PTEN and bcl2 overexpression by the use of anti-PTEN and anti-bcl2 antibody through immunohistochemistry as directed by the manufacturer. There was progressive loss of PTEN expression from inflammatory lesion to OSCC ( $p < 0.05$ ). Significant differences were found for PTEN expression between inflammatory lesion and OSCC. The difference in expression pattern of PTEN in gender did not reach statistical significance ( $p > 0.05$ ). The expression of bcl2 was found to be restricted to tumor cells in well and moderately differentiated tumors. The intense expression of bcl2 was observed throughout the tumor cell in poorly differentiated tumors. The Overexpression of bcl2 and loss of PTEN expression were correlated to poor differentiation, lymph node involvement and late stages. Thus, alteration of PTEN and bcl2 is likely an important molecular event in pathogenesis and carcinogenesis of oral carcinoma.

**Keywords:** Bcl-2, apoptosis, squamous cell carcinoma, IHC

## Introduction

Oral cancer is one of the most formidable health problems in terms of morbidity and mortality facing the mankind today. It is the sixth most common cancer worldwide accounting for 4% of all malignancies in men and 2% in women [1]. The major etiologic factors in the genesis of carcinoma of oral constitute tobacco chewing/smoking and alcohol consumption [2]. The high risk of human papillomaviruses (HPVs), have been implicated as important etiologic agents, particularly in the oral cancers with no tobacco or alcohol associations. Squamous cell carcinoma of oral (OSCC) is the most common type of oral cancer, is often preceded by a premalignant lesion. Premalignant lesions of oral show features of epithelial, moderate and severe epithelial dysplasia carry the highest risk for malignant transformation [3]. Identification of high risk premalignant lesions with increased sus-

ceptibility to oral cancer and consequent aggressive follow up for change of habit and early detection may help in down staging of the cancer and better prognosis [4]. Human oral cancers show a variety of genetic changes, with different changes in different tumors. PTEN (phosphatase and tensin homolog deleted on chromosome TEN), is a tumor suppressor gene mutated in a variety of human cancers including prostate, breast, brain [5], endometrial [6], glioblastoma [7], and melanoma [8]. PTEN expression has been down regulated in many malignancies such as cervical carcinoma (9), oral carcinoma [1] colorectal adenocarcinoma [11], breast cancer [12], colon cancer [13], and renal cell carcinoma.

Earlier investigator showing that induction of apoptosis by low levels of PIP-3 and phosphorylated Akt has been associated with high levels of PTEN in the genesis of breast carcinoma

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[14]. Conversely, loss of PTEN expression results in increased Akt activity and continued cell survival and cell proliferation.

Proliferation, apoptosis and differentiation are the fundamental aspects of tumor biology. Earlier studies have reported that mutation and overexpression of bcl2 in many tumors [15-18]. There is no evidence that bcl2 is expressed in normal oral mucosa [19]. It was only detected in basal cell, frequently seen adjacent to oral cancers and in dysplastic epithelium [20]. It cannot be excluded that bcl2 protein acts as a marker of a neoplastic transformation threatening in precancerous states. This study attempts to study the differential expression pattern of PTEN and bcl-2 protein for its relevance in development and progression of oral carcinoma.

### Materials and methods

#### *Study population*

A total of 60 (46 males and 14 females) of histopathologically confirmed cases of carcinoma of oral and 15 cases of inflammatory lesions of oral were assessed for PTEN and bcl2 expression. The age of the patients ranged from 24 – 76 years with a mean age of 58 years. The majority of the patients (n = 52) were diagnosed in advanced stages III/IV, with only eight patients in stages I/II, according to TNM classification. Histopathologically, the oral cancers were categorized as well differentiated –15 cases, moderately differentiated –20 cases and poorly differentiated –25 cases. The size of the tumor varied from 3cm (38 patients) to > 3cm (22 patients). The inflammatory lesions of oral (10 males and 05 females), 20–64 years of age (median age, 39 years) were taken as control.

Keeping the marker profile in view the cases were further divided according to age into two groups: Less than 50 years &  $\geq$  50 years.

#### *Immunohistochemical analysis*

Formalin fixed paraffin-embedded tissue blocks were cut in 5 microns thick serial sections. The sections were deparaffinized, rehydrated and rinsed in phosphate buffer saline (PBS). An Immunohistochemical assay for PTEN and bcl2 was performed on consecutive paraffin sections using streptavidin–biotin method

Monoclonal mouse antihuman antibody (PM 278, Biocare Medical, USA and PM 003 Biocare Medical, USA) used as primary antibodies for PTEN and bcl2 respectively. After antigen retrieval slides were incubated with primary antibody, followed by secondary biotinylated antibody. Sections were washed in PBS and then incubated with streptavidin peroxidase. Finally chromogen Diaminobenzidine (DAB) was used and section were counterstain with hematoxylin.

#### *Scoring method*

Tumors were classified as PTEN and bcl2 negative (i.e. low expression) if less than 10% of cells displayed positivity. If equal to or greater than 10% of cells were positive for PTEN and bcl2 (i.e. high expression) were considered as positive.

A total of 5-6 fields from each tissue section were chosen, and 100 cells from each field were counted at final magnification at 400X. With every batch of staining a positive and negative control were used to verify the standard of staining.

The percentage of PTEN and Bcl-2 positive cells was calculated independently by two pathologists. The correlations between PTEN and Bcl-2 expression and patient age, sex and histological grading were studied.

#### *Statistical analysis*

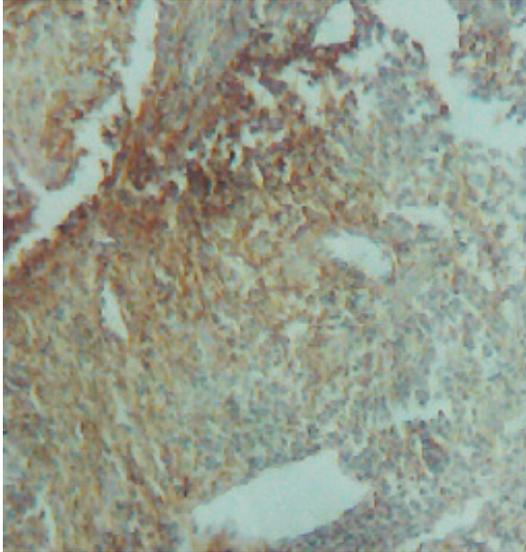
Chi square ( $\chi^2$ ) test was performed to find out the possible correlation among PTEN, bcl2 and other clinical parameters in oral cancer and inflammatory lesions of oral tissue. Statistical significance was defined as  $P < 0.05$ .

### Results

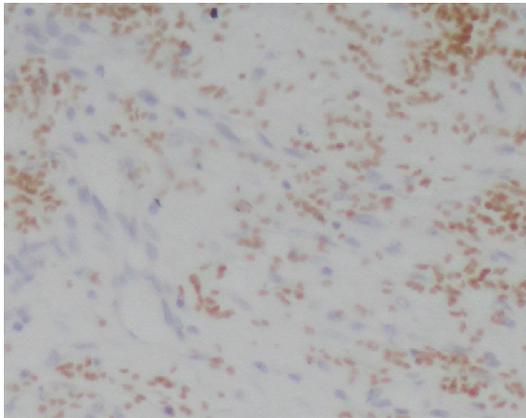
#### *Loss of PTEN protein expression in oral squamous cell carcinoma*

The expression of PTEN protein in inflammatory lesions of oral tissue and OSCC was primarily cytoplasmic and, less frequently, nuclear (**Figure 1** and **2**). We observed PTEN expression in 15 of 15 (100%) in inflammatory lesions of oral tissue and loss of PTEN expression in 34 of 60 (56.6%) in OSCC specimens (**Figure 3**, **Table 1**). Out of the total 60 cases of carcinoma: 10 cases (66%) of well differentiated carcinoma,

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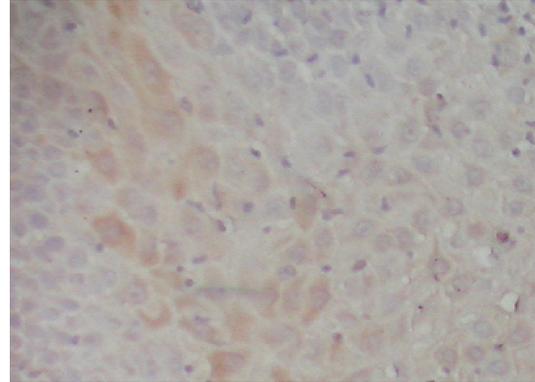


**Figure 1.** PTEN showing cytoplasmic expression in inflammatory lesions of oral (Original magnification x400).

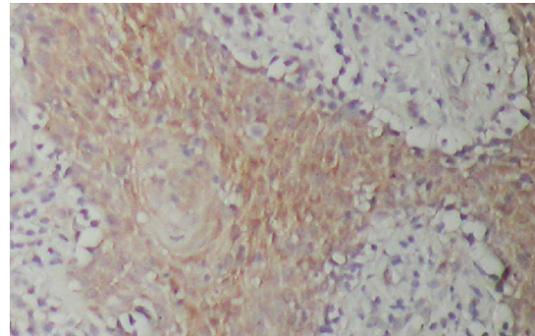


**Figure 2.** PTEN showing nuclear positivity in oral carcinoma (Original magnification x400).

12 cases (60%) of moderately differentiated carcinoma and 04 cases (16%) of poorly differentiated carcinoma was positive for PTEN (**Table 1**). When we compared the expression of PTEN of OSCC to inflammatory lesions of oral tissue, the loss of frequency of PTEN expression was statistically significant ( $P < 0.001$ ). With regard to tumor characteristics, PTEN expression was associated with poor differentiation, lymph node involvement, distant metastasis and late stages (**Table 1**). Keeping the marker profile in view the cases were further divided according to age into two groups: Less than 50 years &  $\geq 50$  years. The expressions of



**Figure 3.** Loss of PTEN expression in oral squamous cell carcinoma (Original magnification x400).



**Figure 4.** Bcl2 immunostaining showing expression in cytoplasm of carcinoma of oral (Original magnification x400).

PTEN were not associated with age, gender, and histological grade.

### *Immunohistochemical detection of bcl2 protein*

Cytoplasmic immuno reaction for bcl2 was considered positive and found in 31/60 cases (51.6%) of Oral carcinoma (**Figure 4**). Expression of bcl2 was found in tumor cell but not in the normal transitional epithelium. The result reveals the significant difference between the normal epithelium (0%) and cancerous tissue (51.6%) for the bcl2 protein ( $p < 0.05$ ). With the progression in tumor grade, the rate of bcl2 expression significantly increased ( $p < 0.05$ ).

Bcl2 expression was further correlated on the basis of sex and age of the patients. The expression of bcl2 was modified with gender and age. The patients based on the above, were divided into two groups  $< 50$  years and  $\geq 50$  years.

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**Table 1.** Correlation between PTEN, bcl2 and clinicopathological factors

Factors	Total no.	Pten+ve(%)	P-value	Bcl2+ve	P-value
Age					
≤50	17	9(52)	p>0.05	8(47)	p>0.05
>50	33	17(51)		23(69.6)	
Male	46	20(43.4)	p>0.05	26(56.5)	p<0.05
Female	14	6(42.8)		5(35.7)	
Well differentiated carcinoma	15	10(66)	p<0.05	5(33)	p<0.05
Moderately differentiated	20	12(60)		10(50)	
Poorly differentiated carcinoma	25	4(16)		16(64)	
Stage					
I	3	1(33)	P<0.05	0(0)	p<0.05
II	5	2(40)		1(20)	
III	24	10(41.6)		13(54)	
IV	28	13(46.4)		17(60)	
Lymphnode involvement No	28	10(35.7)	P<0.05	11(39)	p<0.05
N1+N2	32	16(50)		20(62)	

**Table 2.** Intensity of Bcl-2 expression in Carcinoma of oral

Serial no.	Cases	No of positive cases	10-25%	26-50%	>50%
1.	Well differentiated carcinoma	5	2	2	1
2.	Moderately differentiated carcinoma	10	2	3	5
3.	Poorly differentiated carcinoma	16	4	4	8

**Table 3.** Relationship between bcl2, sex and age

Sex	Age	Bcl2 Positivity			P-Value
		Total cases	Positive cases	Percentage	
Male	<50	12	6	50%	P<0.05
	≥50	23	20	86%	
Female	<50	5	02	40%	P>0.05
	≥50	10	03	33%	

Among females, there was no significant difference of bcl2 expression between the two age groups (< 50 and ≥ 50 years). However; among males with the age ≥ 50 years the expression of bcl2 was significantly higher (86%) as compared to the < 50 years of age group (50%) (p<0.05) (**Table 3**).

The expression of bcl-2 was found to be restricted to tumor cells in well and moderately differentiated tumors. There is no expression or undetectable expression of bcl2 in basal cells. The intense expression of bcl2 was observed throughout the tumor cell in poorly differentiated tumors. The bcl2 overexpression were found in 31/60 oral cancers that were bcl-2 positive, 8(26%) cases were positive with 10-25%, 9(29%) cases were 26-50% and 14 (45%) cases were more than 50 percentage ; while in 15/15 oral lesions that were bcl-2 negative, all the cases were less than 10% (**Table 2**). The

association between Bcl-2 expression and some clinicopathological features are presented in **Table1**.

### *The relationship between PTEN and bcl2 expression*

When we compared bcl2 and to tumor characteristics, bcl2 and PTEN expression were associated with poor differentiation, lymph node involvement and late stages (**Table 1**). A significant negative correlation (P<0.05) was observed between bcl2 overexpression and loss of PTEN expression. Therefore, we speculate that tumor prognostic features correlated with bcl2 and PTEN expression in OSCC.

### **Discussion**

Oral cancers represent 4% of all cancers in the West, whereas, in developing countries, it accounts for up to 45% of all cancers [21].

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Though there are many studies on the etiology of cancer but the exact pathogenesis still remains uncertain. The major etiologic factors in the development of oral cancer constitute tobacco chewing/smoking and alcohol consumption [22, 23]. The incidence of oral cancer has been reported to increase with the increase in age. In our study the peak incidence of oral cancer was observed in the age group of 50-70 years. The reasons for this remain unclear, but it might be due to the cumulative effects of long time exposures to carcinogens, the failings of DNA repair mechanisms and aging [24].

In our study, much higher incidence of tumors was noted in males compared with females. The variation of incidence of oral cancer in gender may be due to the variation in the environmental, dietary exposures, innate sexual characteristics and tobacco chewing, smoking and alcohol intake are higher in men than women; it is also known that men acquire these habits earlier than women.

The identification of prognostic and predictive markers is clinically important, because oral cancer is a group of heterogenous diseases with various biological and clinical characteristics. C-erbB2 status as determined by IHC, have been used as predictive markers and prognostic factors. Recently, molecular studies showed that a strong prognostic power, but immunohistochemistry remains a convenient and powerful means of prognostication in a clinical setting as it is less expensive and easier to perform. The present study highlights the importance of PTEN and bcl-2 in the genesis of carcinoma of oral. Loss of PTEN function and/or overexpression of Bcl-2 occur frequently in prostrate cancer and OSCC [25-30]. Our present understanding of the molecular mechanisms underlying the role of Bcl-2 and its overexpression in oral carcinoma is very scanty. In this study, we analyse the expression of PTEN and Bcl-2 in oral squamous cell carcinoma and inflammatory lesions of oral. Earlier investigator showed that expression status of Bcl-2 or PTEN in oral cancer [31, 32]. However, to our knowledge, this is the first study to report that Bcl-2 expression is inversely correlated with PTEN loss in oral carcinoma. This observation show that a mechanism by which Bcl-2 is up-regulated in the development and progression of oral carcinoma. Such a mechanism would include loss of PTEN activity, with the concomi-

tant activation of Akt. The upregulation of AKT ultimately reduces the apoptotic induction thereby contributing to the tumorigenesis [33]. The present study reveals that loss of PTEN expression in OSCC clinical specimens is significantly correlated with bcl2 overexpression. The study also shows that loss of PTEN expression and bcl2 overexpression are significantly correlated with oral cancer staging, suggesting that both of the activities may play an important role in oral cancer development and progression. Earlier investigator showed that loss of PTEN increased remarkably according to disease stage and lymph node metastasis. Our study showing that *PTEN* gene alteration is associated with advanced stage and lymph node metastasis. Our results suggest that *PTEN* may play an important role in the regulation of tumor progression and metastasis during the development of oral carcinoma. But the exact role of PTEN in the genesis of oral carcinoma remains unknown. Studies on PTEN in OSCC showed frequent genetic alterations and loss of expression but still there are a lot of discrepancies toward the drawing of the final conclusion. Keeping the marker profile in view the expression pattern was analyzed according to age, gender and histological grade. The difference in expression pattern of PTEN in gender, age and histological grade did not reach statistical significance ( $p \geq 0.05$ ). However; in males with the age  $\geq 50$  years the expression of bcl2 was significantly higher (86%) as compared to the  $< 50$  years of age group (50%) ( $p < 0.05$ ). This analysis showed bc2 expression is increased in high grade tumors in comparison with tumors of low grade malignancy and this was statistically significant. Earlier investigator reported similar observation that apoptosis was high in high grade of tumors [34], suggesting that although tumor show high proliferative activity, leading to cellular turnover in these tumors. In the present study, the loss of PTEN expression was significantly associated with overexpression of bcl2 and a similar finding was reported in glioblastoma [35]. Earlier study demonstrates that loss of PTEN leads to up-regulation of the Bcl2 gene, thus contributing to survival of cancer cells in prostate cancer [36]. Our results also showed the strong association of loss PTEN expression with Bcl2 positive expression ( $p < 0.05$ ) in oral carcinoma.

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