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Original Article

Oncology Section

Correlation of BCL-2 and Ki-67 Expression with Clinicopathological Parameters in Oral Squamous Cell Carcinoma

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ABSTRACT

Introduction: Oral Squamous Cell Carcinoma (OSCC) is the most common oral malignancy. Despite advancements in cancer therapeutics, early diagnosis, and prognosis of OSCC remains challenging. Evaluating clinicopathological characteristics, molecular biomarkers and their expression helps comprehend biological traits and tumour behaviour, which is crucial for early diagnosis and favourable prognosis.

Aim: To evaluate the immunohistochemical expressions of B-cell Lymphoma 2 (BCL-2) and Ki-67 in OSCC and to correlate their molecular expressions with clinicopathological tumourigenic parameters.

Materials and Methods: This cross-sectional study was conducted at Ramaiah Hospital, Bangalore, Karnataka, India, over a period of two years from June 2017 to May 2019 on 60 radical resection specimens of OSCC. After gross specimen examination, multiple representative tissue bits were obtained and subjected to Haematoxylin and Eosin (H&E) staining and staged. Immunohistochemical detection of BCL-2 and Ki-67 expression, semi-quantitative and intensity assessment of staining was performed. Data were analysed using statistical software R-version 4.0.1. The $p \leq 0.05$ was considered statistically significant.

Results: The mean age was 57.88±13 years, and a large proportion were females (49, 81.67%). Mean BCL-2 and Ki-67 expression (%) were 24.58±11.29 and 29.5±17.21, respectively. The correlation of BCL-2 ($p<0.001$) and Ki-67 ($p<0.001$) expression with histological grades was statistically significant. The correlation of BCL-2 and Ki-67 expression with gross/clinicopathological features was not significant. A positive moderately strong significant correlation existed between BCL-2 and Ki-67 expression ($p<0.001$).

Conclusion: The BCL-2 and Ki-67 expression is independent of clinicopathological features of OSCC but is directly associated with increasing tumour grades. There is a direct moderately strong correlation between BCL-2 and Ki-67, indicating adverse clinical outcome. Their early identification is critical for effective therapeutic intervention and desirable prognosis.

Keywords: B-cell lymphoma-2 protein, Immunohistochemistry, Ki-67 antigen, Oncoprotein, Oral cancer

INTRODUCTION

Oral cancer, the sixth most common type of cancer, presents 12% and 8% of all malignancies in men and women, respectively [1,2]. Over 90% oral cancers manifest as OSCC, which is the eighth most frequent oral cancer, often affecting the head and neck region [2,3]. Reportedly, prevalence and mortality rate associated with OSCC are 6.6 and 3.1 in 100,000 men, respectively, and 2.9 and 1.4 in 100,000 women, respectively [2]. Primary aetiological factors of OSCC include tobacco abuse, alcohol consumption, human papilloma virus infections, and dietary intake low in fresh vegetables and fruits [2].

The OSCC is preceded by premalignant or Potentially Malignant Lesions (PMLs), which are classified as epithelial hyperplasia, mild, moderate and severe epithelial dysplasia likely to transform into malignant cancers. Thus, early diagnosis of PMLs is crucial for curtailing tumour progression and achieving better prognosis [4]. The composite development of OSCC is a result of multiple genetic alterations arising from extended exposure to carcinogens and progression of epithelial dysplasia to invasive tumours [3]. Tumourigenic genetic abnormalities are known to include activation of growth-promoting oncogenes, inactivation of tumour-suppressor genes, and alterations in apoptosis-regulating genes [3]. Thus, detailed understanding of biological processes observed during preneoplastic stages is paramount for early identification of high risk patients and better therapeutic intervention for desirable prognosis [3]. However, despite remarkable advancements in cancer therapeutics, the prognosis of OSCC remains undesirable [5]. Additionally, the current diagnostic approach comprises subjective clinicohistological analysis. Consequently, in patients with absence of clinically distinguishable signs, the diagnosis of OSCC remains challenging [3].

Nevertheless, considering that molecular alterations occur prior to clinicohistopathological manifestations, molecular biomarkers of genomic alterations and cell proliferation have recently been reported beneficial for assessing high risk PMLs and their potential to transform to malignancy [3]. Lately, immunohistochemistry is gaining attention from scientific community as a crucial tool for diagnosis and prognosis of OSCC as it helps detect protein expression at molecular levels. Several immunohistochemical markers, including p53, BCL-2, and Ki-67, are currently under investigation [3]. However, there exists a huge lacuna in identification of specific and sensitive biomarkers for better diagnosis and prognosis of OSCC to increase overall survival rate among the patients [6].

The BCL-2 is an anti-apoptotic protein overexpressed during early epithelial carcinogenesis; it prevents caspase activation, ultimately impeding apoptosis. This induces cellular immortalisation by stimulating cell survival and facilitates permanent mutations and malignant transformation of a tumour [7,8]. BCL-2 is often expressed in a malignant state. Its overexpression in neoplastic state emphasises its involvement in the pathogenesis of prostate, colorectal, oesophageal, and breast cancers [9,10]. Although some studies have proposed a potential correlation of BCL-2 with overall survival rate and treatment modalities, the biological role of BCL-2 in progression of OSCC remains to be fully elucidated [9,10]. Conversely, Ki-67 is a nuclear protein involved in cellular proliferation [3]. Expressed predominantly during cell proliferation, Ki-67 disappears rapidly as soon as a cell enters resting phase. This feature promotes the implication of Ki-67 to demonstrate the cellular growth fraction of proliferating cells in malignant neoplasms, wherein high index value of Ki-67 indicates metastatic or carcinoma stage [3,11,12]. Moreover, Ki-67 can be employed

to assess cell proliferation by monitoring mitotic activity as it is not influenced by internal or external factors [12]. This suggests that percentage fraction of Ki-67 can help estimate the clinical stage and aggressiveness of tumours and distinguish proliferating cells from resting cells in OSCC [11].

Only few studies have evaluated the correlation between BCL-2 and Ki-67 in OSCC [3,13]. Thus, the present study aimed to evaluate the immunohistochemical expressions of BCL-2 and Ki-67 in patients with OSCC and correlate expressions of these biomarkers with clinicopathological parameters of the tumour.

MATERIALS AND METHODS

Present prospective, cross-sectional study was conducted at a Ramaiah Hospital, Bangalore, Karnataka, India, over a period of two years from June 2017 to May 2019. Study population consisted of 60 resected specimens collected after routine histopathological evaluation of patients with OSCC. The study was approved by the Institutional Ethics Committee (SS-1/EC/003/2017).

Inclusion and Exclusion criteria: Inclusion criteria included the resected specimens representing OSCC, whereas exclusion criteria included cases exhibiting extensive tumour necrosis without sufficient viable tumour cells for accurate evaluation of immunohistochemical results. Demographic characteristics of the patients, such as age, sex, clinical presentation, history of tobacco consumption, and any previous clinical investigation findings, were documented from the case files of patients.

Sample size calculation: The following formula was used for sample size calculation:

$$n = \frac{p\,(100-p)Z^2}{E^2}$$

Where, 'n' is the sample size required, 'p' is the percentage occurrence of a state or condition (proportion or prevalence), 'E' is the percentage maximum error required, and 'Z' is the value corresponding to level of confidence required. The prevalence of OSCC was reported to be 19% at the institution where the present study was conducted. With 95% of confidence level and 10% maximum error, the minimum sample size required for the present study was calculated to be 59. Thus, a total of 60 specimens were included.

All oral biopsies and resected specimens were preserved in 10% formalin. The standard protocol was followed for surgical grossing of resected specimens. After detailed gross specimen examination, multiple representative tissue bits were obtained from the tumour, surgical margins, adjacent mucosa (abnormal areas if any), and lymph nodes [14]. Subsequently, the tissues were embedded in paraffin blocks. The paraffin-embedded tissue was then sliced into sections of 3-5-µm and stained with H&E stains for assessment of depth of invasion, tumour type, histological grade, and lymphovascular invasion [15]. Features, such as the size of tumour and lymph node metastases, were recorded. The tumours were then staged according to the American Joint Committee on Cancer staging system and categorised into well-differentiated, moderately differentiated, poorly differentiated, and verrucous OSCC [16].

BCL-2 and Ki-67 detection by immunohistochemistry: Immunohistochemical detection of BCL-2 and Ki-67 expression was performed using 4 µm thick sections cut from paraffin-embedded tissue blocks. The sections were then smeared on a glass slide coated with adhesive poly-L-lysine. The immunohistochemistry technique followed in the study was "super sensitive link-label HRp detection system," which includes antigen retrieval in ethylenediaminetetraacetic acid buffer in a microwave oven at >200°C, three 15-minute-long cycles, and blocking endogenous peroxidase with 3% hydrogen peroxide [17]. The slides were then incubated with primary mouse monoclonal antibody against BCL-2 and Ki-67 and linked with rabbit anti-mouse secondary antibody (Biogenex, lot number QD000517). Streptavidin-horseradish peroxidase was used for enzyme labelling, whereas deaminobenzidine and haematoxylin were used as chromogen and counterstain, respectively. Positive and negative controls were run with each batch of slides. The positive controls for both BCL-2 and Ki-67 comprised tonsillar tissues.

Stained sections were microscopically evaluated. Specific cytoplasmic brown stain and nuclear stain were considered positive for BCL-2 and Ki-67, respectively. In each case, the percentage of stained tumour cells (number of stained tumour cells divided by total number of tumour cells) was calculated. Semi-quantitative and intensity assessment of staining for BCL-2 and Ki-67 expression were performed for all samples. The following immunohistochemical grading protocol was followed for BCL-2: a) 1 if ≤10% cells showed positive membranous staining; b) 1+ if 11-30% cells showed positive staining; c) 2+ if 31-60% cells showed positive staining; d) 3+ if >61% cells showed positive staining. The following immunohistochemical grading protocol was followed for Ki-67: a) 1 if ≤10% cells showed positive membranous staining; b) 2+ if 11-50% cells showed positive staining; c) 3+ if >51% cells showed positive staining [18,19].

STATISTICAL ANALYSIS

Data were analysed using statistical software R version 4.0.1 and Microsoft Excel. Categorical variables were represented in the form of frequency. Continuous variables were represented in the form of mean±Standard Deviation (SD). Chi-square test and Spearman's rank correlation coefficient was used to analyse the association between attributes. The p≤0.05 was considered statistically significant.

RESULTS

Out of 60 patients, 49 (81.67%) were females, whereas 11 (18.33%) were males. The patients had mean age of 57.88±13 years. [Table/Fig-1] presents the demographic characteristics of the patients and histological findings of the stained sections.

Demographic variables	Sub-category	Number of patients n (%)/Mean±SD
Age (years)	≤30	1 (1.67%)
	31-40	11 (18.33%)
	41-50	6 (10%)
	51-60	11 (18.33%)
	61-70	21 (35%)
	71-80	9 (15%)
	>80	1 (1.67%)
Tobacco use	No	7 (11.67%)
	Yes	53 (88.33%)
Type of tobacco use	Smoking	6 (10%)
	Smokeless	33 (55%)
	Both	14 (23.33%)
	None	7 (11.67%)
Duration of use (years)	Mean±SD	26.2±13.02
Histological findings	**Sub-category**	**Number of patients**
Histological grades	Well differentiated OSCC	17 (28.33%)
	Moderately differentiated OSCC	38 (63.33%)
	Poorly differentiated OSCC	1 (1.67%)
	Verrucous OSCC	4 (6.67%)
Site of tumour	Buccal mucosa	28 (46.67%)
	Gingivobuccal sulcus	13 (21.67%)
	Retro molar trigone	11 (18.33%)
	Tongue	8 (13.33%)
Size of tumour (cm)	<2	12 (20%)
	2-3	17 (28.33%)
	≥4	31 (51.67%)
	Mean±SD	2.94±1.28

Depth of tumour invasion (cm)	<1	23 (38.33%)
	1-2	32 (53.33%)
	2-3	5 (8.34%)
	Mean±SD	1.16±0.5
Gross features	Ulceroproliferative	46 (76.67%)
	Ulceroinfiltrative	12 (20%)
	Nodular	2 (3.33%)
Pathological stage	Stage 1/2	18 (30%)
	Stage 3/4	42 (70%)
BCL-2 expression	<10%	13 (21.67%)
	11-30%	29 (48.33%)
	31-60%	18 (30%)
	Mean±SD	24.58±11.29
Ki-67 expression	<10%	17 (28.33%)
	11-50%	33 (55%)
	>50%	10 (16.67%)
	Mean±SD	29.5±17.21

[Table/Fig-1]: Classification of patients based on demographic characteristics and histological findings.
OSCC: Oral squamous cell carcinoma

In terms of lymph node involvement, only eight cases out of 60 exhibited lymphovascular invasion; three of which showed perinodal spread. Based on Chi-square test, the association of BCL-2 and Ki-67 with histological grades was statistically significant. According to Cramer's V, the strength of both associations was moderate. [Table/Fig-2] presents the association of BCL-2 and Ki-67 with gross features of OSCC. Association of BCL-2 and Ki-67 with gross features of OSCC was not statistically significant [Table/Fig-3].

Immune markers		Histological grades				p-value (Cramer's V)
		WD n (%)	MD n (%)	PD n (%)	V n (%)	
BCL-2	<10%	8 (47.06%)	1 (2.63%)	0	4 (100%)	<0.001^{MC*} (0.5118)
	11-30%	6 (35.29%)	23 (60.53%)	0	0	
	31-60%	3 (17.65%)	14 (36.84%)	1 (100%)	0	
Ki-67	<10%	10 (58.82%)	3 (7.90%)	0	4 (100%)	<0.001^{MC*} (0.5075)
	11-50%	6 (35.30%)	27 (71.05%)	0	0	
	>50%	1 (5.88%)	8 (21.05%)	1 (100%)	0	

[Table/Fig-2]: Association of BCL-2 and Ki-67 with histological grades.
WD: Well differentiated; OSCC: Oral Squamous Cell Carcinoma; MD: Moderately differentiated; PD: Poorly differentiated; V: Verrucous; MC: Chi square test with monte carlo simulation; *Depicts statistical significance

Immune markers		Gross features			p-value
		Ulceroproliferative n (%)	Ulceroinfiltrative n (%)	Nodular n (%)	
BCL-2	<10%	10 (21.74%)	3 (25%)	0	0.7816^{MC}
	11-30%	24 (52.17%)	4 (33.33%)	1 (50%)	
	31-60%	12 (26.09%)	5 (41.67%)	1 (50%)	
Ki-67	<10%	13 (28.26%)	4 (33.33%)	0	0.7421^{MC}
	11-50%	26 (56.52%)	6 (50%)	1 (50%)	
	>50%	7 (15.22%)	2 (16.67%)	1 (50%)	

[Table/Fig-3]: Association of BCL-2 and Ki-67 with gross features of OSCC.
MC: Chi-square test with Monte Carlo simulation

The difference in BCl-2 and Ki-67 expression across different histological grades was statistically significant, while across different gross features of OSCC it was not statistically significant [Table/Fig-4]. No correlation between BCL-2 or Ki-67 with age, duration of tobacco use, size of tumour, and depth of tumour invasion was observed. A positive, moderately strong correlation was observed between BCL-2 and Ki-67 (p<0.001) [Table/Fig-5].

The association between BCL-2 and Ki-67 expression was statistically significant and moderately strong [Table/Fig-6]. [Table/Fig-7] depicts

Variables		BCL-2 Mean±SD	Ki-67 Mean±SD
Histological grades	Well differentiated OSCC	18.82±12.93	19.12±14.06
	Moderately differentiated OSCC	27.63±9.28	35.39±15.74
	Poorly differentiated OSCC	35	55
	Verrucous OSCC	17.50±11.90	11.25±2.50
	p-value	0.0121^{FP*}	<0.001^{FP*}
Gross features	Ulcer proliferative	23.80±11.16	29.02±16.89
	Ulcer infiltrative	25.83±11.45	30.83±18.07
	Nodular	35.00±14.14	32.50±31.82
	p-value	0.3736^{FP}	0.9402^{FP}

[Table/Fig-4]: Comparison of BCL-2 and Ki-67 in different histological grades and gross features of OSCC.
FP: Fisher pitman permutation test; *Depicts statistical significance

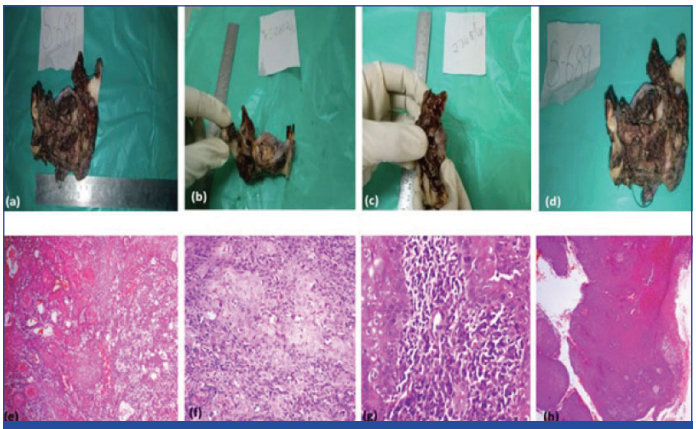
Variables	Correlation coefficient	p-value
BCL-2 and age	0.03204	0.808
Ki-67 and age	-0.01	0.9395
BCL-2 and duration of tobacco use	0.2288	0.0787
Ki-67 and duration of tobacco use	0.1824	0.1631
BCL-2 and size of tumour	-0.0862	0.5124
Ki-67 and size of tumour	-0.039	0.7676
BCL-2 and depth of tumour invasion	-0.0227	0.8633
Ki-67 and depth of tumour invasion	-0.0402	0.7603
BCL-2 and Ki-67	0.4398	<0.001^{S*}

[Table/Fig-5]: Correlation of BCL-2 and Ki-67 with study variables.
S: Spearman rank correlation; *Depicts statistical significance

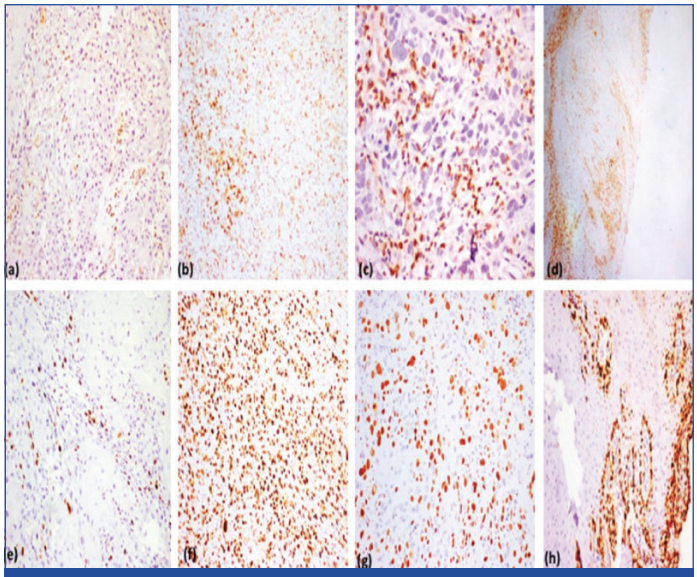
the gross features and histological grades of OSCC. [Table/Fig-8] depicts the expression of BCL-2 and Ki-67 in specimens with different histological grades of tumour.

Immune markers		Ki-67			p-value
		<10%	11-50%	>50%	
BCL-2	<10%	11 (64.71%)	2 (6.06%)	0	<0.001^{MC*} (0.4938)
	11-30%	5 (29.41%)	20 (60.61%)	4 (40%)	
	31-60%	1 (5.88%)	11 (33.33%)	6 (60%)	

[Table/Fig-6]: Association between BCL-2 and Ki-67.
MC: Chi-square test with Monte Carlo simulation; * Depicts statistical significance



[Table/Fig-7]: Gross features and histological grade of specimens. (a) Specimen photograph showing ulceroproliferative type of Oral Squamous Cell Carcinoma (OSCC) arising from buccal mucosa; (b) Specimen photograph showing ulceropro-liferative and nodular type of OSCC arising from the buccal mucosa; (c) Specimen photograph showing ulceroinfiltrative type of OSCC arising from buccal mucosa; (d) Specimen photograph showing ulceroinfiltrative type of OSCC arising from gingivobuccal sulcus; (e) Photomicrograph showing well differentiated squamous cell carcinoma with plenty of keratinisation and keratin pearls {Haematoxylin and Eosin (H&E) stain, 10X}; (f) Photomicrograph showing moderately differentiated squamous cell carcinoma with few areas of keratinisation and tumour cells with vesicular nucleus and abundant eosinophilic cytoplasm (H&E stain, 10X); (g) Photomicrograph showing poorly differentiated squamous cell carcinoma with plenty of tumour cells with hyperchromatic nucleus, pleomorphism, scant eosinophilic cytoplasm. Also, seen are few mitotic figures. (H&E stain, 20X); (h) Photomicrograph showing verrucous type of squamous cell carcinoma showing frond-like papillary processes (H&E stain, 10X).



[Table/Fig-8]: Immunohistochemical expression of BCL-2 and Ki-67 with different histologic grades of tumour. (a) Photomicrograph showing very low expression of BCL-2 in a case of well differentiated squamous cell carcinoma (SCC) {Immunohistochemistry (IHC), 10X}; (b) Photomicrograph showing 30-40% expression of BCL-2 in a case of moderately differentiated SCC (IHC, 10X); (c) Photomicrograph showing moderate (40-50%) expression of BCL-2 in a case of poorly differentiated SCC (IHC, 20X); (d) Photomicrograph showing low expression of BCL-2 in a case of verrucous type of SCC (IHC, 10X); (e) Photomicrograph showing very low expression of Ki-67 in a case of well differentiated SCC (IHC, 10X); (f) Photomicrograph showing moderate-high (>50%) expression of Ki-67 in a case of moderately differentiated SCC (IHC, 10X); (g) Photomicrograph showing moderate-high expression of Ki-67 in a case of poorly differentiated SCC (IHC, 20X); (h) Photomicrograph showing very low expression of Ki-67 in a case of verrucous type of OSCC (IHC, 20X).

DISCUSSION

In India, oral cancers are the most common form of malignancies in men with 12% prevalence rate and one of the five most commonly observed malignancies in women with 8% prevalence rate [1]. Despite therapeutic furtherance, the diagnosis and prognosis of OSCC remain challenging [3,5]. Analysing only clinicopathological features is not sufficient for studying biological traits of OSCC. The identification and investigation of molecular biomarkers, such as BCL-2, Ki-67, and p53, provide detailed insights into potential behaviour/aggressiveness of tumours [1]. The present study evaluated the immunohistochemical expressions of BCL-2 and Ki-67 in patients with OSCC and correlated the molecular expressions with clinicopathological parameters of the tumour.

Concurrent with the literature, the present study suggests that the most common site of tumour invasion is buccal mucosa as observed in 28 (46.67%) patients [1,20]. Despite majority of patients with OSCC are known to exhibit well differentiated carcinoma, in the present study, moderately differentiated OSCC was predominantly observed in 63.33% patients. The present study suggests that the expression of BCL-2 and Ki-67 is independent of age, duration of tobacco use, size of tumour, and depth of tumour invasion. According to the literature, BCL-2 expression is reportedly independent of various clinicopathological characteristics investigated in the study, except histological grading of OSCC [1,21,22]. However, regarding Ki-67, the findings in the literature are reportedly inconsistent [23-25]. Kim J et al., suggested that the expression of Ki-67 is inversely correlated to age, as younger patients in their cohort had poorer recurrence free survival than older patients with OSCC [24]. Conversely, Naderi NJ et al., evaluated the correlation of Ki-67 expression with age and gender in patients with laryngeal Squamous Cell Carcinoma (SCC) and concluded that Ki-67 expression is indeed independent of age and gender [25,26]. This inconsistency could be due to several factors, such as geographic-demographic characteristics of patients, genetic mutations, distinct aetiological and risk factors, immunohistochemistry techniques followed, and allele loss of genes [1]. Moreover, research has primarily centered around immunohistochemistry and molecular characteristics of OSCC. Thus, further research with a large sample size is essential to comprehend the influence of age and gender on molecular alterations in the development and progression of OSCC [25].

In the present study, BCL-2 and Ki-67 expressions were directly correlated to the histologic grading of OSCC. BCL-2 overexpression is indicative of the early stages of development and progression of tumour [2,27]. BCL-2 expression is upregulated with increasing tumour grade, i.e., from well to moderate and to poorly differentiated OSCC [1]. However, according to some studies, BCL-2 significantly declines as the histological grade increases [28,29]. These inconsistencies in the literature suggest that in OSCC, a clear relationship between BCL-2 overexpression and poor diseases prognosis is yet to be fully established [1,2,27]. These differences in BCL-2 expression could be attributed to inherent distinct characteristics in upstream genetic events between different population groups and environmental characteristics, which need further investigations [1,2,27].

The present study findings, wherein BCL-2 consistently increased with tumour grade, could be due to Ki-67 overexpression directly correlated with increasing tumour grades. Ki-67 induces active proliferative potential to tumour cells, and highly proliferating tumours are known to express BCL-2 proto-oncogene [2,27]. Bcl-2 is topographically confined to proliferating cells and those with longer life span with impaired apoptosis [2,27]. In the present study, moderately strong positive correlation was observed between BCL-2 and Ki-67 expression, suggesting that although BCL-2 is overexpressed in the early stages of OSCC, its overexpression is influenced by the proliferation potential induced by Ki-67. Additionally, the study suggests that this correlation between Ki-67 and BCL-2 is directly associated with increasing histological grade. Nevertheless, taking into account the gaps and inconsistencies in the literature, future studies with large sample size are needed to validate the hypothesis. Reportedly, Ki-67 protein is known to be upregulated in several tumours, including OSCC. Tumour proliferative ability induced by Ki-67 is directly correlated with tumour progression, i.e., with increasing tumour grade and stage [23]. Ki-67 expression also increases from mild-severe dysplasia and is associated with lymph node metastasis and higher worst pattern of invasion [23].

Alterations at molecular levels, including anti-apoptotic activity and cell proliferation, are strong predictors of malignant potential in OSCC, and their early identification could lead to desirable prognosis [28]. Altered BCL-2 favours cell accumulation by impairing cellular apoptosis, whereas overexpressed Ki-67 encourages aggressive cell proliferation [28]. Identification of proliferative index and anti-apoptosis using various biomarkers early in the development of OSCC helps achieve better patient outcome [28]. Nevertheless, future studies with large sample size can be conducted to validate the findings and aid in therapeutic advancement.

Limitation(s)

However, the present study possesses certain limitations, such as low sample size and unequal classification of patients as the study was conducted with patients from a restricted geographic area in Bangalore, Karnataka, India.

CONCLUSION(S)

The most common site of the tumour invasion in OSCC was the buccal mucosa. BCL-2 and Ki-67 expressions are independent of clinicopathological features of OSCC but directly associated with intensifying aggressive histopathological behaviour of the tumour. Additionally, despite inconsistencies in the literature, there exists a direct moderately strong correlation between BCL-2 and Ki-67, denoting adverse clinical outcome or poor prognosis of OSCC. Although BCL-2 and Ki-67 expression is independent of clinicopathological features, their early identification is critical for effective therapeutic strategies

and desirable prognosis. Future multicentric studies including patients from all parts of India and different clinicopathologic features should be sampled to establish a precise understanding regarding the expression and correlation of molecular biomarkers with clinicopathological characteristics in OSCC.

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