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Expression of Bcl-2 in Squamous Cell Carcinoma of Oral Cavity

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Author`s Contribution

[1,2]Substantial contributions to the conception or design of the work; or the acquisition, [3]Final approval of the version to be published [5]Drafting the work or revising it critically for important intellectual content,[4, 6]Active participation in active methodology, critical review

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ABSTRACT

Objectives: To assess Bcl-2 Immuno-histochemical expression in oral squamous cell carcinoma.

Methodology: This descriptive cross-sectional study was carried out at the "Department of Histopathology, Pakistan Institute of Medical Sciences, Islamabad", spanning from December 2021 to June 2022. Sixty individuals, ranging in age from 30 to 85 and representing both genders, diagnosed with squamous cell carcinoma of the oral cavity based on histopathological assessments, were included. Bcl-2 expression was assessed through immunohistochemistry, and subsequent analysis of all findings was conducted using Version 20 of SPSS software.

Results: Out of the 60 cases studied, 36 were males (60%) and 24 were females (40%). The patients' mean age was 55 years. The predominant histological grade observed was well-differentiated squamous cell carcinoma, constituting 26 out of 60 cases (43.3%), with moderately differentiated squamous cell carcinoma following closely at 21 out of 60 cases (35%). Poorly differentiated squamous cell carcinoma was the least common grade, found in 13 out of 60 cases (21.7%). Bcl-2 expression was positive in 23 patients (38.3%). In relation to the histological grade, there was a progressive increase in Bcl-2 expression across different grades of oral squamous cell carcinoma: 19% expression in "well-differentiated squamous cell carcinoma", 38% in "moderately differentiated squamous cell carcinoma", and 77% in "poorly differentiated carcinomas". This demonstrated a significant correlation between Bcl-2 expression and histopathological grades (p value <0.001).

Conclusion: In summary, Bcl-2 expression was detected in 38.3% of oral squamous cell carcinoma cases. Its immuno-expression is significantly associated with the histopathological grades of oral squamous cell carcinoma. Therefore, Bcl-2 can be considered an important prognostic marker as well as a potential target for chemoresistance cases through Bcl-2 inhibition.

Keywords: Squamous cell carcinoma, oral cavity, Bcl-2, Immunohistochemistry.

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Introduction

Conventional squamous cell carcinoma stands as one of the top ten most prevalent cancers worldwide. Its incidence has been on the rise since last few decades.[1] The recent incidence of this cancer showed 354,864 new cases in 2018. According to this statistical analysis, 177,384 deaths occurred because of this carcinoma.[2] Oral squamous cell carcinoma (OSSC) exhibits increased prevalence within specific geographic regions of developing countries. Globally one third of these cases occur in Southeast Asia.[3]Within this geographic area, oral squamous cell carcinoma ranks as the most prevalent cancer in India, Pakistan, Bangladesh and Sri

Lanka.[4] Among females in Pakistan, OSSC is the second most frequent cause of cancer-related fatalities.[5]

Within Western populations, smoking and alcohol consumption account for 74% of cases of this cancer, whereas in Asian populations, chewing of tobacco, including or excluding areca nut areca nut, emerges as the primary cause of OSCC.[6] A varying proportion of cases has been due to Epstein Barr Virus (EBV) infection.[6] The various subsites of OSCC are tongue, buccal mucosa, gingiva 1and floor of mouth. Out of these anatomic subsites, tongue OSCC is the most common and having the worse prognostic outcome. Despite the advancement of medical technologies the overall 5 year survival is <50%.[7]

Based on Broder's Criteria (mitotic activity, pleomorphism, and degree of differentiation), the most recent WHO Classification system in 2017 divides OSCC into two categories: keratinizing and non-keratinizing SCC. [8,9] Keratinizing SCC is further divided into three grades depending on the degree of keratinization as well, moderately & poorly differentiated histopathological grades. [9] Although the recognition is there that this 3 tier grading system and prognosis doesn't always correlate with each other.[10]

Tumor suppressor genes, oncogenes and cellular proliferation markers have been considered as the causative factors in tumorigenesis and as the potential predictive markers of tumor behavior. Bcl-2 gene is an anti-apoptotic gene (oncogene) that encodes into Bcl-2 protein which regulates cell cycle. It resides within mitochondria, the endoplasmic reticulum, and the peri-nuclear membrane, typically demonstrating expression in the basal layer of epithelium. Nevertheless, its atypical expression has been noted in a variety of solid tumors, encompassing those impacting the "lung, thyroid, breast, ovaries, stomach, and colon".

Functionally, Bcl-2 plays a pivotal role in cell survival by impeding apoptosis, thereby promoting tumor development. Consequently, its overexpression in many tumors has been linked to a poor response to therapy, as it inhibits apoptosis initiated by chemotherapy or radiotherapy.[11] On the contrary, research suggests that decreased Bcl-2 expression levels are linked to a positive prognosis, whereas elevated levels are associated with a less favorable outlook.[12] This connection is frequently explained by the reciprocal relationship between "Bcl-2" and "Bax" protein expression; reduced "Bcl-2" levels typically correspond with elevated "Bax" levels, subsequently facilitating apoptosis.[13]

A study conducted in India showed 37% Bcl-2 positivity[14] while an Egyptian study depicted 30% positivity of this antiapoptotic protein in OSCC. [15] Also Bcl-2 expression is decreased in terminally differentiated cells. [13]

Management of this carcinoma is mainly surgical resection with or without neoadjuvant therapy. The indication of neoadjuvant therapy is majorly dependent on histopathological grade and stage of OSCC.[16] However, TNM staging and the biological behavior of this tumor are not always in concordance with each other. So there comes the need of an immunohistochemical marker to help predict tumor behavior.[17] Many studies indicate that this anti-apoptotic protein is expressed in OSCC making it a prognostic indicator as well as an alternative option in chemoresistance cases in form of "Bcl-2 inhibition". [18] The presence of "Bcl-2 expression" has been shown to possess substantial predictive value for the response to chemotherapy. [19]

This study aims to assess the "Bcl-2" immuno-expression in OSCC. This anti-apoptotic protein will give prognostic idea about high grade OSCC. Thus, detecting the expression of this marker will assist in early identification and management of OSCC's adverse biological characteristics. Bcl-2 expression will also give an option of target therapy with anti-Bcl-2 in chemo resistant cases, as the number of OSCC responding to "Cisplatin" chemotherapy are unfortunately low.[20] Thus, Bcl-2 expression will also help in alleviating tumor burden and increasing disease-free period of patients of OSCC.

Methodology

The descriptive cross-sectional study took place at the "Pakistan Institute of Medical Sciences, Islamabad," spanning from December 2021 to June 2022. Following approval from the hospital's ethical committee, oral mucosal biopsies and resection specimens from sixty patients diagnosed with OSCC were selected. Patients with metastatic carcinomas to the oral cavity, as well as those with histopathological diagnoses other than oral squamous cell carcinoma, were excluded from this study.

The specimens were first fixed in 10% formalin, then subjected to gross examination, followed by sectioning, embedding in paraffin blocks, cutting,

preparation of slides, and finally staining the tissue with Eosin and Hematoxylin. The slides were examined under light microscope by the consultant pathologist along with postgraduate resident and diagnosis was recorded. Immunohistochemistry was applied for Bcl-2 expression on duplicate slides for each case. The slides underwent deparaffinization and rehydration before antigen retrieval. Subsequently, they were incubated with a Bcl-2 monoclonal antibody. Visualization of the antigen-antibody complexes was achieved using the Advance detection system (Dako). Following several steps of slide preparation, positive results were indicated by nuclear and cytoplasmic brown staining. Evaluation involved assessing the percentage of stained tumor cells and the intensity of staining.

Light brown staining observed in the nuclei and cytoplasm of epithelial cells was regarded as positive, with staining in lymphocytes serving as an internal control. Positivity percentage was determined by counting 100 cells across 10 fields. Staining intensity was categorized as follows: no stain, mild, moderate, and intense, as detailed in Table I.[21]

Table I: Staining criteria for Bcl-2 expression.

Staining intensity	Scoring
"No stain (±)"	"<10%"
"Mild (+)"	"10% - 25%"
"Moderate (++)"	"26% - 50%"
"Intense (+++)"	">50%"

The data underwent computerized entry and analysis via SPSS version 20. Quantitative variables such as age were summarized using mean and standard deviation, while qualitative variables like gender, histopathological subtype, tumor grades, and Bcl-2 expression were presented as frequencies and percentages. Stratification was employed to adjust for effect modifiers such as age, gender, histopathological subtypes, and tumor grade. Subsequent post-stratification Chi-square tests were conducted, with statistical significance set at $P \leq 0.05$.

Results

Total number of OSCC cases were 60. Out of these 60, 36 were males (60%) while 24 were females (40%). The age span ranged from "30 to 85 years", with a mean age of "55 years". As far as histological sub-types were considered, conventional OSCC was the most dominant subtype causing for 48/60 (80%) of cases followed by Verrucous carcinoma i.e. 5/60 (8.3%) and Adeno-squamous carcinoma 3/60 (5%). The least common subtypes were Basaloid squamous cell carcinoma 2/60 (3.3%) and Acantholytic cell carcinoma 2/60 (3.3%). Coming to the histopathological grades of OSCC, most common grade was "well differentiated SCC" i.e. 26/60 (43.3%) followed by "moderately differentiated SCC" 21/60 (35%) while "poorly differentiated SCC" was the least common grade accounting for 13/60 (21.7%) cases. Bcl-2 expression was found to be positive in 23 patients (38.3%).

Regarding grade-wise Immunohistochemical expression of Bcl-2, five out of twenty-six (19%) cases of "well differentiated SCC" showed Bcl-2 positivity (Figure 1) while eight out of twenty-one (38%) cases of "moderately differentiated SCC" expressed Bcl-2 (Figure 2). Regarding "poorly differentiated SCC" , ten out of thirteen (77%) were Bcl-2 Positive (Figure 3).

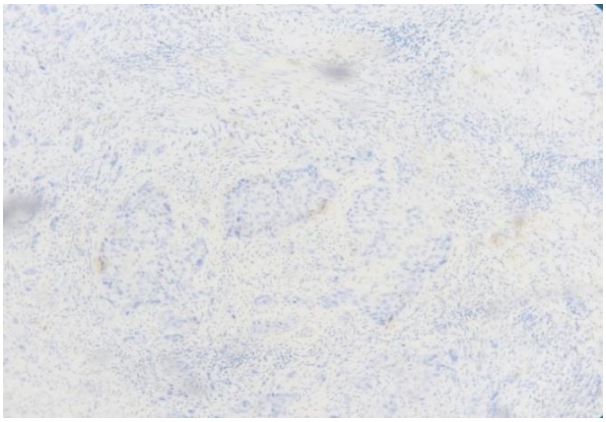

Figure 1. "Bcl-2 in Well differentiated SCC" showing no stain (±) in ≤10 % of tumor cells.

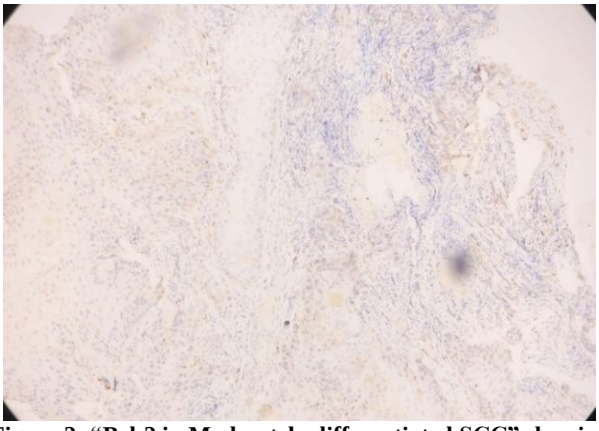

Figure 2: "Bcl-2 in Moderately differentiated SCC" showing mild intensity staining in 26-50 % of tumor cells.

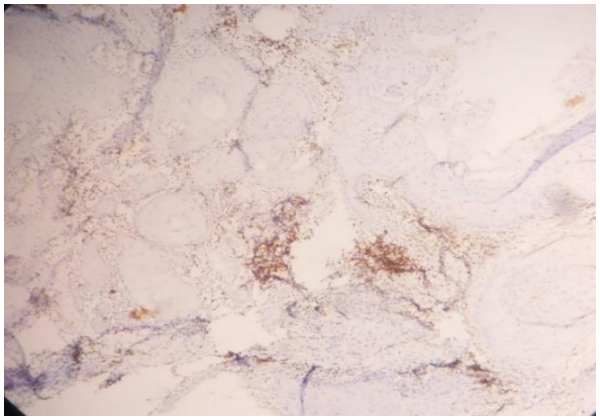

Figure 3: "Bcl-2 in Poorly differentiated SCC" showing moderate to severe intensity staining in 26-50 % of tumor cells.

A significant correlation was observed between Bcl-2 and histopathological grade (p value <0.001). (Table II).

Table II: "Correlation of Bcl-2 with Histopathological Grades of OSCC".

Grade of tumour	Bcl-2 expression		Total	P value
	Positive	Negative		
Well differentiated	5	21	**26**	
Moderately differentiated	8	13	**21**	**<0.001**
Poorly differentiated	10	3	**13**	
Total	**23**	**37**	60	

Discussion

The number of OSCC responding to "Cisplatin" chemotherapy are unfortunately low.[20] A detailed explanation of the molecular mechanisms behind OSCC is essential for early detection and treatment, ultimately leading to better patient survival outcomes.[22]The key step in tumorigenesis is the inhibition of apoptosis, characterized by Bcl-2 overexpression. .[23] The presence of Bcl-2 expression has been shown to have considerable predictive value for the response to chemotherapy.[19]

Certain studies suggest a correlation between reduced Bcl-2 expression and a positive prognosis, whereas heightened expression is associated with a less favorable outlook.[12] Decreased "Bcl-2" levels commonly coincide with elevated "Bax protein" expression, directing cells towards apoptosis.[13] In our present investigation, we enrolled 60 cases of OSCC, with a higher prevalence observed among males compared to females, having 1.5:1 ratio of male-to-female gender. This increased incidence among males is attributed to factors such as smoking and betel nut chewing, aligning with findings from an Indian study.[24]

The youngest patient in our study of 60 patients of OSCC aged 30 years while the oldest one is 85 years of age giving the age range of 30-85 years. An Egyptian study showed the most frequent age group of OSCC was 40–60 years.[25] In terms of histological subtypes, conventional OSCC accounted for the majority (80%), consistent with findings reported by "CIUCA et al".[(26)] When histo-pathological grades of our study were assessed, Grade 1 (Well differentiated) cases accounted for the most common ones. Same is the case by an Indian study carried out in Karnatka. [27]

Nuclear and cytoplasmic immuno-expression of Bcl-2 was assessed which came out to be positive in 38.3% cases (23/60). "In our study, we observed low overall Bcl-2 expression, which correlates with a good prognosis. This finding is corroborated by histological evidence. The majority of the tumors analyzed displayed well-differentiated features having abundant areas of keratinization, with only sporadic cells showing Bcl-2 labeling.[11] A study conducted in India showed 37% Bcl-2 positivity [14] and another Indian study showed 33% Bcl-2 positivity [17] Another Indian study carried out by Teni et al showed 56% Bcl-2 positivity in OSCC[13] while an Egyptian study depicted 30% positivity of this antiapoptotic protein in OSCC.[15] Correlation of Bcl-2 with age, gender and histological subtypes was insignificant (p value >0.05). As the tumor grade advanced, there was a notable rise in Bcl-2 expression rates: 19% in "well-differentiated", 38% in "moderately differentiated", and 77% in "poorly differentiated OSCC" cases. This underscores a significant association between Bcl-2 expression and WHO grades (p-value < 0.001) which aligns with findings from another study conducted by Rahmani et al (p value <0.05). [28]

Conclusion

In 38.3% of oral squamous cell carcinoma cases, "Bcl-2 expression" was identified. A significant association was observed between "Bcl-2 expression" and WHO histopathological grades (p-value < 0.001). Therefore, the anti-apoptotic "Bcl-2" positivity may serve as a valuable prognostic indicator and could potentially offer an alternative approach in cases of chemoresistance through Bcl-2 inhibition.

References

1. Kolokythas A. Oral squamous cell carcinoma in the young patient: an emerging unique cohort of patients. Oral Oral Surg. Oral Med. Oral Radiol. 2022;133(6):617. https://doi.org/10.1016/j.oooo.2022.02.016
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. https://doi.org/10.3322/caac.21492
3. García-Martín JM, Varela-Centelles P, González M, Seoane-Romero JM, Seoane J, García-Pola MJ. Epidemiology of oral cancer. Oral Cancer Detection: Springer; 2019. 81-93. https://doi.org/10.1007/978-3-319-61255-3_3
4. Tandon A, Bordoloi B, Jaiswal R, Srivastava A, Singh RB, Shafique U. Demographic and clinicopathological profile of oral squamous cell carcinoma patients of North India: A retrospective institutional study. SRM J Res Dent Sci. 2018;9(3):114-8. https://doi.org/10.4103/srmjrds.srmjrds_21_18
5. Javed A, e Zahra G, Qureshi AM. Epidemiology of Oral Cancer in Pakistan. American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS). 2020;72(1):118-27.
6. Perera M, Al-Hebshi N, Perera I, Ipe D, Ulett G, Speicher D, et al. Inflammatory bacteriome and oral squamous cell carcinomaJ. Dent. Res. 2018;97(6):725-32. https://doi.org/10.1177/0022034518767118
7. Farhood Z, Simpson M, Ward GM, Walker RJ, Osazuwa-Peters N. Does anatomic subsite influence oral cavity cancer mortality? A SEER database analysis. The Laryngoscope.2019;129(6):1400-6. https://doi.org/10.1002/lary.27490
8. Boxberg M, Jesinghaus M, Dorfner C, Mogler C, Drecoll E, Warth A, et al. Tumour budding activity and cell nest size determine patient outcome in oral squamous cell carcinoma: proposal for an adjusted grading system. Histopathology.2017;70(7):1125-37. https://doi.org/10.1111/his.13173
9. Mattavelli D, Ferrari M, Taboni S, Morello R, Paderno A, Rampinelli V, et al. The 8th TNM classification for oral squamous cell carcinoma: What is gained, what is lost, and what is missing. Oral Oncology. 2020;111:104937. https://doi.org/10.1016/j.oraloncology.2020.104937
10. El-Naggar AK, Chan JK, Grandis JR, Takata T, Slootweg PJ. WHO classification of head and neck tumours: International Agency for Research on Cancer (IARC); 2017.
11. Popović B, Jekić B, Novaković I, Luković L, Tepavčević Z, Jurišić V, et al. Bcl-2 expression in oral squamous cell carcinoma. Ann N Y Acad Sci. 2007;1095(1):19-25. https://doi.org/10.1196/annals.1397.003
12. Stoll C, Baretton G, Ahrens C, Löhrs U. Prognostic significance of apoptosis and associated factors in oral squamous cell carcinoma. Virchows Archiv. 2000;436:102-8. https://doi.org/10.1007/PL00008207
13. Teni T, Pawar S, Sanghvi V, Saranath D. Expression of bcl-2 and bax in chewing tobacco-induced oral cancers and oral lesions from India. Pathology Oncology Research. 2002;8:109-14. https://doi.org/10.1007/BF03033719
14. Pallavi N, Nalabolu GRK, Hiremath SKS. Bcl-2 and c-Myc expression in oral dysplasia and oral squamous cell carcinoma: An immunohistochemical study to assess tumor progression. J. Oral Maxillofac. JOMFP. 2018;22(3):325. https://doi.org/10.4103/jomfp.JOMFP_197_18
15. Aziz MMA, Zaki MM, Farg DA, El Kourdy KA. Immunohistochemical expression of Bcl-2 in oral squamous cell carcinoma: a clinicopathological correlation. Egyptian Journal of Pathology. 2019;39(2):257. https://doi.org/10.4103/EGJP.EGJP_33_19
16. Almangush A, Mäkitie AA, Triantafyllou A, de Bree R, Strojan P, Rinaldo A, et al. Staging and grading of oral squamous cell carcinoma: An update. Oral Oncology. 2020;107:104799. https://doi.org/10.1016/j.oraloncology.2020.104799
17. Pavithra V, Kumari K, Haragannavar VC, Rao RS, Nambiar S, Augustine D, Sowmya S. Possible role of Bcl-2 expression in metastatic and non metastatic oral squamous cell carcinoma. J Clin Diagn Res.2017;11(9):ZC51. https://doi.org/10.7860/JCDR/2017/29363.10601
18. Muthukumaran R, Vezhavendhan N, Vidyalakshmi S, Sivaramakrishnan M, SanthaDevy A, Suganya R. Immunohistochemical Expression of Bcl-2 in Oral Squamous Cell Carcinoma.
19. Moreno-Galindo C, Hermsen M, García-Pedrero JM, Fresno MF, Suárez C, Rodrigo JP. p27 and BCL2 expression predicts response to chemotherapy in head and neck squamous cell carcinomas. Oral oncology. 2014;50(2):128-34. https://doi.org/10.1016/j.oraloncology.2013.10.018
20. Cheng Y, Li S, Gao L, Zhi K, Ren W. The molecular basis and therapeutic aspects of cisplatin resistance in oral squamous cell carcinoma. Frontiers in oncology. 2021;11:761379. https://doi.org/10.3389/fonc.2021.761379
21. Bhattacharya I, DawSon L, Sharma S. Prognostic significance of p53, Ki-67 and Bcl-2 in leukoplakia and squamous cell carcinoma of the oral cavity. Natl J Lab Med. 2017;6:16-21.
22. Sasahira T, Kirita T. Hallmarks of cancer-related newly prognostic factors of oral squamous cell carcinoma. Int. J. Mol. Sci.2018;19(8):2413. https://doi.org/10.3390/ijms19082413
23. Jain A. Molecular pathogenesis of oral squamous cell carcinoma. Squamous cell carcinoma-hallmark and treatment modalities. 2019. https://doi.org/10.5772/intechopen.85650
24. Suresh GM, Koppad R, Prakash B, Sabitha K, Dhara P. Prognostic indicators of oral squamous cell carcinoma. Ann. Maxillofac. Surg. 2019;9(2):364. https://doi.org/10.4103/ams.ams_253_18
25. Aziz MMA, Zaki MM, Farg DA, El Kourdy KA. Immunohistochemical expression of Bcl-2 in oral squamous cell carcinoma: a clinicopathological

correlation. Egypt. J. Pathol.2019;39(2):257-62. https://doi.org/10.4103/EGJP.EGJP_33_19

26. CIUCĂ FI, MARASESCU P-C, MATEI M, FLORESCU A-M, MARGARITESCU C, Petrescu S, Dumitrescu C. Epidemiological and histopathological aspects of tongue squamous cell carcinomas-retrospective study. Curr. Health Sci. J. 2018;44(3):211.
27. Kumar GK, Abidullah M, Elbadawi L, Dakhil S, Mawardi H. Epidemiological profile and clinical characteristics of oral potentially malignant disorders and oral squamous cell carcinoma: A pilot study in Bidar and Gulbarga Districts, Karnataka, India. Journal of oral and maxillofacial pathology: JOMFP. 2019;23(1):90. https://doi.org/10.4103/jomfp.JOMFP_116_18
28. Rahmani A, Alzohairy M, Babiker AY, Rizvi MA, Elkarimahmad HG. Clinicopathological significance of PTEN and bcl2 expressions in oral squamous cell carcinoma. Int J Clin Exp Pathol. 2012;5(9):965.