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ORIGINAL ARTICLE

Evaluation of Immunohistochemical Expression of BCL-2 in Oral Squamous Cell Carcinoma

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ABSTRACT

Introduction: Immunohistochemical (IHC) expression of Bcl-2 oncogene is helpful in characterization and prognosis of oral cancer. In the present study, we evaluated the immunoexpression of Bcl-2 oncogene in oral squamous cell carcinoma (OSCC) tissue specimen and correlated it clinicohistopathologically.

Material and Method: A total of 42 OSCC tissue specimen were subjected to evaluation for immunohistochemical expression of Bcl-2 oncogene. IHC expression was scored as "-" (<5% positive cells), + (5–24% positive cells), ++ (25–50% positive cells) and +++ (>50% positive cells). Pathological T stage, nodal status, histopathological grade, lymphovascular invasion (LVI), perineural invasion (PNI) and mitotic index (MI) were noted. Data was analysed using SPSS 21 software. Chi-square, ANOVA and Kruskall-Wallis tests were used to compare the data.

Results: Mean age of patients was 49.05 ± 13.28 years, majority of them were males (83.3%). There was a dominance of well/moderately differentiated (81%) cases. Lymphovascular and perineural invasion were seen in 16.7% and 26.2% cases. Majority had mitotic index 2 (66.7%), pT stage 3/4 (73.8%) and no nodal involvement (54.8%). Bcl-2 expression was seen in 34 (81%) specimen. It was +, ++ and +++ in 6 (14.3%), 15 (35.7%) and 13 (31.0%) cases. A significant correlation of Bcl-2 expression was seen with higher histopathological grades, mitotic index and pT stage. There was no correlation of Bcl-2 expression with age, sex, LVI, PNI, MI and pN status.

Conclusion: There was a high IHC expression of Bcl-2 oncogene which was significantly associated with histopathological grade and pT stage. Bcl-2 expression could have a prognostic value.

Keywords: Oral SCC, Bcl-2 family protein, Lymphovascular invasion, Perineural invasion, Mitotic index, Immunohistochemistry.

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INTRODUCTION

Oral cancer is one of the most common cancer forms globally. In the year 2020, cancers of oral cavity and pharynx collectively contributed to substantial morbidity and mortality worldwide, with an estimated 476,125 new cases and a total of 225,900 deaths.[1] In India, oral cancer is one of the major causes of total cancer burden owing to high chewing tobacco and gutkha use. It ranks number one in terms of incidence among men and third among women.[2] In India, the age standardized incidence rate of oral cancer is 12.6 per 100,000 population.[3] Proliferation, apoptosis and differentiation are the fundamental aspects of tumorigenesis and its progression[4]. Growing evidence suggestive of correlation between oral carcinogenesis and progressive accumulation of genetic alterations in molecules having a dominant role during apoptosis is emerging.[5] In the recent years, a number of proto-oncogenes have been identified that are known to prevent apoptosis or programmed cell death. It has also been highlighted that their altered expression could result in dysregulation of this process.[6] Bcl-2 (B-cell lymphoma/leukemia-2 gene) has been identified to play a role in regulating apoptosis.[7-9] Increased expression of protein product of Bcl-2 gene appears in the early phase of carcinogenesis leading to apoptosis deterioration and in consequence to the progression of neoplastic changes.[10] In many earlier studies, overexpression of Bcl-2 protein has been demonstrated in carcinomas of the nasopharynx, lung, colorectum, prostate, stomach, and esophagus. In addition, overexpression has also been observed in pre-cancerous lesions of the colorectum, oral cavity, stomach, and esophagus. This suggests that Bcl-2 may be associated with early oncogenesis in these organs.[10] Evidence has shown that overexpression of Bcl-2 was reported in oral dysplastic lesions and was suggested to play an important role in oral tumorigenesis.[11] Hence, the present study was carried out to evaluate immunohistochemical expression of Bcl-2 in oral squamous cell carcinoma in order to

correlate expression of Bcl-2 with different grades of Oral squamous cell carcinoma, mitotic index, lymphovascular invasion, perineural invasion, pathological stages and lymph node status.

MATERIAL AND METHOD

The study was carried out in the Department of Pathology of SRMS IMS, Bareilly over a period of 1.5 years from November 2019 to April 2021 after obtaining permission to carry out the study from the Institutional Ethics Committee. A total of 42 histopathologically confirmed wide local excision cases of OSCC with neck dissection submitted in the department of pathology were included in the study. Punch biopsy and wide local excision without neck dissection were excluded from the study. Sample size estimation was based on a previous study by Solomon *et al.*[12] that has reported the immunoexpression rate of Bcl-2 in OSCC to be 83.3%. The sample size projection was done at 95% confidence, 5% precision and after 10% contingency provision.

Histopathologically, the lesions were graded as well-differentiated (WD), moderately differentiated (MD) and poorly differentiated (PD) respectively according to the differentiation of cells and the resemblance of neoplastic cells to that of epithelial cells using Broder's classification.[13] Lymphovascular and perineural invasions, if any, were recorded. The tumor mitotic index and index of atypical mitoses was assessed semi quantitatively using the following criteria described by Sulkowska *et al*. (2003)[14]

Score 0	No mitoses.
Score 1	1–5 mitoses.
Score 2	6–10 mitoses.
Score 3	more than 10 mitoses.

The mitotic ratio was evaluated in at least 100 neoplastic cells per sample, using alight microscope at x 400 magnification.

IHC was done using Quartett GmBH Immunodiagnostica kit. The immunohistochemical assay was done as per instructions of the kit-manufacturer. A total of 100 cells were counted in ten fields and the percentage of positive cells was derived. The staining was graded using the following scoring criteria:

The percentage of positive cells was classified as:
If more than 50% of cells were positive (+++);
If 25–50% cells were positive (++);
If 5–24% cells were positive (+),
and if fewer than 5% positive or no staining (–).

Data Analysis

Data analysis was done using IBM Statistical Package for Social Sciences version 21.0. Bcl-2 expression was correlated with different histopathological grades of oral squamous cell carcinoma, mitotic index, lymphovascular invasion, perineural invasion, pathological stage and lymph node status. Chi-square test, ANOVA and Kruskal-Wallis tests were used to compare the data.

RESULTS

Age of patients ranged from 27 to 75 years. Mean age of patients was 49.05 ± 13.28 years. Majority of cases were males (83.3%). There was a dominance of well/moderately differentiated (81%) cases. Lymphovascular and perineural invasion were seen in 16.7% and 26.2% cases. Majority had mitotic index 2 (66.7%), pT stage 3/4 (73.8%) and no nodal involvement (54.8%). Bcl-2 expression was seen in 34 (81%) specimen. It was +, ++ and +++ in 6 (14.3%), 15 (35.7%) and 13 (31.0%) cases. There was no significant association of age and sex with Bcl-2 expression ($p>0.05$). With increasing histopathological grade, mitotic index and pT stage, there was a significant increase in Bcl-2 positive expression and its score ($p<0.05$), however, LVI, PNI and pN status did not show a significant association with Bcl-2 expression scores ($p>0.05$) (Table 1).

DISCUSSION

In the present study, Bcl-2 positive expression was seen in 81% of specimen. Two-third of the cases (66.7%) had Bcl-2 expression score 2/3. Similar to the present study, Solomon *et al.*[12] and Sutariya *et al.*[15] also reported high Bcl-2 expression (83%) in their study. There were some workers who reported Bcl-2 expression in all the SCC cases in their studies.[11,16] Rahmani *et al.*[17] in their study reported Bcl-2 immunoexpression in 51.7% of their cases with 1+, 2+ and 3+ expression in 15.0%, 15% and 23.3% cases respectively. Juneja *et al.*[18] on the other hand reported Bcl-2 positivity in only 30% of OSCC cases with 1+, 2+ and 3+ expression in only 16.7%, 10% and 3.3% cases respectively. However, a number of other workers observed IHC expression of Bcl-2 in only 30-40% of their cases.[19-21] Compared to most of the previous studies, the IHC expression of Bcl-2 in present study was higher. However, this overexpression pattern could not be termed as unusual as some other workers also report high immunoexpression of Bcl-2 as seen in present study.[15,17,22-23] Keeping in view the fact that Bcl-2 is associated with cellular apoptosis, the differences in Bcl-2 overexpression in different studies could be owing to difference in nature and type of cellular activity level in the cases.

In the present study, we did not find a significant association of Bcl-2 expression with age, sex, LVI, PNI and pN stage. Only histopathological grade, pathological T stage and mitotic index showed a significant association

with IHC expression of Bcl-2. Poorly differentiated grade was significantly associated with strongest expression of Bcl-2. With increase in mitotic index, a significant increase in Bcl-2 expression was observed.

The association of Bcl-2 expression with different clinicopathological factors have been assessed using different approaches and has shown a considerable variability across different studies.

Contrary to the findings of present study, Rahmani *et al*.[17] in their study found Bcl-2 positivity rate in males to be significantly higher as compared to that of females. They also found that lymph node positivity status was significantly associated with Bcl-2 positivity. Similar to present study, they also found that Bcl-2 expression and its strength was significantly associated with histopathological grade. Association of Bcl-2 overexpression with histopathological grade was also seen by other workers too.[18,19,21] Pavithra *et al*.[20] in their study did not find a significant association of Bcl-2 expression with metastatic status. In present study, we also did not find any such association with nodal status. However, Abdel Aziz *et al*.[24] in their study did not find a significant association of Bcl-2 overexpression with histopathological grade but found it to be significantly associated with lymph node metastasis status and clinicopathologic stage. As far as evidence related with association of Bcl-2 overexpression with histopathological grade is concerned, it is perhaps the most widely documented evidence[17-19,21] while relationships with other histopathological characteristics did not show a consistency across different studies. One of the reasons for inconsistencies in association of Bcl-2 overexpression with other clinicopathological factors, it could be attributable primarily to small sample size of different studies. It would be pertinent to mention here that most of these studies were carried out with sample size of 30

Table 1: Bcl-2 Immunoexpression in OSCC and its clinicopathological correlation (n=42)

		Bcl-2 Expression Score					*Statistical*
SN	*Variable*	*Total*	*-*	*1+*	*2+*	*3+*	*significance*
1.	No. of cases	42	8 (19%)	6 (14.3%)	15 (35.7%)	13 (31.0%)	-
1.	Mean age ± SD (Yrs)	49.05 ± 13.28	44.50 ± 13.10	44.17 ± 8.21	51.87 ± 14.04	50.85 ± 14.32	F=0.880; p=0.460 (ANOVA)
2.	Sex						
	Male	35 (83.3%)	8 (100%)	4 (66.7%)	12 (80%)	11 (84.6%)	χ^2=2.935; p=0.402
	Female	7 (16.7%)	0 (0.0%)	2 (33.3%)	3 (20%)	2 (15.4%)	
4.	Grade						
	WD	16 (38.1%)	5 (62.5%)	2 (33.3%)	9 (60.0%)	0	χ^2=21.77; p<0.001
	MD	18 (42.9%)	3 (37.5%)	4 (66.7%)	6 (40.0%)	5 (38.5%)	
	PD	8 (19.0%)	0	0	0	8 (61.5%)	
5.	LVI						
	Yes	7 (16.7%)	0	0	5 (33.3%)	2 (15.4%)	χ^2=5.67; p=0.128
	No	35 (83.3%)	8 (100%)	6 (100%)	10 (66.7%)	13 (84.6%)	
6.	PNI						
	Yes	11 (26.2%)	0	2 (33.3%)	5 (33.3%)	2 (15.4%)	χ^2=5.67; p=0.128
	No	31 (73.8%)	8 (100%)	4 (66.7%)	10 (66.7%)	13 (84.6%)	
7.	Mitotic Index						
	1	10 (23.8%)	5 (62.5%)	2 (33.3%)	1 (6.7%)	2 (15.4%)	χ^2=8.04; p=0.045
	2	28 (66.7%)	3 (37.5%)	3 (50.0%)	13 (86.7%)	9 (69.2%)	
	3	4 (9.5%)	0	1 (16.7%)	1 (6.7%)	2 (15.4%)	
8.	pT Stage						
	1	1 (2.4%)	0	1 (16.7%)	0	0	χ^2=11.92; p=0.008
	2	10 (23.8%)	5 (62.5%)	1 (16.7%)	4 (26.7%)	0	
	3	19 (45.2%)	3 (37.5%)	2 (33.3%)	8 (53.3%)	6 (46.2%)	
	4	12 (28.6%)	0	2 (33.3%)	3 (20.0%)	7 (53.8%)	
9.	pN Status						
	1	23 (54.8%)	7 (87.5%)	3 (50.0%)	7 (46.7%)	6 (46.2%)	χ^2=3.83; p=0.281
	2	5 (11.9%)	0	1 (16.7%)	3 (20.0%)	1 (7.7%)	
	3	13 (31.0%)	1 (12.5%)	2 (33.3%)	5 (33.3%)	5 (38.5%)	
	4	1 (2.4%)	0	0	0	1 (7.7%)	

or less.[18,21,23,24] Moreover, except for histopathological grading, which was generally chosen as a differentiating factor in all these studies, there is no consensus regarding other histopathological characteristics for which Bcl-2 overexpression was correlated.

In present study, we found a significant association of mitotic index apart from histopathological grade with Bcl-2 overexpression. A higher mitotic index is an indicator of increased cellular mitotic activity and is often related with poor histopathological grade and thus the association of Bcl-2 overexpression with both decreasing histopathological differentiation and higher mitotic index could be inferred as apoptosis inhibitory effect of Bcl-2.

The findings in present study mostly endorse the findings of previous study and underscore the role of Bcl-2 in carcinogenesis and its progression in oral SCC. However, like previous studies, the present study also suffers from the problem of a small sample size owing to which other clinicopathological relationships could not be extensively explored. Hence, further studies with a larger sample size are recommended.

CONCLUSION

There was a high IHC expression of Bcl-2 oncogene which was significantly associated with histopathological grade and pT stage. Bcl-2 expression should be considered as a progressive marker of tumorigenic activity and hence could have a prognostic value. Further studies on a longitudinal study design are recommended.

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