Section: Pathology

Original Research Article

ROLE OF BCL-2 AS A PROGNOSTIC MARKER IN ORAL SQUAMOUS CELL CARCINOMA

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Abstract

Background: To study the expression of Bcl-2 in Oral Squamous Cell Carcinoma by Immunohistochemistry. **Materials and Methods:** Fifty cases of OSCCs were studied in the Department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar for a period of 18 months [Nov 2019 - April 2021] for the Immunoexpression of Bcl-2 with relation to histopathological grade and various clinical parameters such as Age, Gender, History of Smoking, Alcohol Consumption, Betel Nut Chewing, Tobacco Chewing, Anatomical Site Involvement, Lymph Node Status and Stage of the cancer wherever available. **Result:** The present study found no significant correlation of Bcl-2 with respect to duration of Smoking, chewing Tobacco and Betel nut chewing (p=0.092, p=0.546 and 0.297respectively). There was no significant differing immunoexpression of Bcl-2 with respect to Major Anatomical Sites (p=0.351). Bcl-2 correlated well with histopathological grade of OSCCs ($p < 0.001$). Out of 50 cases, in only thirty four of the 50 study cases, radical neck dissection was performed. Of these 4 cases (12%) showed metastatic deposits in the lymph nodes. The study of Bcl-2 immunoexpression in these 4 cases showed equal distribution of immunoreactive cells and showed no statistical significance. Of the 42 cases in which pathological staging could be assessed, only 37 cases showed immunopositivity where >50% immunopositivity of Bcl-2 was found increased in tumors of stage IV with, [5 cases,62.5 %] followed by stage III tumors with p value of 0.035 and is significantly correlated. **Conclusion:** The present study identified correlation of immunoexpression of Bcl-2 with respect to Gender, History of Smoking, History of Alcohol Consumption, Histopathological Grade and Tumor Stage.

INTRODUCTION

Currently Oral cancer is one of the most alarming health problems facing mankind in terms of morbidity and mortality.[1] According to World Health Organization, Oral Squamous Cell Carcinoma is the 8th most common type of cancer worldwide, with geographical variations.17 More than 90% of all oral cancers are Oral Squamous cell carcinoma which is often preceded by a pre-malignant lesion. Oral SCC more frequently affects men than women (M: F = 1.5:1), most probably because more men than women indulge in high-risk habits.[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 3.1/10,000 in men and 2.9/100,000 and 1.4/10,000 in women respectively.[3] Most commonly these Squamous cell carcinomas arise in the mucosal epithelial cells as Metaplasia followed by Dysplasia, then Carcinoma in situ and finally Invasive cancers.1 Recently, various studies are identifying Novel markers representing molecular pathways in Squamous cell carcinomas of the Head and Neck region. Among them, the over expression of BCL-2, anti-apoptotic gene is documented in the HNSCCs [Head and Neck Squamous Cell Carcinomas].[4]

Apoptosis is a genetically regulated process involved in tissue size regulation, morphogenesis, and elimination of genetically damaged cells. A pallet of genes is involved in the control of apoptosis, such as Bcl-2 family whose oncogenic potential has been demonstrated in oral tumorigenesis4. The Bcl family consists of the following two opposing groups of proteins: pro- apoptotic (death agonists) and anti-apoptotic proteins (death antagonists). One of the anti-apoptotic proteins, Bcl-2, with a crucial role in apoptosis regulation is the object of our study. B-cell lymphoma/leukemia-2 (Bcl-2) is an anti-apoptotic

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protein that interacts with and is regulated by p53. Bcl-2 gene on chromosome 18q21 maintains membrane integrity of mitochondria and involved in apoptotic pathway. It evades apoptosis and facilitates cell survival. The earliest step in the carcinogenic process involves the emergence of the "initiated" cell populations that are genetically altered. The enhanced expression of Bcl-2 and/or Bcl-xl in the human premalignant and malignant oral keratinocytes suggests the development of oral cancer. Association between low Bcl-2 expression and good prognosis and adversely high expression with poor prognosis has been documented by few.[5]

This study points to know the association between Bcl-2 expression and prognosis by immunohistochemistry. Antibodies used in the Evaluation of Oral Cavity Specimens are Bcl-2-BioCare (1:200). The advent of increasing evidence-based therapy in medicine today aided by advancing research in molecular pathogenesis of cancers has led us to a new era of targeted chemotherapy for the cancers.[6] Overexpression of anti-apoptotic Bcl-2 family members has been associated with chemotherapy resistance in various human cancers, and pre-clinical studies have shown that agents targeting anti-apoptotic Bcl-2 family members have pre-clinical activity as single agents and in combination with other anti-neoplastic agents.[7]

MATERIALS AND METHODS

The present study was conducted in the Department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences, Bommakal, Karimnagar. It was a prospective study for the period of 18 months from November 2019 to April 2021 comprising of samples from 50 patients. Institutional ethical committee clearance was obtained.

Inclusion Criteria

All cases diagnosed as Primary Squamous Cell Carcinoma on routine biopsies and radical specimens of oral cavity lesions irrespective of age and gender of patient.

Exclusion Criteria

Secondaries or metastatic lesions, Histological variants of Oral cavity other than Squamous Cell Carcinoma and Oral cavity lesions diagnosed as Carcinoma in situ.

Sample Collection

Biopsy and tissue samples of patients submitted for histopathological examination, diagnosed as Squamous cell carcinomas of the Oral cavity were evaluated for Prospective study.

Sample Processing

Biopsies and tissue samples were fixed in 10 % Neutral buffered formalin solution for an average period of 24 hours. Resection specimens were also fixed as said above and bits were given from representative areas and processed in Microm histokinette, paraffin embedded using an automated instrument and sections (4-5 microns thickness) were cut using Lyca Microtome. Sections were taken onto slides and stained by routine Hematoxylin and Eosin (H&E) stain.

Analysis of Immunoreactivity of Bcl-2: Ten random fields were selected under 100x magnification. For the immuno quantification of Bcl-2, a semi quantitative evaluation system consisting of Sudha et al8 criteria was followed; hundred cells were counted in ten fields and the percentage of positive cells were derived.

Criteria for Analysis of Immunoreactivity of Bcl-2: The percentages of positive cells were classified as:

- More than 50% of cells positive scored as (+++);
- 25%-50% positive (++);
- 10%-25% (+);
- Fewer than 10% (±), i.e., by counting the number of cells per 1mm2 field.

The histological grade on the H&E sections and the expression of Bcl-2 were correlated along with other clinical parameters.

Statistical Analysis

Descriptive statistical analysis such as mean, median, proportion was calculated using Microsoft Excel. Chi-square test of independence and goodness of fit was employed to determine the Probability (P) value. A P value of < 0.05 was considered as statistically significant.

RESULTS

Fifty cases of Primary Oral Squamous Cell Carcinomas were studied at the Department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar, over a period of 18 months (November 2019 to April 2021). All 50 cases were studied histopathologically, evaluated for immunoexpression of Bcl-2 expression for prognostication and correlated with clinical parameters.

The Age of patients ranged from 25 to 82 years with a mean age of 54 years. Maximum number of cases were seen in the age group of 41 to 50 years (32%) and least number of cases in the age group of 21-30 years (4%). Only two cases were seen in the young age group of 21 to 30 years. Out of 50 cases, 32 (64%) were males and 18 (36%) were females. Male to Female ratio was 1.7:1 [Table 1].

35 cases had a history of smoking, accounting for 70% of total number of cases with 82.8% of them being males and 17.1% being females. Among the category of Non-smokers (15 cases, 30%), 3 were males and 12 were females. 32 cases (64%) of Oral Squamous cell carcinomas gave a positive history of alcohol consumption, 93.7% of whom were males. Of the non- alcoholics, 88.9 % of them constituted females. 27 cases had a positive history of tobacco chewing accounting for 54% of cases of which almost 74% were males and 26% were females. 30 cases had a positive history of Betel nut chewing

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accounting for 60% of cases of which almost 35% were females and 65% were males. [Table 2]

Majority of the cases seen in the Oral cavity, with Buccal mucosa (40%) being the commonest site, followed by the Tongue (30%). Among 6% of Pharyngeal Squamous cell carcinoma, the Tonsillar fossa was found to be the most involved site (4%). Laterality was not considered in the assessment of site as laterality is not applicable in most of these unpaired organs of head and neck region. [Table 3]

Well-differentiated type was the predominant histologic grade accounting for (23 cases 46%), followed closely by Moderately differentiated type (21 cases, 42%) and then by Poorly differentiated type (6 cases, 12%). [Table 4]

The expression of Bcl-2 was in the form of granular cytoplasmic staining with an accentuation around the nuclear membrane. This is due to the cellular localization of Bcl-2 in the outer membrane of the mitochondria, endoplasmic reticulum and the nuclear membrane.

Immunopositivity of Bcl-2 was seen in 45 cases of which 25-50% staining of tumour cells was seen in a majority of 21 cases (46.7%), followed by 13 cases (28.8%) showing 10-25% immunostained cells, >50% of tumor cells were seen in 6 cases (13.3%). The remaining 5 cases (11.1%) showed <10% of immune positivity [Table 5].

Out of 23 Well differentiated Squamous cell carcinomas,18 stain positive of which majority [13 cases (72.2%)] of cases showed Bcl-2 positivity index of 10-25%. Among Moderately differentiated type, 15 cases (71.4%) showed Bcl-2 positivity index of 25-50%. Hundred percent (6 out of 6 cases) of Poorly differentiated type of Squamous cell carcinoma showed Bcl-2 positivity index of > 50%. The correlation of Bcl-2 immunoexpression was found to be statistically significant with respect to the various histopathological grade of OSCC (p=<0.001). [Table 6].

As is evident from the above [Table 7], there is no significant association between immunoexpression of Bcl-2 and Age but there is significant association between immunoexpression of Bcl-2 and Gender in Oral SCC.

Positive smoking history and alcohol consumption found to be significantly associated with Bcl-2 expression. The duration of smoking history where applicable, Betel nut chewing and Tobacco chewing history were not found to be significantly associated with Bcl-2 expression [Table 8].

As evidenced by the above Table [Table 9], immunoexpression of Bcl-2 was found to be more in tongue and buccal mucosa affected cases.

In only 34 of the 50 study cases, radical neck dissection was performed. Of these 4 cases (12%) showed metastatic deposits in the lymph nodes. The study of Bcl-2 immunoexpression in these 4 cases showed equal distribution of immunoreactive cells. [Table 10]

Of the 42 cases in which pathological staging could be assessed among which only 37 cases showed immunopositivity, where >50% immunopositivity of Bcl-2 was found increased in tumors of stage IV with, [5 cases,62.5 %] showing followed by stage III tumors. Immunopositivity of Bcl-2 was found to be significantly associated with increasing tumor stage.

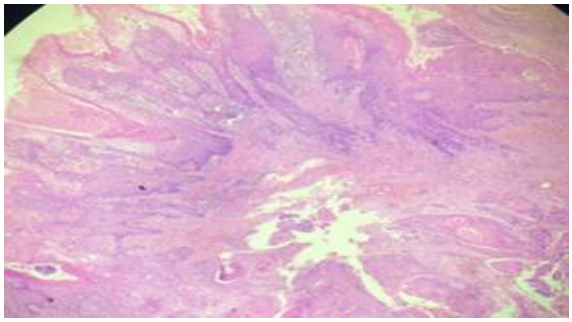

Figure 1: Microphotograph showing Well Differentiated Squamous Cell Carcinoma with Tumour Invading Subepithelium (H&E 10x4)

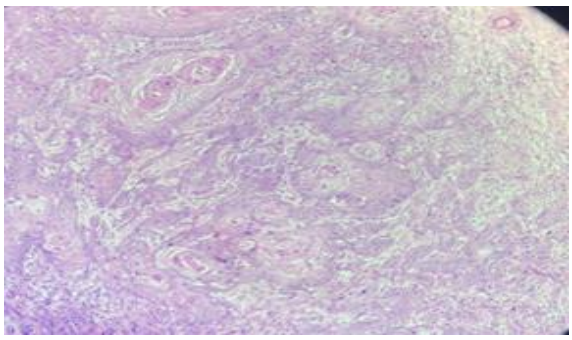

Figure 2: Microphotograph showing Well Differentiated Squamous Cell Carcinoma showing keratin pearls (H&E 10x10)

Figure 3: Microphotograph showing cytoplasmic immunoexpression of Bcl-2 in Well differentiated squamous cell carcinoma (score 10 %) (IHC, 10x4)

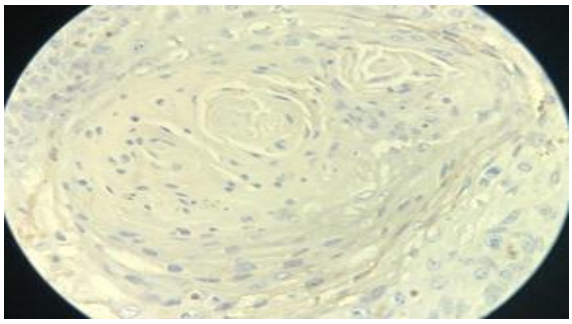

Figure 4: Microphotograph showing cytoplasmic immunoexpression of Bcl-2 in Well Differentiated Squamous Cell Carcinoma showing positive staining at periphery (IHC,10x40)

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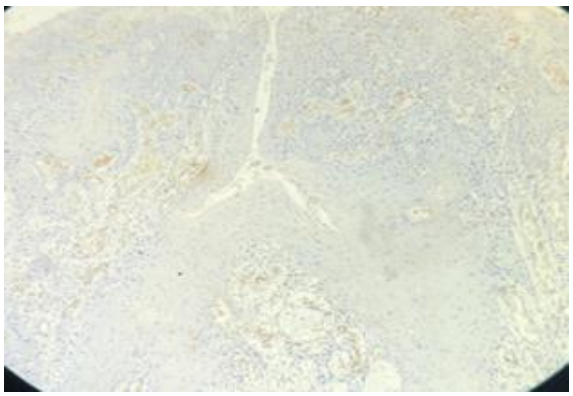

Figure 5: Microphotograph showing cytoplasmic immunoexpression of Bcl-2 in Moderately Differentiated Squamous Cell Carcinoma showing Bcl-2, (score 30%), (IHC 10x10).

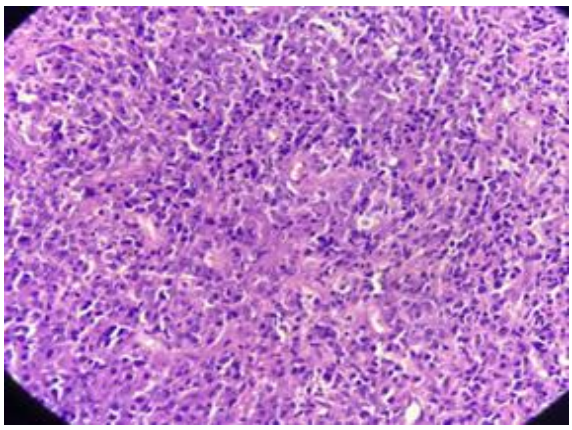

Figure 7: Microphotograph showing Poorly Differentiated Squamous Cell Carcinoma (H&E 10x40)

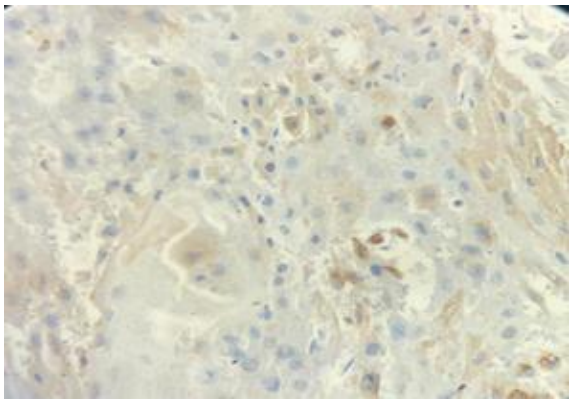

Figure 6: Microphotograph showing cytoplasmic immunoexpression of Bcl-2 in Moderately Differentiated Squamous Cell Carcinoma (IHC,10x40)

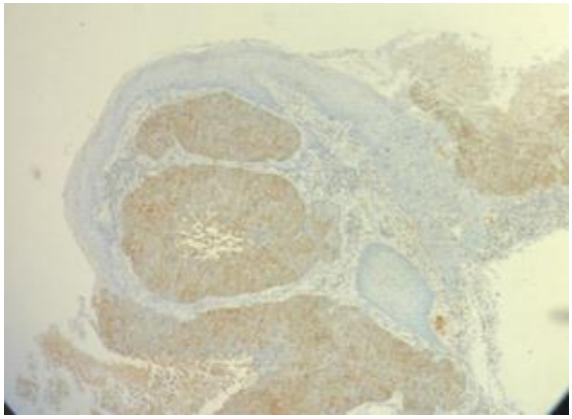

Figure 8: Microphotograph showing cytoplasmic immunoexpression of Bcl-2 in Poorly Differentiated Squamous Cell Carcinoma (score >50%) (IHC,10x4)

Table 1: Demographic distribution in Oral Squamous cell carcinomas.

Age group in Years	Number of cases	Percentage
21-30	2	4.00
31-40	7	14.00
41-50	16	32.00
51-60	12	24.00
61-70	5	10.00
71-80	5	10.00
81-90	3	6.00
Gender		
Male	32	64
Female	18	36

Table 2: Distribution of cases in patients with history of risk factors associated

Risk Factor	Number of cases	
Smoking history		
Yes	35 (70%)	M= 29 (82.8%)
		F = 6 (17.1%)
No	15 (30%)	M= 3 (20.0%)
		F = 12 (80.0%)
Alcohol consumption history		
Yes	32 (64%)	M= 30 (93.7%)
		F=2 (6.3%)
No	18 (36%)	M=2(11.1%)
		F= 16 (88.9%)
Tobacco chewing history		
Yes	27(54%)	M= 20 (74%)
		F= 7 (26%)
No	23 (46%)	M= 12 (52.1%)
		F= 11 (47.8%)
Betel nut chewing		
Yes	30 (60 %)	M= 19 (63.4%)

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		F= 11 (36.6%)
No	20 (40%)	M= 13 (65%)
		F= 07 (35%)

Table 3: Distribution of cases with respect to site of the lesion

Sites and regions of Oral squamous cell carcinoma			Number of cases n [%]	Males	Females
Pharynx (n=3) - (6%)	Oropharynx	Tonsillar fossa	2 [4]	2	0
		Pyriform fossa	1 [2]	1	0
Oral cavity (n=59) – (39.3%)	Tongue		15 [30]	8	7
	Buccal mucosa		20 [40]	13	7
	Lower Alveolus		4 [8]	2	2
	Hard palate		1 [2]	0	1
	Soft palate		2 [4]	2	0
	Gingivobuccal sulcus		3 [6]	1	2
	Retromolar trigone		1 [2]	1	0
Lip			1 [2]	1	0

Table 4: Distribution of cases with respect to histopathological grade of Squamous cell carcinoma

Histopathological grade	Number of cases	Percentage
Well differentiated	23	46
Moderately differentiated	21	42
Poorly differentiated	6	12

Table 5: Immunoexpression of Bcl-2

Antibody	Number of cases				Total
	<10%	10-25%	25-50%	>50%	
Bcl-2	5 (11.1%)	13 (28.8%)	21 (46.7%)	6 (13.3%)	45 (90%)

Table 6: Correlation of Bcl-2 immunoexpression in relation to histopathological grade of Oral SCC.

Grade of SCC	Bcl-2 expression				Total	P-Value
	<10%	10-25%	25-50%	>50%		
Well Differentiated	5 (21.7%)	13 (72.2%)	0	0	18 (40%)	
Moderately Differentiated	0	3 (14.2%)	15 (71.4%)	3 (14.2%)	21 (46.6%)	<0.001
Poorly Differentiated	0	0	0	6 (100%)	6 (13.3%)	
Total	5	16	15	9	45	

Table 7: Correlation of Bcl-2 expression in relation to Age and Gender

Variables	Bcl-2 expression				P value
	<10%	10-25%	25-50%	>50%	
Age <50yrs (n=21) >50 yrs (n=24)	1 (22.5%) 4 (20.8%)	6 (28.5%) 10 (41.7%)	9 (51.0%) 6 (54.4%)	5(23.8%) 4(16.6%)	0.344
Gender Males (n=31) Females (n=14)	5 (24.1%) 0	11 (35.5%) 5 (35.7%)	10(52.8%) 5 (54.8%)	5(16.1%) 4(28.8%)	0.009

Table 8 Correlation of Bcl-2 with Smoking history, Duration of Smoking, Alcohol consumption, Betel nut chewing and Tobacco chewing

Variables	Bcl-2 immuno expression				P value
	<10%	10-25%	25-50%	>50%	
Smoking History (n=45) Yes(n=33) No (n=12)	5 (14.3%) 0	9 (27.3%) 7 (58.3%)	12 (34.3%) 3 (52.2%)	7 (20%) 2(13.3%)	0.0008
Duration of smoking(n=33) <20 yrs (n=21) >20 yrs (n=12)	4 (18.2%) 1 (7.7%)	5(23.8%) 4 (33.3%)	9 (40.1%) 3 (23.1%)	3(13.6%) 4(30.8%)	0.092
Alcohol Consumption (n=45) Yes (n=31) No (n=14)	5 (15.6%) 0	10 (32.2%) 6 (42.8%)	10 (31.2%) 6(33.3%)	6(18.7%) 3(16.7%)	0.015
Tobacco chewing (n=45) Yes (n=24) No (n=21)	3 (11.5%) 2 (8.3%)	7 (29.2%) 9 (42.8%)	8 (30.8%) 7 (29.2%)	6(23.1%) 3(12.5%)	0.546
Betel Nut chewing (n=45) Yes(n=27) No(n=18)	3(10.3%) 2(9.5%)	10(37%) 6(33.3%)	8(27.6%) 7(33.3%)	6(20.7%) 3(14.4%)	0.297

Table 9: Correlation of Bcl-2 with Major anatomical sites: Table 5.14: Correlation of Bcl-2 with Major anatomical sites

Sites			Bcl-2 expression				P-Value
			<10%	10- 25%	25- 50%	>50%	
Pharynx (n=3) - (6%)	Oro pharynx	Tonsillar fossa	0	0	1	1	
		Pyriform fossa	0	0	0	1	
	Tongue		1	4	5	1	

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Oral cavity (n=59)-(39.3%)	Buccal mucosa	4	3	4	3	0.351
	Lower Alveolus	0	1	2	1	
	Hard palate	0	0	0	1	
	Soft palate	0	0	1	1	
	Gingivobuccal sulcus	0	2	1	0	
	Retromolar trigone	0	0	1	0	
Lip		0	1	0	0	
Total		5	16	15	9	

Table 10: Correlation of Bcl-2 with Pathological stage of OSCC.

	Bcl-2 expression				P-Value
	≤10%	10-25%	25-50%	>50%	
Antibody					
Bcl-2 (n=4)	1 (25%)	1 (25%)	1 (25%)	1 (25%)	
Stage (n=42)					
Stage I (n=5)	1 (20%)	1 (20%)	3 (60%)	0	
Stage II (n=10)	1 (10%)	6 (60%)	3 (30%)	0	
Stage III (n=14)	2 (14.3%)	6 (42.8%)	4 (28.5%)	2 (14.3%)	0.035
Stage IV (n=8)	0	3 (37.5%)	0	5 (62.5%)	

Table 11: Comparison of Distribution of Bcl-2 immuno positivity in various studies

Studies	Number of cases	Inference
Sudha et al,[8]	60	The role of Bcl-2 in Oral potentially malignant disorders (oral leukoplakia, OSMF and OLP) and in OSCC was studied by using IHC staining. The results of positive topographic expression between OSCC and potentially malignant disorders were statistically significant (P value—0.0144).
Suri et al,[23]	38	In OSCCs, the number of cells expressing Bcl-2 increased from Well differentiated to Poorly differentiated, showing an inverse relationship with the degree of differentiation of the tumor.
Solomon et al,[12]	30	Showed an inverse relationship between Bcl-2 and levels of differentiation of OSCCs.
Juneja et al,[24]	30	Stronger expression of Bcl-2 oncoprotein was seen in Poorly differentiated OSCC. The cells peripherally located within infiltrating tumor nests were more intensely stained, while fully keratinized neoplastic cells showed diminished or absence of Bcl-2 immunoreactivity in OSCC cases. Alterations In expression Of Bcl-2 Protein, creating a favorable environment for malignant transformation.
Jessica et al,[25]	. 30	Bcl-2 expression is predominant in Well differentiated than in Moderately and Poorly differentiated OSCC, which suggested that apoptosis probably played a major role in the early stages of carcinogenesis.
Arul et al,[21]	30	Bcl-2 Expression Was Decreased from Well Differentiated to Moderately to Poorly Differentiated Tumors.

Table 12: Comparison of correlation of Bcl-2 with TNM Stage in various studies

Studies	No of cases	Inference
Lehnerdt et al,[9]	133	AJCC stage was not significantly associated with Bcl-2
Rodrigues et al,[10]	380	Over expression of Bcl-2 is significantly associated with advanced tumor stage.
Rahmani et al,[5]	60	Suggest that pathological TNM staging is an important prognostic factor.
Taneja K.et al,[20]	35	No statistical significance between p53+/Bcl2+ co-expression and stage
Present study	50	Immunopositivity of Bcl-2 was found increased in tumors with advanced stage and is significantly associated with advanced tumor stage (p=0.035).

DISCUSSION

All 50 cases were studied histopathologically, evaluated for immunoexpression of Bcl-2 expression and correlated with clinical parameters. These parameters were compared with similar studies conducted by various other authors. Most cases of Oral SCC occur in people older than 50 years of age. The mean age of patients including both men and women in our study of 50 cases was found to be 54 years. Rahmani et al,[5] had a mean age of 58 years. G. F. Lehnerdt,[9] Rodrigues et al,[10] whose patients had a mean age of 57 years. This is well comparable with other studies as shown by Ipshita Bhattacharya et al,[11] and Solomon et al,[12] who found that the mean patient age was 57.8 years and OSCC are most frequently encountered in the male gender owing to increased susceptibility to injurious personal habits.

Various studies conducted by researchers across the globe show significant increase in OSCC in male gender as corroborated. In the present study, a similar male preponderance was seen.[5,9-12]

Tobacco smoking is one of the most important predisposing factors for development of OSCC. Authors like Roda et al,[14] and Lehnerdt et al,[9] who have studied large number of cases have established that majority of their cases had a positive smoking history. Likewise, 70% of our cases also proved to be smokers with a long and continuous smoking history. Recent studies conducted by the above authors indicate that alcohol consumption plays a contributory role in the etio-pathogenesis of OSCC. Rodrigues et al,[10] studied 380 cases and found that 80% of them had a significant alcohol consumption history. The present study also found that two thirds of its study patients (64%) admitted to having a significant history of alcohol consumption.

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Other forms of tobacco consumption like tobacco chewing are also documented as important risk factors in the development and progression of OSCCs. Researchers as shown in the above table found that more than three-fourths of their patients had history of tobacco chewing. The present study highlights that 52% of the cases had a positive history of tobacco chewing in concordance with other authors.

Betel nut chewing also documented as important risk factors in the development and progression of OSCCs. Su et al,[17] Mandiha et al,[18] Quinata et al,[19] found that more than eighty percent of their patients had history of Betel nut chewing. The present study highlights that 52% of the cases had a positive history of Tobacco chewing in concordance with other authors.

According to World Health Organisation, Oral Squamous Cell Carcinoma is the 8th most common type of cancer worldwide, with geographical variations. The major anatomical sites in the head and neck region affected by SCC are the Oral Cavity, Pharynx, and the Larynx, followed by other sites like skin of External Ear, Scalp and the Nasal region. Rodrigues et al,[10] Roda et al,[14] Bouckaert et al,[15] Bhattacharya et al,[11] Abdel Aziz et al,[13] limiting their study to the oral cavity SCCs have found the tongue and the buccal mucosa to be the most common sites involved. Taneja K. et al,[20] who studied all the head and neck SCCs found the Oropharynx to be most frequently involved site.

The present study which included 50 cases of Oral SCCs found that majority of cases (39.3%) clustered in Buccal mucosa (40%) being the commonest site, followed by the Tongue (30%). Among 6% of Pharyngeal Squamous cell carcinoma, the Tonsillar fossa was found to be the most involved site (4%).

Histopathological grade has proved to be of independent prognostic value. The increased Bcl-2 expression in Poorly differentiated carcinomas may reflect the loss of ability of malignant keratinocytes to differentiate terminally. The studies done Solomon et al,[12] Arul et al,[21] Rodrigues et al,[10] Lehnerdt et al,[9] and Rahmani et al,[5] mentioned have used modified Broder's Grading system which sub-classifies SCC into Well- Differentiated, Moderately Differentiated and Poorly Differentiated tumour grades. Different studies conducted among patients from variable geographic areas show differing distribution of tumour grades.

The present study found most cases (46%) showing a Well Differentiation pattern, and the least number of cases (1%) to be Poorly Differentiated. This is different from with Rodrigues et al,[10] Lehnerdt et al,[9] Rahmani et al,[5] where majority of cases were Moderately Differentiated.

Abdel Aziz et al,[13] Stronger expression of Bcl-2 oncoprotein was seen in Poorly differentiated OSCC. Bhattacharya et al,[11] No association was found between Bcl-2 and the percentage of immuno positive cells and the grade of malignancy (P 0.278). Thomas et al,[26] Bcl2 was expressed in 85% of OSCC samples whereas control samples were negative. Taneja K et al,[20] A highly significant association was seen between Bcl-2 expression and tumor grade (p=0.001). Rahmani et al,[5] The expression of Bcl-2 was found to be restricted to tumor cells in Well and Moderately differentiated tumors.

Muthukumaran et al,[27] The number of cells expressing Bcl-2 increased from Well-differentiated to Poorly differentiated OSCCs, showing an inverse relationship with the degree of differentiation.

Bcl-2 Expression intensity was increased from Well Differentiated to Moderately Differentiated to Poorly Differentiated Tumors and tumor cells. The present study found significant correlation of Bcl-2 (p=<0.001) with histopathologic grade and stage of Oral Squamous Cell Carcinoma. In OSCC there was a marked reduction of Bcl-2-positive cells in the basal part, and in the central parts of Well-differentiated and Moderately differentiated and also in Poorly differentiated tumors. The cells peripherally located within infiltrating tumor nests were more intensely stained, while fully keratinized neoplastic cells showed diminished or absence of Bcl-2 immunoreactivity in OSCC cases. Similar results were observed by other authors.[32,33] These observations might be attributed to down-regulation of Bcl-2 expression concomitant with terminal cell differentiation (keratinization).

The present study found significant correlation of Bcl-2 (p=<0.001) with increasing histopathological grade. This is supported by the findings from other studies too such as Bhattacharya et al,[11] Abdel Aziz et al,[13] Solomon et al,[12] which showed, As the degree of differentiation decreases, number of cells in the tumor that express Bcl-2 is greater. These tumor cells evade apoptosis and achieve unrestrained cell survival.

Stronger expression of Bcl-2 oncoprotein was seen in Poorly differentiated OSCC which is consistent with the findings of Suri et al,[23] Solomon et al,[12] Aziz et al,[13] Muthukumaran et al,[27] and Juneja et al.[24] The increased Bcl-2 expression in poorly differentiated carcinomas may reflect the loss of ability of malignant keratinocytes to differentiate terminally. Bcl-2 has previously been seen to actively block the differentiation of cultured keratinocytes in an in vitro experiment as well.

The present study is contradicted by Rahmani et al,[5] Jessica et al,[25] Arul et al,[21] which stated that down regulation of Bcl-2 with increasing grades of carcinoma showed that apoptosis plays a major role in early events of carcinogenesis.

Presence of lymph node metastases is an important prognostic criterion and a key feature of the staging system. LNs are most often present in levels III. Bilateral metastases are frequently encountered in tumors of the base of tongue.According to N. Bhutania et al,[23] who conducted study on 75 cases of Oropharyngeal squamous cell carcinoma observed that Bcl-2 expression did not correlate with the grading of tumor. Present study also showed no

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correlation of lymph node involvement with grading of tumor or Bcl-2 immunopositivity. The present study found no statistically significant correlation with respect to Bcl-2 index which is in accordance other studies.[5]

The present study found no significant correlation of Bcl-2 [p=0.344] and expression with age. This is supported by the findings from other studies such as Bhattacharya et al,[6] Abdel Aziz et al,[13] Solomon et al,[12] Lehnerdt et al,[9] and Rodrigues et al.[10] The present study found significant correlation of Bcl-2 (p=0.009) with Gender with predominance of males. This is in concordance with Roda et al.[14] The present study found significant association of Bcl-2 [0.0008] with Smoking history which is in accordance with Roda et al,[14] but discordant with Lehnerdt et al,[9] but there was no correlation with duration of smoking (p=0.092). This finding is supported by study done by Mai et al,[29] that stated Nicotine induces activation of PKC alpha and the MAPKs ERK1 and ERK2, which are physiological Bcl2 kinases thus promoting cell survival and therefore increased Bcl-2 expression. The present study found significant correlation of Bcl-2 (p=0.015) with Alcohol consumption history which is in concordance with the findings of Lehnerdt et al,[9] Taneja K et al.[20]

The present study found expression of Bcl-2 in OSCCs, however but there was no significant correlation between Tobacco use(p=0.546), Betel Nut Chewing (p=0.297) and the expression pattern of Bcl-2. The present study found no significant correlation of Bcl-2 with major anatomical sites. This is supported by the findings from other studies too such as Bhattacharya et al,[11] Abdel Aziz et al.[13] As highlighted in the table below, the present study found a higher immunopositivity of Bcl-2 in tumors with advanced stage and is significantly associated. This is consistent with the studies done by other authors Rodrigues et al,[10] Roda et al,[14] Rahmani et al,[3] who obtained positive correlation of Bcl-2 with respect to advanced tumour stage. These conclusions reflect that the biology of undifferentiated tumours is usually associated with increased mitotic activity and invasiveness [Table 12].

Limited study sample: Further studies should be done with a larger sample size, so that proper precautionary measures can be advised for early detection and treatment.

CONCLUSION

This study highlights the immunohistochemical use of important marker, the anti-apoptotic Bcl-2 which represents neoplastic progression in routine diagnostics of Oral Squamous Cell Carcinomas. Hence "Bcl-2 immunoexpression can prove to be of tremendous beneficial value in the early assessment of neoplastic behavior in Oral squamous cell carcinoma and may serve as independent prognostic markers with therapeutic implications."

We conclude that, in OSCC, there is an increased Bcl-2 expression with increasing tumour grade. The expression of Bcl-2 correlates with histological tumor grading in Oral Squamous Cell Carcinoma and has prognostic implications. Further investigation is needed to collaborate the expression of various oncogenes during development and progression of oral neoplasia, so as to open avenues for therapeutic modalities.

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