BCL2 expression in OPMD and OSCC - An immunohistochemical study on 70 samples.

Manish Kumar Munjal[1], Karthikeyan Ramalingam [2*], Sandeep Singh Sihmar[3], Kaajal Gill[4], Mohd Ikhlas Khan[5].

[1]Senior lecturer, Department of Oral Pathology and Microbiology, Surendera Dental College and Research Institute, Sriganganagar, Rajasthan, India. Email: manish_munjal29@yahoo.com

[2]Professor and Head, Department of Oral Pathology and Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamilnadu, India, Email:karthikeyanr.sdc@saveetha.com

[3]Senior lecturer, Department. of Oral Pathology and Microbiology, Daswani Dental College, Kota, Rajasthan, India, Email:sssihmar10@yahoo.co.in

[4]Department of Oral Pathology and Microbiology, Surendera Dental College and Research Institute, Sriganganagar, Rajasthan, India.Email:kaajalgill@gmail.com

[5]Senior lecturer, Department. of Oral Pathology and Microbiology, Daswani Dental College, Kota, Rajasthan, India, Email:drikhlaskhan2512@gmail.com

Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common malignancy in India. Majority of OSCC occurs in relation to various oral potentially malignant disorders (OPMD). Most patients report in advanced stages due to lack of awareness and delay in biopsy. Immunohistochemistry (IHC) could be used to identify the genetic alterations influencing proliferation and apoptosis that accumulate in OPMD and may transform it into OSCC. Bcl2 is one such molecule that enhances cell survival and reduces apoptosis.

Aims & Objectives: Our study aimed to determine and compare the expression of Bcl-2 in OPMD and OSCC.

Materials and methods: 70 samples were retrieved from the department archives including 10 normal mucosa, 30 cases of OPMD and 30 cases of OSCC. The number of Bcl-2 positive cells was counted in 50 cells of each field by two independent observers. Cases were assigned to one of the following categories: 0% positive cells (-), 10% positive cells (+), 10-25% positive cells (++), 26-50% positive cells (+++) or more than 50% positive cells (++++). They were scored as: 0% positive cells (-), 10% positive cells (+), 10-25% positive cells (++), 26-50% positive cells (+++) or more than 50% positive cells (++++). The data were tabulated and analyzed using CROSSTABS procedures, Mean ± SD, Mann-Whitney's U-test and Chi Square test using SPSS 15.0 (SPSS INC, Chicago, IL).

Results: Mean Bcl-2 expression in 10 Normal Mucosa (N) samples with the Mean values of 0.0824 with the Standard Deviation values of 0.127. In 30 OPMD samples the Mean value was 0.0397 with the Standard Deviation value of 0.083 and in the 30 OSCC samples the Mean value was 0.0301 with the Standard Deviation value of 0.060.

Summary: We found increased Bcl2 expression in basal and parabasal cells of normal epithelium but progressive decrease in its expression in OPMD and in OSCC. Though our study did not achieve statistical significance, multi-centre studies on this expression will be helpful to understand the role of this anti-apoptotic molecule.

Keywords: OSCC, OPMD, Bcl2, Apoptosis

DOI: 10.47750/pnr.2022.13.S04.138

INTRODUCTION

The oral cavity reflects personal health as a mirror. It can reveal deleterious habits like tobacco usage and systemic diseases like diabetes. The oral mucosa is under constant renewal and many cells undergo programmed cell death termed as Apoptosis derived from a Greek word that means "falling off" as leaves from a tree and ptosis that means "to fall". 1,2

The term apoptosis (AP) was proposed by Kerr and colleagues in 1972 to describe a specific morphological pattern of cell death observed and characterized by nuclear and cytoplasmic condensation and cellular fragmentation into membrane-bound apoptotic bodies, that were taken up by other cells and degraded within phagosomes2. AP occurs normally during development, aging and as a part of homeostasis. It can also be a defense mechanism such as in immune reactions or cell damage by disease or noxious agents. Fas or TNF receptor expression can induce AP through ligand binding and protein cross-linkage. Some cells are programmed to survive only with the presence of certain hormones or growth factors. AP should be differentiated from

necrosis, two processes that can occur independently, sequentially, as well as simultaneously. AP is an energy-dependent mechanism that involves the activation of caspases and a complex chain of events that link the initiating stimuli to the final demise of the cell3.

Excessive or insufficient AP can be a significant component of diseases such as cancer, AIDS, ischemia, and neuro-degenerative diseases 3. BCL2 promotes cell survival and it was discovered as the chromosomal translocation at t14;18.4 Tumor cells can resist AP by the expression of anti-apoptotic proteins such as Bcl-2 or by the down-regulation or mutation of pro-apoptotic proteins such as Bax. The expression of both Bcl-2 and Bax is regulated by the p53 tumor suppressor gene3.

Pro- and antiapoptotic BCL-2 family proteins have functions other than regulating apoptosis and that these functions are important for normal physiology of healthy cells 4. BCL2 family members possess up to four conserved BCL-2 homology (BH) domains designated BH1, BH2, BH3, and BH4 .5

The Indian subcontinent accounts for one third of the global burden of cancers of lip and oral cavity. Cumulative Incidence Rate, Cumulative Risk and Possibility of one in a number of Persons Developing Cancer of Any Site (ICD-10) is 6.1 to 8.1 in people aged 0-64 years, 10.5 to 11.7 in aged 0-74 years. 38.2% of males and 13% of females had tobacco-related cancers with higher incidence of cancer involving tongue and mouth. As per National Cancer Registry, Age adjusted incidence rates (per 100000) for people in Patiala district are, Tongue cancer is 4.4 in males, 1.2 in females. Mouth cancer is 4.4 in males and 1.3 in females.6

Oral squamous cell carcinoma (OSCC) is the most prevalent type of cancer accounting for 91% of the diagnosed cases of oral malignancies. The risk factors for oral cancers are closely related to lifestyle, such as tobacco use, alcohol use, poor oral hygiene and constant irritation. Clinico-pathologically, malignant transformation of oral potentially malignant disorders (OPMD) is observed in up to 17.5% of the cases. The prognosis for early treatment of patients with OSCC is much better, with 5-year survival rates as high as 80%. In addition, the quality of life also improves after early treatment7.

A significant proportion of OSCC develops from OPMD like leukoplakia, erythroplakia, oral sub mucous fibrosis, palatal lesions in reverse smokers, lichen planus, and discoid lupus erythematosus. Molecular biological markers of proliferation, epithelial differentiations and genomic changes could potentially improve the prognostic evaluation of OPMD and OSCC. The latest WHO monograph on head and neck tumors (2005) uses the term ‘epithelial precursor lesions’ and defined it as altered epithelium with an increased likelihood for progression to squamous cell carcinoma. 5% - 43% of leukoplakia cases progress to invasive OSCC and it is related to severity of the dysplastic changes. Patients with OSMF are at least 19 times more likely to develop OSCC than healthy people 8-14. OSCC can occur on a syphilitic gumma as well as on a leukoplakia but is very rare15.

Immunohistochemistry (IHC) utilizes antibodies (monoclonal/polyclonal) to determine the distribution of tissue antigens of interest in both health and disease. Using specific markers, IHC is used for cancer diagnosis, tumor staging and grading, typing of metastatic tumors16.

OSCC pathogenesis is due to progressive accumulation of genetic alterations in AP mechanism. Abnormal bcl-2 protein expression is an indicator of blocked apoptosis thus promoting prolonged cell survival, which facilitates acquisition of mutations and malignant transformation17. Bcl-2 is expressed in basal cell layers of normal epithelium and stem cell zones. Bcl-2 overexpression has been noted in the initial stage of malignant transformation of cells18.

OPMDs are often missed due to lack of awareness, minimal symptoms, neglect and lack of diagnostic biopsy. Detection of apoptotic abnormalities before the consequences become clinically or histologically detectable will greatly enhance the potential for early diagnosis.

Hence, we studied the differential expression of bcl-2 protein in OPMD and in OSCC cases that could throw light on carcinogenesis.

Materials And Methods

OPMD and OSCC cases from the Archives of Department of Oral and Maxillofacial Pathology and Oral Microbiology, Surendera Dental College and Research Institute, Sriganganagar, Rajasthan were retrieved.

The inclusion criteria were Histopathologically diagnosed cases of OPMD and OSCC with tissue blocks of adequate size (minimum of 5mm). The exclusion criteria were OPMD of sites other than oral cavity proper like oropharynx, maxillary sinus etc, Carcinomas of sites other than oral cavity proper like oropharynx, maxillary sinus etc and Tissue blocks without adequate size (less than 5 mm).

A total of 70 samples including 10 normal oral mucosa, 30 OPMD and 30 OSCC cases were included in the study.

Formalin fixed paraffin embedded tissue sections was considered for immunohistochemical analysis using monoclonal antibodies to Bcl-2 protein (Polymer IHC Detection Kit (Biogenex; CA, USA) using EZ Retriever system V.2.1, Biogenex; USA.

3µ sections were made with a Yorco YSI-062 Fully automatic rotary microtome. It was stained routinely with Hematoxylin and Eosin as per manufacturer instructions. The stained slides were reevaluated and reconfirmed for the histopathological diagnosis of OPMD and OSCC by 2 independent oral pathologists. Immunohistochemical staining for BCL-2 was performed with Primary Antibody: Anti BCL-2 100 Monoclonal (AM287-5M), Biogenex; CA, USA and Chromogen: Liquid DAB (3.3'-diaminobenzidine) chromogen solution; 4 ml, Biogenex; CA, USA. For each batch of staining, a positive control and a negative control was used by replacing the primary antibody with a negative control buffer.

The stained slides were interpreted by two oral pathologists for positivity shown as brown staining due to DAB chromogen. H and E stained slides were used for comparison of IHC stained slides results. The fields containing artifactual changes such as chatter, tears etc were omitted from the study. Any non-specific positivity was not taken into account.

The slides were analysed using the binocular Labomed research microscope (LX400), digital camera, Desktop or a Laptop computer, PIXELPRO software (LABOMED Inc, USA) and DIGIMER image analysis software (Medcalc software BVBA, Belgium)

Quantitative evaluation of Bcl-2 positive cells

Briefly, in an optical microscope, hot-spot areas for Bcl-2 expression within epithelial cells were initially identified by scanning the entire section in low power (10x). Based on the hotspot areas under low power, the number of Bcl-2 positive cells in five of these areas was then counted in high power magnification (40x).

Presence of brown-colored end product at the site of target antigen was indicative of positive reactivity. The negative control does not show specific staining. The positive control used was a section of tonsil. The number of Bcl-2 positive cells was counted in 50 cells of each field by two independent observers.

Cases were assigned to one of the following categories: 0% positive cells (-), 10% positive cells (+), 10-25% positive cells (++), 26-50% positive cells (+++) or more than 50% positive cells (++++).

The data were tabulated and analyzed using CROSSTABS procedures, Mean ± SD, Mann-Whitney's U-test and Chi Square test. A probability of $p<0.05$ was considered as significant. SPSS 15.0 (SPSS INC, Chicago, IL) was used for statistical analysis.

Results

Table 1: shows that the study group comprised a total of 70 samples which included 10 normal oral mucosa (N), 30 potentially malignant disorders (PMD) and 30 Oral squamous cell carcinoma (OSCC). It was observed that all the samples tested for the expression of Bcl-2, showed Immunopositivity.

S. No	Normal Mucosa (n=10)	Potentially Malignant Disorders (n=30)	Oral Squamous Cell Carcinoma (n=30)	Score (BCL-2)
1	0	0	0	-
2	08	27	28	+
3	01	02	01	++
4	01	01	01	+++
5	0	0	0	++++

(- negative, + <10%, ++10-25%, +++26-50%, ++++>50% Positive cells)

Table 2: shows the comparison between N and OPMD. Among 40 samples, 35 samples scored 2 (N-8, OPMD-27), 3 samples scored 3 (N-1, OPMD-2) and 2 samples scored 4 (N-1, OPMD-1).

Count				
		VAR00002		Total
		1.00	2.00	
VAR00001	2.00	8	27	35
	3.00	1	2	3
	4.00	1	1	2
Total		10	30	40

Table 3: shows the Pearson Chi-Square Test for the N Vs PMD showed the value of 0.863 with Degree of freedom value as 2. The P value was 0.649 which did not achieve statistical significance.

Chi-Square Tests			
	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.863a	2	.649
Likelihood Ratio	.767	2	.681
Linear-by-Linear Association	.831	1	.362
N of Valid Cases	40		

a. 4 cells (66.7%) have expected count less than 5. The minimum expected count is .50.

Table 4: shows a comparison between N and OSCC. Out of a total 40 samples, 36 samples scored 2 (N-8, OSCC-28), 2 samples scored 3 (N-1, OSCC-1) and 2 samples scored 4 (C-1, OSCC-1).

Count				
		VAR00002		Total
		1.00	2.00	
VAR00001	2.00	8	28	36
	3.00	1	1	2
	4.00	1	1	2
Total		10	30	40

Table 5: shows the Pearson Chi-Square Test for the between N and OSCC showed the value of 1.481 with Degree of freedom value as 2. The P value was 0.477 which did not achieve statistical significance.

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.481a	2	.477
Likelihood Ratio	1.303	2	.521
Linear-by-Linear Association	1.286	1	.257
N of Valid Cases	40		
4 cells (66.7%) have expected count less than 5. The minimum expected count is .50.			

Table 6: shows the comparison between OPMD and OSCC. Out of 60 samples, 55 samples scored 2 (OPMD-27, OSCC-28), 3 samples scored 3 (OPMD-2, OSCC-1) and 2 samples scored 2 (OPMD-1, SCC-1).

Count				
		VAR00002		Total
		1.00	2.00	
VAR00001	2.00	27	28	55
	3.00	2	1	3
	4.00	1	1	2
Total		30	30	60

Table 7: shows the Pearson Chi-Square Test for the between PMD and OSCC showed the value of 0.352 with Degree of freedom value 2. The P value is 0.839 which did not achieve statistical significance.

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.352a	2	.839
Likelihood Ratio	.358	2	.836
Linear-by-Linear Association	.097	1	.756
N of Valid Cases	60		
4 cells (66.7%) have expected count less than 5. The minimum expected count is 1.00.			

Table 8: shows the comparison of Score between the Control, Potentially Malignant Disorders and Oral Squamous Cell Carcinoma using the Chi-Square Test. The X2 value for the Control Vs Potentially Malignant disorder was 0.863 and P value was 0.649 which did not achieve the significant statistical values. The x2 value for the Control Vs OSCC was 1.481 and the P value was 0.477 which also did not achieve the significant statistical values. The x2 value for the Potentially Malignant Disorders Vs OSCC was 0.352 and the P value was 0.839 which also did not achieve the significant statistical values.

Comparison	x2 value	P value	Significance
Normal Mucosa vs Potential Malignant	0.863	0.649	Not Significant
Normal Mucosa vs OSCC	1.481	0.477	Not Significant
Potential Malignant vs OSCC	0.352	0.839	Not Significant

Table 9: shows that out of total 40 samples, 10 N samples showed the Mean value as 24.55 and 30 PMD samples showed the Mean 19.15.

Mann-Whitney Test

Ranks				
	Group	N	Mean Rank	Sum of Ranks
BCL_Avg	Controls	10	24.55	245.50
	Pot. Malignant	30	19.15	574.50
	Total	40		

Table 10: shows the Mann- Whitney U test to compare the BCL2 expression in the samples of N and PMD. The average value was 109.500.P value was 0.205 which did not achieve statistical significance.

Test Statistics b	
	BCL_Avg
Mann-Whitney U	109.500
Wilcoxon W	574.500
Z	-1.268
Asymp. Sig. (2-tailed)	.205
Exact Sig. [2*(1-tailed Sig.)]	.209a
a. Not corrected for ties.	
b. Grouping Variable: Group	

Table 11: shows that out of a total 40 samples, 10 N samples showed the Mean value as 25.10 and 30 OSCC samples showed the Mean 18.97.

Ranks				
	Group	N	Mean Rank	Sum of Ranks
BCL_Avg	Controls	10	25.10	251.00
	OSCC	30	18.97	569.00
	Total	40		

Table 12: shows the Mann-Whitney U test to compare the BCL2 expression in the samples of N and OSCC. The average value was 104.000. The P value was 0.150 which did not achieve statistical significance.

Test Statistics b	
	BCL_Avg
Mann-Whitney U	104.000
Wilcoxon W	569.000
Z	-1.441
Asymp. Sig. (2-tailed)	.150
Exact Sig. [2*(1-tailed Sig.)]	.158a
a. Not corrected for ties.	
b. Grouping Variable: Group	

Table 13: shows that out of total 60 samples, 30 PMD samples showed the Mean value as 30.87 and 30 OSCC samples showed the Mean 30.13.

Ranks				
	Group	N	Mean Rank	Sum of Ranks
BCL_Avg	Pot. Malignant	30	30.87	926.00
	OSCC	30	30.13	904.00
	Total	60		

Table 14: shows the Mann-Whitney U test to compare the BCL2 expression in the samples of PMD and OSCC. The average value was 439.000. The P value was 0.870 which did not achieve statistical significance.

Test Statistics a	
	BCL_Avg
Mann-Whitney U	439.000
Wilcoxon W	904.000
Z	-.163
Asymp. Sig. (2-tailed)	.870
a. Grouping Variable: Group	

Table 15: Descriptive Table

Group	N	Mean BCL-2	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Controls	10	0.0824	0.127	0.040	-0.008	0.173	0.0020	0.4260
Potentially Malignant	30	0.0397	0.083	0.015	0.009	0.071	0.0020	0.4120
OSCC	30	0.0301	0.060	0.011	0.007	0.053	0.0020	0.3220
Total	70	0.0417	0.082	0.009	0.022	0.061	0.0020	0.4260

Table-15 shows the Mean Bcl-2 expression in 10 Normal Mucosa (N) samples with the Mean values of 0.0824 with the Standard Deviation values of 0.127. In 30 Potentially Malignant Disorders (PMD) samples the Mean values was 0.0397 with the Standard Deviation value of 0.083 and in the 30 Oral Squamous Cell carcinoma (OSCC) samples the Mean value was 0.0301 with the Standard Deviation value of 0.060. For the total 70 samples, the Mean value for Bcl-2 expression was 0.0417 with the Standard Deviation value of 0.082.

Table 16: Descriptive table

Anova

		Sum of Squares	df	Mean Square	F	Sig
Between People		.940	69	.014		
Within People	Between Items	.000	1	.000	.100	.753
	Residual	.042	69	.001		
	Total	.042	70	.001		
Total		.982	139	.007		

Grand Mean = .0417

Table-16 shows the one-way ANOVA test which was conducted to compare the Bcl-2 expression between Normal Mucosa, Potentially Malignant Disorders and Oral Squamous cell carcinoma. It was not significant with the P value of 0.753 [F (1,69) = 0.100].

Table 17: Descriptive table

Intraclass Correlation Coefficient							
	Intraclass Correlationa	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df 1	df 2	Sig
Single Measures	.915b	.866	.946	22.427	69	69	.000
Average Measures	.955c	.928	.972	22.427	69	69	.000

Table 17 shows the Intraclass Correlation Coefficient of Bcl-2 expression among the study samples between the two observers was high with the P value of 0.000.

Two-way mixed effects model where people effects are random and measured effects are fixed.

a. Type C intraclass correlation coefficients using a consistency definition-the between-measure variance is excluded from the denominator variance.

b. The estimator is the same, whether the interaction effect is present or not.

c. This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

T-Test

Table 18: Descriptive table

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	BCL_Ob1	.0410	70	.09243	.01105
	BCL_Ob2	.0423	70	.07541	.00901

Table 19: Descriptive table

Paired Samples Correlations				
		N	Correlation	Sig.
Pair 1	BCL_Ob1 & BCL_Ob2	70	.934	.000

Table-19 shows Paired Sample Correlation which revealed that Bcl-2 expression in the study samples as scored by both observers was significantly positively correlated.

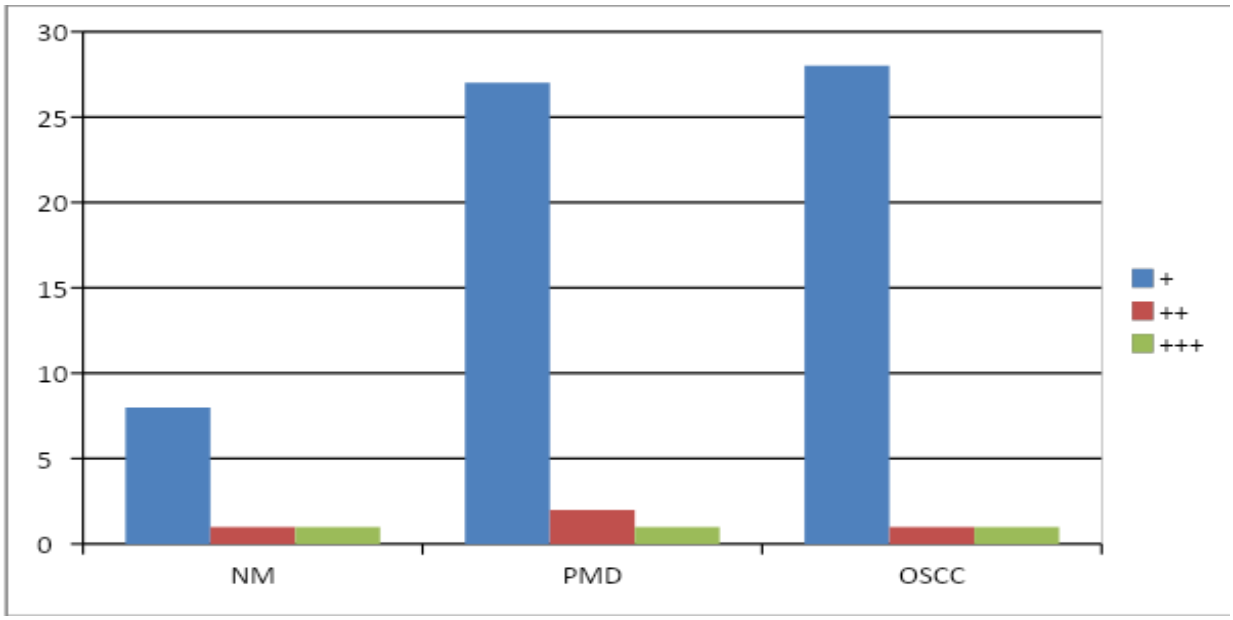
Table 20: Descriptive table

Observers	Mean±SD	T -test	P value
Ob-1	0.41±0.92	-0.315	0.753
Ob-2	0.42±0.75		

Table shows-20 the Paired Sample Test, it reveals that the Mean Bcl-2 expression in the study samples found by both observers were 0.41, 0.42 respectively, but it was statistically insignificant with the P value of 0.753.

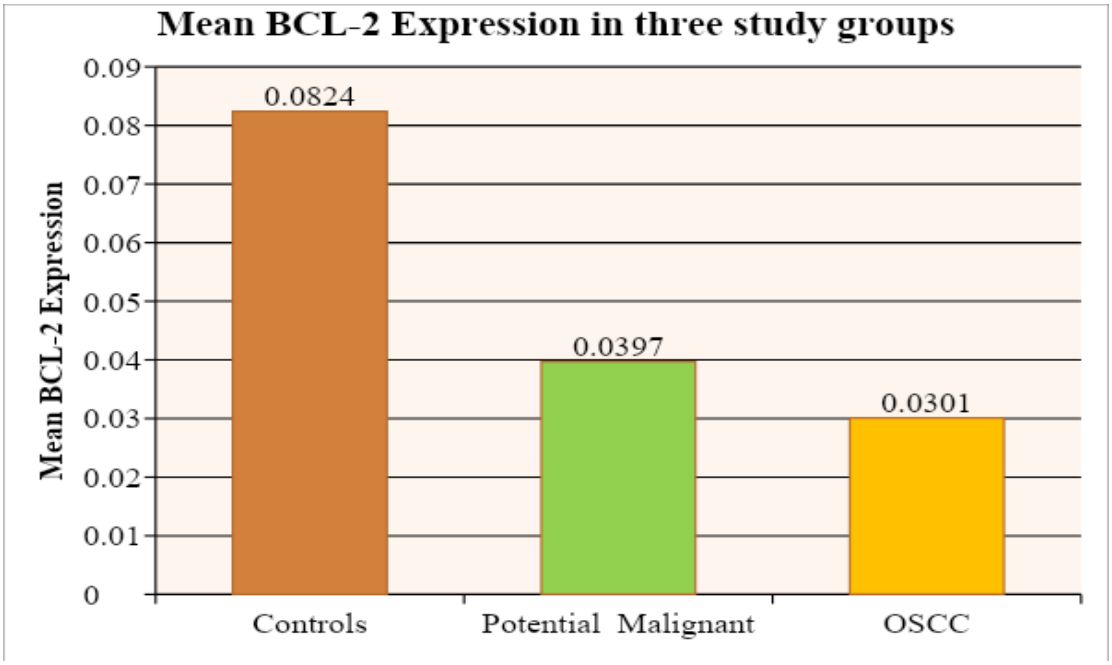
Table 21: Descriptive table

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	BCL_Ob1 - BCL_Ob2	-.00131	.03486	.00417	-.00963	.00700	-.315	69	.753



(NM- Normal oral mucosa, PMD- Potentially malignant disorder, OSCC- Oral squamous cell carcinoma, + <10%, ++ 10-25%, +++26-50%, ++++>50% positive cells)

Graph-1 shows the Bcl-2 positivity in the Normal Oral mucosa, PMD and OSCC



Graph-2 shows the mean Bcl-2 expression in the c Normal Mucosa, OPMD and OSCC.

Discussion

Apoptosis may be induced via transmembrane receptors of the tumour necrosis factor (TNF) receptor family such as Fas (CD95/APO-1 and tumor necrosis factor receptor 1 (TNFR1/TNFRp55). Bcl-2 protein is demonstrated in a wide variety of normal tissues, including normal oral epithelium. In human solid tumors, bcl-2 is described in a wide variety of cancers, including head and neck carcinomas and oral carcinomas 18-22.

The current Working Group, therefore, did not favour subdividing precancer to lesions and conditions and the consensus view was to refer to all clinical presentations that carry a risk of cancer under the term 'potentially malignant disorders' to reflect their widespread anatomical distribution11.

In the cancerization field, multiple oral cancers may develop from independent cell clones. This hypothesis has been supported by data from chromosome X inactivation studies, microsatellite analysis, and p53 mutational analysis. However, more recent genetic studies suggested that multiple cancers can be clonally related and derived from expansion of an original clone 10.

Recent studies on cancer have shown that the process of carcinogenesis may involve not only increased cell proliferation but also decreased cell death (apoptosis) or increased cell survival. Apoptosis is regulated by a number of genes and plays an important role in morphogenesis, homeostasis and cancer regression. Apoptosis regulatory genes include the Bcl-2 gene family, which codes for both pro-apoptosis and anti-apoptosis proteins. However, in the literature, there are conflicting reports on the clinical significance and prognostic value of these proteins in cancers. The purpose of this study was to investigate the expression of the apoptosis-related proteins bcl-222.

This retrospective study was carried out in the Department of Oral & Maxillofacial Pathology and Oral Microbiology, Surendera Dental College and Research Institute, Sriganganagar, Rajasthan, India on Paraffin embedded blocks of histologically diagnosed cases of Normal Oral Mucosa (N), OPMD and OSCC with Bcl-2 monoclonal antibody from Biogenex; CA, USA. Loro LL et al22, Nair R G et al30, Thomas S A et al45 and Sadiq H et al38 had performed similar studies.

Popovic B et54 al, Camisasca DR et al53, Ahmed MM et al52, Suri C et al51, Desosa FACG et al44, Ranganathan K et al24, Sudha VM et al17, Leyva- Huerta ER et al29 , Martinez AB et al42, Arreaza AJ et al41, Shailaja G et al39, Arya V et al25, Helal DS et al18, Camillo CMC et al50 and Pavic I et al19 have used the Bcl-2 antibody from DAKO, Denmark in their studies.

Rehmani A et al47, and Grewal J et al46 used the Bcl-2 antibody from Biocare, USA. Xie X et al58 and Loro LL et al22 used the Bcl-2 antibody from Santa Cruz Biotechnology, Santa Cruz, CA and Nafarzadeh S et al27 used the Bcl-2 antibody from Sigma-Aldrich, St. Louis, Missouri, USA in their research work.

The present study comprised a total retrieved 70 Paraffin embedded blocks of histologically diagnosed samples, 10 of Normal

Oral mucosa and 30 PMD (7 mild, 6 moderate, 15 severe Dysplasia) and 30 OSCC (13 Well Differentiated, 17 Moderately Differentiated). Similarly, Pavic I19 et al studied 20 Normal oral mucosa, 20 OSCC and 50 Oral lichen planus (OLP) samples. Nafarzadeh S et al27 studied 29 Normal, 10 OSCC and 30 cases of OLP. Leyva- Huerta ER et al29 studied 4 Normal, 16 OSCC and 21 OLP cases. Kipli NP et al20 studied 4 Normal, 10 OSCC and 50 Leukoplakia cases. Fan Y et al21 studied 10 Normal, 22 OSCC, 23 Leukoplakia and 18 OLP, Piattelli A et al32 studied 10 Normal, 12 Leukoplakia, 12 Epithelial Dysplasia (6 mild, 6 severe) and 36 OSCC (12 Well-differentiated, 12 Moderately Differentiated, 12 Poorly differentiated) and Schoelchg ML et al35 studied 90 Paraffin embedded blocks of 25 patients which included 8 Normal, 8 severe Dysplasia to Moderately differentiated OSCC, 17 cases of Focal Keratosis, Mild and Moderate Dysplasia.

Ranganathan K et al24 included 10 Normal, 50 Oral submucous fibrosis (OSF) and 10 OSCC patients with the written consent in the study. Shailaja et al39 carried out a cross sectional study among 70 samples, including 10 Normal healthy mucosae, 30 OLP and 30 Oral epithelial dysplasia (OED).

In the present study the slides were subjected to IHC staining for the BCL-2 immunoexpression. In an optical microscope, hot-spot areas for Bcl-2 expression within basal and parabasal epithelial cells were initially identified. Similarly, Jordan R C K et al61, Singh BB et al23, Loro LL et al22, Piattelli A et al32, sudha VM et al17, Ranganathan K et al24, Nafarzadeh S et al27, Juneja S et al26, Pigatti FM et al40 and Shailaja G et al39 also identified Bcl-2 positive cells in the Basal and Parabasal layers of epithelium. Arreaza AJ et al41 assessed the positive Bcl-2 cells in the Basal, suprabasal and submucosa. Helal DS et al18 assessed the positive Bcl-2 cells at both tissue and cellular level.

To eliminate the subjective bias, two observers independently evaluated the expression of Bcl-2 in the study sample. Similarly, Jordan R C K et al61, Suri C et al51 and Rahmani A et al47 also applied the same criteria in their study. Shailaja G et al39 and Juneja S et al26 evaluated the positive cells by a single observer. In the present study based on the hotspot areas under low power, the number of Bcl-2 positive cells in five of these areas was then counted in high power magnification (40x) using the binocular microscope attached to a computer with image analysis software. The number of Bcl-2 positive cells was counted in 50 cells of each field by two observers. Similarly, Garewal J et al46 in 2014 counted 50 cells of each field. Suri C et al51, Nafarzadeh S et al27 and Shailaja G et al39 counted the positive cells in five high power fields.

Birchall M. A et al36, Piattelli A et al32 and Popovic B et al54 counted 1000 cells per slide to evaluate the positive Bcl-2 cells. Suri C et al51, Rahmani A et al47, and Arya V et al25 evaluated 100 cells per sample.

In the present study the percentage of stained cells were estimated in 5 randomised microscopic fields and classified as (-): 0%, (+): <10%, (++): 10-25%, (+++): 26-50%, (++++): >50% positive cells. In the Normal mucosa out of 10 samples, 8 (80%) showed +, 1 (10%) showed ++ and 1 (10%) showed +++ grading, none of sample showed ++++ grading. In OPMD out of 30 samples, 27 (90%) showed +, 2 (6.66%) showed ++, 1 (3.33%) showed +++ and none of the sample showed ++++ grading. In OSCC out of 30 samples 28 (93.33%) showed +, 1 (3.33%) showed ++, 1 (3.33%) showed +++ and none of the sample showed ++++ grading. Nafarzadeh S et al27 used the same criteria.

Some other authors used a different gradient for the percentage of stained cells. Nair R G et al30, Popovic B et al54, Juneja S et al26, Jordan R C K et al61 in 1996 and Desousa FACG et al44 in 2009 classified as (-) Negative: <5%, (+) Low: 5-24%, (++) Moderate: 25-50%, (+++) High: >50% positive cells.

Sami SM et al49 and Jordan RCK et al61 graded the tumour cells as Score 0 (Negative) No staining or staining in <5% Tumour cells, Score 1 (Weak Positive) staining in 5-24%, Score 2 (Moderate Positive) staining in 25-50%, Score 3 (Strong Positive) staining in >50% Tumour cells.

In our present study, the above-mentioned data was recorded on a master chart for analysis. SPSS 15.0 (SPSS INC, Chicago, IL) was used for statistical analysis. A descriptive statistical analysis was performed (FREQUENCIES Procedure), expressing qualitative data as frequencies and rates. Contingency tables were constructed (CROSSTAB procedure) to determine relationships among qualitative variables. The comparison of scores between the N, PMD and OSCC was done using the Chi-Square Test. The X2 value for the N Vs PMD was 0.863 and P value was 0.649 which did not achieve the significant statistical values. The x2 value for the N Vs OSCC was 1.481 and the P value was 0.477 which also did not achieve the significant statistical values. The x2 value for the PMD Vs OSCC was 0.352 and the P value was 0.839 with Degree of freedom (df) value as 2 which also did not achieve the significant statistical values.

Sudha VM et al17 in 2011 did not report statistical significance when compared between OSCC and PMD overall. Desousa FACG et al44 in 2009 used the Chi-square test which was not statistically significant with the P value as 0.349. Similarly, Ranganathan K et al24 in 2011 used ANOVA and Chi-Square tests. Pittalli A et32 al in 2002 and Ahmed MM et al52 in 2009 used the Chi-Square and MANN-WHITNEY U Test. Birchall MA et36 al in 1997 and Helal DS et al18 in 2016 used the Chi-Square and T-Test. Pavic I et al19 in 2017 studied the difference in bcl-2 expression in the epithelium between OLP, OSCC

and Healthy oral mucosa using Chi-Square test. The X2 value noted in their study for the comparison are as follows between, OLP and OSCC was 22.489 with P value <0.001, OLP and healthy oral mucosa was 53.667 with P value <0.001, and OSCC and healthy oral mucosa was 11.613 with the P value 0.009. The Degree of freedom (df) value was 3. Martinenz AB et al42 in 2013 used the Pearson Chi-square test and CROSSTABS procedures but reported with the significant p value (<0.05).

The Pearson Chi- Square test, Mann-Whitney U test, T-test and ANOVA Test were applied with the statistically significant P value of < 0.05 and Mean ± SD to analyze independent qualitative variables. Similarly, Ahmed MM el al52 in 2009 statistically analyzed the score using SPSS version 15 and Mean ± SD, Chi-Square and Mann-Whitney U test were applied for the analysis with the significant P value ≤0.05.

In the present study Mann-Whitney U test applied to compare the BCL2 expression in the samples of N and PMD. The average value was 109.500 with P value as 0.205 which did not achieve statistical significance. To compare the BCL2 expression in the samples of N and OSCC. The average value was 104.000 with P value as 0.150 which did not achieve statistical significance. To compare the BCL-2 expression in the samples of PMD and OSCC. The average value was 439.000 with P value as 0.870 which did not achieve statistical significance.

The Mean Bcl-2 expression in 10 Control (N) samples with the values found in our study is 0.0824 with the Standard Deviation values of 0.127. In 30 OPMD samples the Mean value was 0.0397 with the Standard Deviation value of 0.083 and in the 30 OSCC samples, the Mean value was 0.0301 with the Standard Deviation value of 0.060. For the total 70 samples, the Mean value for Bcl-2 expression was 0.0417 with the Standard Deviation value of 0.082. Similarly, Helal D S et al18 observed in their study that the mean number of Bcl-2+ve cells was significantly higher in control sections of normal skin (89.35 ± 2.55) than studied cases of nonspecific dermatitis (37.45 ± 34.21). Within the SCC cases; poorly differentiated cases of SCC showed higher Bcl2 positivity (113.27±4.26) followed by moderately differentiated tumors (93.08 ± 2.71). Well differentiated SCC (34.14 ± 44.10) showed the least Bcl-2 positivity. This difference was found to be statistically significant. One-way ANOVA test was conducted to compare the Bcl-2 expression between Normal Mucosa, OPMD and OSCC was not significant with the P value of 0.753 [F (1,69) = 0.100].

We observed higher expression of Bcl2 in the basal and parabasal layer of the normal oral mucosa. Suri C 51 and Loro LL et al 22 had also observed a similar finding in his study. This could be related to the preservation of an adequate reservoir of proliferating stem cells in this region to maintain the oral epithelium.51

The differences in immunolocalization of Bcl-2 in various epithelia and neoplastic tissues might be attributed to geographic distribution, genetic discrepancies as well as different etiological factors involved in oral cavity carcinogenesis. Most studies on Bcl-2 and bax have been conducted on formalin fixed, paraffin-embedded tissue sections. Fixation and antigen retrieval methods such as enzyme digestion, microwave treatment, or a combination of both are bound to affect the sensitivity of the antibodies used. The staining of the basal compartments of well differentiated and moderately differentiated OSCC was stronger, suggesting that carcinogenesis in oral epithelium may involve an early suppression of Bcl-2 expression in the basal cells. Compared with normal oral mucosa, the Bcl-2 expression in oral squamous cell carcinoma was decreased22.

Generally, terminally differentiating (keratinizing) cells showed diminishing immunoreactivity for bcl-2. Increased bcl-2 expression in sequentially progressing epithelial dysplasia and down-regulation of bcl-2 in differentiating carcinomas indicate that this oncoprotein may play a key role in relatively early events in the development and progression of oral neoplasia. Demonstration of bcl-2 expression in non-dysplastic basal and parabasal cells contiguous to neoplastic epithelium raises the possibility that bcl-2 alterations precede the overt appearance of oral pre-neoplastic and neoplastic lesions.23

We conclude that bcl-2 may play a key role in the early stages of oral tumorigenesis and appears to be inversely related to the degree of epithelial cell differentiation.23

Cluster analysis has been used, allowing one to make a more objective interpretation of immunoprofiles, based on staining with multiple antibodies, and holding great promise for the immunohistochemical classification of tumors. Tissue microarrays can screen for protein expression patterns in a large number of tumors and these data could be used to classify tumors. Camillo CMC et al50 in 2017 did a study in which a hierarchical cluster analysis was performed to identify protein profiles, which could distinguish different subtypes of OSCC, and how they relate to patient outcome.50

The cluster analysis was also performed by Chung et al, Walter et al and Mendez et al. They analyzed the gene expression profile of different cases of oral cancer and classified their samples into different prognostic groups. Though the results were conflicting between studies, their cluster analysis revealed that apoptosis is linked to tumor behavior in oral cancer.50

Polverini PJ and Nor JE have stated that Activation of Bcl-2 through inactivation or loss of p53 might also enhance the growth of metastatic tumor cells. Metastatic foci often translate to an environment with limited nutrients. In normal cells, nutrient deprivation is a potent stimulus for apoptosis. However, tumor cells that have a decreased dependency on exogenous growth

factor or that overexpress survival genes such as Bcl-2 would no longer be subjected to the selection pressure normally associated with nutrient or growth factor deprivation. 64

We have evaluated Bcl-2 oncoprotein expression during the progression of oral tumors, including mild, moderate, and severe epithelial dysplasia and squamous cell carcinoma of varying grades. Most studies reveal that overexpression of Bcl2 was related to poorly differentiated lesions. But, our study showed low Bcl-2 expression which is in contrast to reported literature.

Xi et al 58 have emphasized that the in-situ technique for detection of apoptosis is not entirely specific, because overlap between apoptotic and necrotic cell death has been reported. This may explain why some morphologically apoptotic cells do not stain, which may result in the underestimation in some cases. Popovic B et al 54 have reported that low expression of Bcl2 was associated with higher survival rates in their study samples. We are also planning to perform a follow-up study on patients with potentially malignant disorders and oral cancer to correlate the Bcl2 expression with clinical progress of such lesions and validate the prognostic significance of our findings. Low sample size, unequal distribution of sample, and use of only one molecular marker may be considered as limitations of the present study. As the study mainly included the patients from a limited geographic area Sriganganagar, Rajasthan, Western India, further multi-centre studies from all parts of the country may be required to establish a more precise correlation between clinicopathologic features and expression of BCl2 family genes in oral cancer and precancer.

Conclusion

Increased Bcl-2 expression in sequentially progressing epithelial dysplasia and down regulation of Bcl-2 in differentiating carcinomas indicate that this oncoprotein may play a key role in relatively early events in the development and progression of oral neoplasia. Bcl-2 expression appears to be inversely related to the degree of epithelial cell differentiation. Deregulation of bcl-2 is observed in Dysplasia and OSCC. Further investigations of its interaction with other proto-oncogenes that might be involved in oral neoplasia will improve our understanding of its behaviour and clinical implications.

Novel anticancer therapy targeting the Bcl-2 family will decide the tumor progression and its response to radiation or cytotoxic drugs. Our study results suggested that apoptosis is present in our samples of PMD and OSCC. This information may be of value in establishing new approaches to investigate the complex molecular roads that lead to uncontrolled cell proliferation in malignant neoplasms.

Financial support and sponsorship

Nil

Conflicts of interest

There are no conflicts of interest

Source of funding

The author of this review has no funding sources to declare.

Acknowledgements

I thank Dr. Pratibha Ramani, Dr. Murugesan. K and Dr. Arvind. M, Saveetha Dental College for their valuable inputs in preparation of this manuscript.

REFERENCES

1. Alberts B, Johnson A, Lewis J. Molecular Biology of the cell. 4th edition. New York: Garland Science; 2002.
2. Fink S L and Cookson B T. Apoptosis, Pyroptosis and Necrosis: Mechanistic Description of Dead and Dying Eukaryotic Cells. Infect. Immun. 2005; 73(4): 1907-1916.
3. Elmore S. Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol. 2007; 35(4): 495-516.
4. Hardwick JM and Soane L. Multiple Function of BCL-2 Family Proteins. Cold Spring Harb Perspect Biol. 2013; 5(2): a008722.
5. Gross A, McDonnell JM and Korsmeyer SJ. BCL-2 Family members and the mitochondria in apoptosis. Genes & Dev. 1999.13: 1899-1911.

6. Coelho KR. Challenges of the Oral Cancer Burden in India. J Cancer Epidemiol. 2012- 701932.
7. Birajdar SS, Radhika MB, Paremala K, Sudhakara M, Soumya M and Gadivan M. Expression of Ki67 in normal oral epithelium, Leukoplakic oral epithelium and oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2014; 18(2): 169-176.
8. Llu SC, Klein-Szanto AJP. Markers of Proliferation in normal and leukoplakic epithelia. Oral Oncology. 2000; 36(2): 145-151.
9. Reibel J. Prognosis of oral pre-malignant lesions: significance of clinical, histopathological, and molecular biological characteristics. Crit Rev Oral Biol Med. 2003; 14(1): 47-62.
10. Markopoulos A K. Current Aspects on Oral Squamous Cell Carcinoma. Open Dent J. 2012; 6: 126–130.
11. Warnakulasuriya S, Johnson NW, Van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007; 36: 575-80.
12. Sarode SC, Sarode GS, Tupkari JV. Oral potentially malignant disorders: A proposal for terminology and definition with review of literature. J Oral Maxillofac Pathol. 2014; 18(Suppl 1): S77-80.
13. Van der Waal I. Oral potentially malignant disorders: Is malignant transformation predictable and preventive? Med Oral Patol Oral Cir Bucal; 2014; 19(4): e386-e390.
14. Mortazavi H, Baharvand M and Mehdipour M. Oral Potentially Malignant Disorders : An Overview of more than 20 Entities. J Dent Res Dent Clin Dent Prospects. 2014; 8(1): 6-14.
15. Rahima S, Riyaz N, Abdul Latheef EN and Shyni PM. Squamous Cell Carcinoma on a syphilitic gumma: A unique presentation. Indian J Sex Transm Dis.2015; 36(1): 89-91.
16. Duraiyan J, Govindarajan R, Kaliyappan K and Palanisamy M. Application of Immunohistochemistry. J Pharm Bioallied Sci. 2012; 4(Suppl 2): S307-S309.
17. Sudha V M, Hemavathy S. Role of Bcl-2 oncoprotein in oral potentially malignant disorders and squamous cell carcinoma: An Immunohistochemical study. Indian J Dent Res 2011; 22: 520-525.
18. Helal DS, Wahba OM. Immunohistochemical Study of CD68, CD3 and Bcl-2 and their role in progression of Head and Neck Squamous Cell Carcinoma. Arch Cancer Res. 2016, 4:1.
19. Pavic I, Balicevic D, Vrdoljak B, Filipovic M, Zekic J and Boras VV. The significance of FHIT AND Bcl-2 in patients with Oral Lichen Planus in comparison to the healthy oral mucosa and Oral Squamous Cell Carcinoma. Libri Oncol. 2017; 45(1): 9-14.
20. Kipli NP, Kumar SKS, Zain RB, Fan CY and Boon PK. Expression of p53, pRb, Ki67 and Bcl-2 in betel quid induced oral mucosal lesions. Oral Oncology 2005; 1: 87-87.
21. Fan Y, Zhan Z, Peng T, Song XL and Feng ZQ. The expression of apoptosis-associated proteins Bcl-2, Bax in oral leukoplakia and lichen planus. Shanghai Kou Qiang Yi Xue. 2004; 13(6): 497-501.
22. Loro LL, Vintermyr OK, Liavaag PG, Jonsson R and Johannessen AC. Oral Squamous Cell Carcinoma is associated with decreased Bcl-2/Bax expression ratio and increased apoptosis. Human Pathology 1999; 30(9): 1097-1105.
23. Singh BB, Chandler FW, Whitaker SV and Forbes-Nelson AE. Immunohistochemical evaluation of bcl-2 oncoprotein in oral dysplasia and carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85(6): 692-698.
24. Ranganathan K and Kavitha R. Proliferation and apoptosis markers in oral submucous fibrosis. J Oral Maxillofac Pathol. 2011; 15(2): 148–153.
25. Arya V, Singh S, Daniel MJ. Clinicopathological correlation of Bcl-2 oncoprotein expression in oral precancer and cancer. J Oral Biol Craniofac Res. 2016; 6(1): 18-23.
26. Juneja S, Chaitanya NB, Agarwal M. Immunohistochemical expression of Bcl-2 in oral epithelial dysplasia and oral squamous cell carcinoma. Indian J Cancer 2015; 52: 505-10.
27. Nafarzadeh S, Jafari S and Bijani A. Assessment of Bax and Bcl-2 Immunoexpression in patients with Oral Lichen Planus and Oral Squamous Cell carcinoma. Int J Mol Cell Med. 2013; 2(3): 136-142.
28. Nunez M A, De Matos F R, Freitas Rde A, Galvao H C. Immunohistochemical study of integrin a5b1, fibronectin, and the Bcl-2 in normal oral mucosa, inflammatory fibro epithelial hyperplasia, oral epithelial dysplasia, and oral squamous cell carcinoma. Appl Immunohistochem Mol Morphol. 2013; 21(4): 354-361.
29. Leyva–Huerta ER, Ledesma-Montes C, Rojo-Botello RE and Vega-Memije E. P53 and Bcl-2 immunoexpression in patients with oral lichen planus and oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal.2012; 17(5): e745-e750
30. Nair RG, Shameen PM, Varghese I, Sudha S. Immunohistochemical Evaluation of Bcl-2 oncoprotein in Oral Dysplasia and Carcinoma. OMPJ. 2011; 2(1): 0976-1225.
31. Loro LL, Johannessen AC, Vintermyr OK. Loss of BCL-2 in the progression of oral cancer is not attributable to mutations. J Clin Pathol 2005; 58: 1157–1162.
32. Piattelli A, Rubini C, Fioroni M, Lezzi G and Santinelli A. Prevalence of p53, Bcl-2, and Ki-67 Immunoreactivity and of Apoptosis in Normal Oral Epithelium and in Premalignant and Malignant Lesions of the Oral Cavity. J Oral Maxillofac Surg. 2002; 60: 532-540.
33. Teni T, Pawar S, Sanghvi V, Saranath D. Expression of Bcl-2 and Bax in chewing tobacco-induced oral cancer and oral lesions from India. Pathol Oncol Res. 2002; 8(2): 109-114.
34. McAlinden RL, Maxwell P, Napier S, Hamilton P, Cowan CG, Lundy FT, Lamey PJ and Marley JJ. Bcl-2 expression in sequential biopsies of potentially malignant oral mucosal lesions assessed by immunocytochemistry. Oral Diseases. 2002; 6: 318-326.
35. Schoelchg ML, Leb QT, Silverman Jrc S, McMilland A, Dekkera NP, Fue KK, Zioverf BL and Regezia JA. Apoptosis-associated proteins and the development of oral squamous cell carcinoma. Oral Oncology.1999; 35(1): 77-85.
36. Birchall MA, Schock E, Harmon BV and Gobé G. Apoptosis, mitosis, PCNA and bcl-2 in normal, leukoplakic and malignant epithelia of the human oral cavity: Prospective, in vivo study. Oral Oncology.1997; 33(6): 419-425.
37. Ravi D, Kumar M, Shukla M and Pandey M. Importance of Bcl-2 protein expression in histological negative margins after excision of oral squamous cell carcinoma. Word J Med Res 2017; 6 :1-7.
38. Sadiq H, Ahuja P, Gupta P, Singh G, Singh N and Anand P. Immunohistochemical expression of p53 and Bcl2 in varying grades of Oral Epithelial Dysplasia. I J Pre Clin Dent Res 2015; 2(4): 17-25.
39. Shailaja G, Kumar J V, Baghirath PV, Kumar U, Ashalata G and Krishana AB. Estimation of malignant transformation rate in cases of oral epithelial dysplasia and lichen planus using immunohistochemical expression of Ki-67, p53, BCL-2 and BAX markers. Dent Res J (Isfahan). 2015; 12(3): 235-42.
40. Pigatti FM, Taveira LA, Soares CT. Immunohistochemical expression of Bcl-2 and Ki-67 in oral lichen planus and leukoplakia with different degrees of dysplasia. Int J Dermatol. 2015; 54(2): 150-155.
41. Arreaza AJ, Rivera H and Correnti M. Expression of COX-2 and bcl-2 in oral lichen planus lesions and lichenoid reactions. Spanish ecancer 2014; 8: 411.

42. Martínez AB, Gutierrez CR, Gomez ER, Gil Montoya JA, Font RG and Gonzalez Moles MA. Evaluation of p53, Caspase-3, Bcl-2, and Ki-67 markers in oral squamous cell carcinoma and premalignant epithelium in a sample from Alava Province (Spain). Med Oral Pathol Oral Cir Bucal. 2013;18(6): e846-850.
43. Nogami T, Kuyama A and Yamamoto H. Histopathological and immunohistochemical study of malignant transformation of oral leukoplakia, with special reference to apoptosis-related gene products and proliferative activity. Acta Otolaryngol. 2003; 123(6): 767-75.
44. Desousa FACG, Paradella TC, Carvalho YR and Rosa LEB. Immunohistochemical expression of PCNA, p53, bax and Bcl-2 protein in oral lichen planus and epithelial dysplasia. J Oral Sci. 2009; 51: 117-121.
45. Thomas S A and Sethupathy. S. Expression of apoptotic markers in patients with oral squamous cell carcinoma (OSCC). IOSR-JDMS. 2014; 13(11): 78-81.
46. Garewal J, Garewal R and Sircar K. Expression of Bcl-2 and MIB-1 Markers in Oral Squamous Cell carcinoma (OSCC)- A Comparative Study. J Clin Diagn Res. 2014; 8(7): QC01-QC04.
47. Rahmani A, Alzohairy M, Babiker AY, Rizvi MA and Elkarimah-Mad HG. Clinicopathological significance of PTEN and bcl2 expressions in oral squamous cell carcinoma. Int J Clin Exp Pathol. 2012; 5(9): 965-971.
48. Zhang B, Liu M, Tang H K, Ma HB, Wang C, Chen X and Huang HZ. The expression and significance of MRP1, LRP, TOPOIIβ, and BCL2 in tongue squamous cell carcinoma. J Oral Pathol Med. 2012; 41(2): 141-148.
49. Sami SM, Al-Muala HD, Kummoona R and Kaptan IA. Bcl 2 Proto oncogene expression correlates with grading of oral carcinoma. Journal of Kerbala University. 2011; 9(2): 101-10.
50. Coutinho-Camillo CM, Lourenco SV, Puga RD, Damascena AS, Teshima T HN, Kowalski LP and Soares FA. Profile of apoptotic proteins in oral squamous cell carcinoma: A cluster analysis of 171 cases. Applied Cancer Research 2017; 37(2).
51. Suri C. The immunohistochemical evaluation of the expression of Bcl-2 in different histological grades of squamous cell carcinoma. Journal of Clinical and Diagnostic Research.2009; (3): 1891-1899.
52. Ahmed MM. Expression profile of Apoptotic Mediators and Proliferative Markers in Oral Squamous Cell Carcinoma. Journal of Egyptian Nat. Cancer Inst. 2009; 21(2): 85-92.
53. Camisasca D R, Honorato J, Bernardo V, Da Silva L E, Da fonseca E C, De faria PAS, Dias FL and Lourenco SDQC. Expression of Bcl-2 family proteins and associated clinicopathologic factors predict survival outcome in patients with oral squamous cell carcinoma. Oral Oncology. 2009; 45(3): 225–233.
54. Popovic B, Jekic B, Novakovic I, Milasin J. Bcl-2 expression in oral squamous cell carcinoma. Ann N Y Acad Sci 2007 Jan; 1095: 19-25.
55. Muzio LL, Mignogna MD, Pannone G, Rubini C, Grassi R, Nocini PF, Ferrari F, Serpico R, Favia G, De rosa G and Maiorano E. Expression of Bcl-2 in oral squamous cell carcinoma: An immunohistochemical study of 90 cases with clinicopathological correlations. Oncology Reports. 2003; 10(2): 285-291.
56. Sulkows M, Famulski W, Sukkowski S, Reszec J, Koda M, Baltaziak M and Koda L K. Correlation between Bcl-2 protein expression and some clinicopathological features of oral squamous cell carcinoma. Pol J Pathol 2003; 54(1): 49-52.
57. Saikrishna P , Sivapathasundharam B , Rafiuddeen IS , Krishnan B. Expression of Bcl-2 oncoprotein in Oral Squamous Cell Carcinoma-An Immunohistochemical study. Indian Journal of Pathology & Microbiology 2002; 45(3): 283-287.
58. Xie X, Clausen OPF, De Angelis P and Boysen M. The prognostic value of spontaneous apoptosis, Bax, Bcl-2, and p53 in oral squamous cell carcinoma of the tongue. TOC 1999; 86(6):913-920.
59. Kannan K, Latha PNL and Shanmugam G. Expression of Bcl-2 oncoprotein in Indian Oral Squamous Cell Carcinomas. Oral Oncology 1998; 34(5): 373-376.
60. Nylander K, Schildt EB, Eriksson M and Roos G. PCNA, Ki-67, p53, bcl-2 and prognosis in intraoral squamous cell carcinoma of the head and neck. Analytical Cellular Pathology 1997; (14): 101-110.
61. Jordan RCK, Catzavelos GC, Barrett AW, Speight PM. Differential expression of bcl-2 and bax in squamous cell carcinomas of the oral cavity. Oral Oncol, Eur J Cancer. 1996; 32(6): 394-400.
62. Jain M, Kasetty S, Sridhara SU, Jain N, Khan S and Desai A. Apoptosis and Its Significance in Oral Diseases: An Update. Journal of Oral Diseases 2013, article ID 401049, 11 pages.
63. Reddy BS, Reddy BM, Shyam NDVN. Tumour Markers in Oral Neolplasia. IJDA 2010; 02(1): 103-114.
64. Polverini PJ, Nor JE. Apoptosis and predisposition to oral cancer. Crit Rev Oral Biol Med 1999; 10 (2): 139-152.