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Original Research Article

Exploring the Role of EZH2 and BCL2 in Demarcating Oral Verrucous Hyperplasia and Verrucous Carcinoma

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Abstract

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Introduction Oral verrucous hyperplasia (OVH) and verrucous carcinoma (OVC) are precursors of oral squamous cell carcinoma exhibiting overlapping histopathological picture which warrants distinction. EZH2 is an epigenetic marker possessing multifaceted function in cellular proliferation, migration, and malignant transformation, whereas BCL2 is an integral part of the antiapoptotic mechanism regulating cellular homeostasis.

Aim The aim was to distinguish OVH and OVC by analysis of immunohistochemical expression of EZH2 and BCL2.

Material and Methods The study sample consisted of 79 formalin-fixed paraffin-embedded tissue sections of normal oral mucosa (10), OVH (10), oral OVC (27), and oral squamous cell carcinoma (32). Immunohistochemical analysis of EZH2 and BCL2 was done and labeling indices were calculated. Additionally, six histopathological parameters were assessed in OVH and OVC. Statistical analysis was done using Kruskal–Wallis test, Tukey honest significant difference test, and Spearman's correlation. Receiver operating characteristic curve was plotted and sensitivity, specificity, and cutoff score of each marker were calculated.

Result and Discussion Labeling indices of EZH2 and BCL2 depicted a gradual incline from normal mucosa to oral squamous cell carcinoma. Significant difference of EZH2 and nonsignificant difference in BCL2 expression between OVH and OVC were noted. Out of the six histopathological parameters, keratin plugging, juxtaepithelial lymphocytic response, and frank endophytic growth yielded a significant difference. EZH2 serves as a superior marker than BCL2 to differentiate OVH and OVC. Juxtaepithelial lymphocytic response can also serve as a histopathological parameter in distinguishing OVH and OVC.

Keywords

- verrucous hyperplasia
- verrucous carcinoma
- BCL2
- EZH2
- oral squamous cell carcinoma
- distinguish
- proliferation
- apoptosis

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Introduction

Oral squamous cell carcinoma (OSCC) constitutes a majority of the worldwide burden of cancer with a 5-year survival rate of 50%.[1] The root cause of OSCC can be pinned to a combination of genetic changes due to long-term exposure of carcinogens and evolution of premalignant lesions to invasive tumors.[2] Verrucous papillary lesions of the oral cavity encompass a spectrum of benign, potentially malignant, and malignant lesions which are also considered as precursors of OSCC. Belonging to this domain, oral verrucous hyperplasia (OVH) and verrucous carcinoma (OVC) are two distinctive verrucous lesions which share homologous clinical and histopathological picture and the existing knowledge to distinguish them seems inadequate.[3]

First described by Ackerman in 1948, OVC is a rare low-grade variant of OSCC which exhibits hyperplastic epithelium with parakeratotic plugging, bulbous rete ridges with an intact basement membrane, and minimal dysplasia.[4,5] Shear and Pindborg first described OVH as epithelial hyperplasia and verrucous surface, no invasion of the hyperplastic epithelium into the lamina propria compared with adjacent normal mucosal epithelium; however, with varying degrees of epithelial dysplasia.[6,7] A properly oriented hematoxylin–eosin stained section is the gold standard for their distinction; however, it is often worsened by very small biopsies, poorly orientated specimens, and most notably, biopsies failing to demonstrate the lesion margin.[8] Several authors have tried to formulate demarcating histopathological parameters which might help us to separate these entities but the data are still disputed.[9–11] Subsequently, the distinction between these lesions could be better acknowledged by utilizing certain supplemental immunohistochemical markers.

Dysplastic, metaplastic, and neoplastic alterations are mostly caused by genetic defects leading to an imbalance in the molecular pathways which regulate apoptosis and cell growth.[12] Enhancer of zeste homolog (EZH2), is a histone – lysin N-methyltransferase which forms a catalytic subunit of polycomb recessive complex (PRC2) for trimethylation of histone H3 at lysine 27 (H3K27me3) which play a vital role in cell proliferation and is a critical factor of pluripotency and differentiation of stem cells as well as aberrant gene expression during malignant transformation.[13,14] EZH2 has been previously associated with histological differentiation, mode of invasion, lymph node metastasis, and prognosis in OSCC.[15] While exploring EZH2 expression in verrucous lesions, possible alternate pathways other than methyltransferase activity have been suggested.[16] Few studies conducted in hematological malignancies and OSCC cell lines have uncovered EZH2 playing a pivotal role in the apoptotic pathway.[1,17–21] Considering the abovementioned facts, it could be hypothesized that delving into the intricacies of apoptotic pathway could unveil an unexplored aspect of the molecular mechanism of EZH2 regulation eventually assisting in treatment modalities. B cell lymphoma 2 (BCL2) localizes itself in the outer membrane of mitochondria, where it promotes cell survival and inhibits apoptosis by blocking cytochrome C followed by inactivation of the caspases.[17,22,23] Although few studies have explored BCL2 expression in OVH and OVC, only a single study was conducted using EZH2 in the same lesions.[16] In the present study, we have analyzed the immunohistochemical expression of EZH2 and BCL2 concomitantly with an attempt to distinguish OVH and OVC. We have also examined and correlated the expression pattern of EZH2 and BCL2 from normal oral mucosa to OSCC to determine their malignant transformation and the possible overlap between EZH2 and the antiapoptotic pathway mediated by BCL2. Six histopathological criteria including surface projection, keratin plugging, atypia, basilar hyperplasia, juxtaepithelial lymphocyte response, and frank endophytic growth were also selected and observed in OVH and OVC.

Materials and Methods

The current observational and cross-sectional study was conducted in the Department of Oral and Maxillofacial Pathology and Microbiology, Post Graduate Institute of Dental Sciences (PGIDS), Rohtak, Haryana, India and approved by Institutional Scientific and Ethical Committee (PGIDS/2021/OP/152 dated 03/03/2021). A total of 79 cases of formalin-fixed paraffin-embedded tissue section as well as new biopsy specimens of OSCC (32), OVC (27), OVH (10), and normal oral mucosa (10) were retrieved from the departmental archives. The clinicopathologic information of all cases, including age, sex, intraoral location, clinical presentation, and habit history, was retrieved from the requisition forms. The following criteria were implemented while selecting the samples.

Inclusion Criteria

- Histopathologically diagnosed cases of:
  - Normal oral mucosa (submitted during orthodontic extractions and operculectomy procedure) – group I
  - OVH (diagnosed by criteria given by Lin et al) – group II
  - OVC (diagnosed by criteria given by Lin et al) – group III
  - OSCC – group IV

  Due to the shortage of OVH, two cutaneous verrucous hyperplasia specimens were included in the study, sharing similar nature of these lesions with different localizations.[8]

Exclusion Criteria

- Recurrent and unconfirmed cases of OVC, OVH, and OSCC.
- Patients who underwent prior chemotherapy or radiotherapy.
- Patients with history or symptoms of systemic illnesses.

Immunohistochemistry

Note that 4 µm sections were obtained from the formalin-fixed paraffin-embedded specimen on polylysine-coated slides. Immunohistochemical staining was performed using the streptavidin-biotin-peroxidase complex method. The slides were incubated in primary antibodies EZH2 (mouse monoclonal antibody, 1 mL concentrated dilute, 1:10, Invitrogen) and BCL2 (mouse monoclonal antibody, 1 mL concentrated dilute, 1:90, cell marque) at room temperature for 1 hour.

Diaminobenzidine was used as chromogen. Negative control sections were done by omission of the relevant primary antibody. Positive controls for EZH2 (testis) and BCL2 (tonsil tissue) were also performed on each run.

Immunohistochemical Analysis

A dark brown nuclear immunoreactivity was considered as positive for EZH2 immunoexpression, whereas brown cytoplasmic or membranous expression was considered as positive for BCL2 immunoexpression. Five hotspots containing maximum number of positively stained cells were selected at the magnification of 400× and 1,000 epithelial cells or epithelial tumor cells were counted. The percentage of positive-staining cells per 1,000 counted cells was regarded as labeling index (LI).

Histopathological Analysis

Hematoxylin and eosin-stained slides of OVH and OVC were independently evaluated by two oral pathologists (S.C. and A. D.) under light microscope for the presence or absence of histopathological parameters including surface projection, keratin plugging, atypia, basilar hyperplasia, juxtaepithelial lymphocyte response, and frank endophytic growth. According to Li et al, juxtaepithelial lymphocytic response was further categorized as weak, intermediate, and strong.[24]

Statistical Analysis

Data was subjected to statistical analysis using SPSS (v 25.0, IBM). Shapiro–Wilk test revealed a nonnormal distribution of data based on which comparison of frequencies between groups was done using Kruskal–Wallis and Tukey honest significant difference test. The receiver operating characteristic (ROC) curve was plotted using the labeling indices of two groups at a time to determine the sensitivity, specificity, and cutoff score. EZH2 and BCL2 were correlated by Spearman's correlation coefficient. The association of the histopathological parameters and OVH and OVC was evaluated using chi-square test. The entire methodology of the study is represented by a consolidated flowchart in ►Supplementary figure 1.

Results

Demographic Details

The observational study was conducted on 79 cases (63 males, 16 females) with an age range of 22 to 91 years (mean age = 52.5 years). Buccal mucosa (28/79, 35.44%) was most commonly involved followed by gingiva (10/79, 12.65%). Mandibular alveolus, lip, and retrocommissural area each had the same number of cases (7/79, 8.86%). The association of site distribution among the various groups yielded a significant difference ($p = 0.000$). Various habits were identified in 47 patients (59.5%), out of which 22 cases (27.8%) showed only smoking habit, 11 cases (13.9%) with tobacco chewing habit alone, and 11 cases (13.9%) with both smoking and tobacco chewing habit. Two cases (2.5%) reported with smoking and alcohol history and one case (1.3%) had a history of smoking, tobacco chewing, and alcohol consumption. Habit history yielded no significant difference ($p = 0.006$) among all study groups. Excluding 10 normal mucosa cases, 25 (31.6%) presented as ulceroproliferative, 13 (16.8%) were ulcerative, 8 (10.1%) presented with swelling, 18 (22.8%) as proliferative growth, and 5 (6.3%) cases showed a whitish patch. The association of clinical presentation among various study groups yielded a significant difference ($p = 0.000$) (►Table 1).

EZH2 and BCL2 Expression in All Groups

The LI of EZH2 was recorded as 11.06 in group I, 37.14 in group II, 63.14 in group III, and 78.66 in group IV with a statistically significant difference ($p = 0.000$). BCL2 showed LI of 11.16 in group I, 23.24 in group II, 33.82 in group III, and 63.70 in group IV along with a significant difference ($p = 0.000$). For EZH2, there was a significant difference obtained between all the groups whereas in BCL2 expression, significant difference was observed between group I and IV ($p = 0.000$), group II and IV ($p = 0.002$), and group III and IV ($p = 0.001$) (►Table 2).

While correlating EZH2 and BCL2 immunoexpression, group I depicted $r = 0.164$ ($p = 0.65$) and group II showed $r = 0.06$ ($p = 0.85$). Group III and IV recorded $r = 0.303$ ($p = 0.12$) and $r = 0.33$ ($p = 0.06$), respectively.

EZH2 and BCL2 as Differentiator between OVH, OVC, and OSCC

ROC curve was plotted to estimate the sensitivity and specificity of EZH2 and BCL2 in different study groups. The point which was closest with maximum sensitivity and specificity score was selected as the cutoff value. For group II versus group III, EZH2 showed a high specificity of 100% and a sensitivity of 85.2% with a cutoff score of 51.7%. BCL2 recorded a low specificity of 50%, sensitivity of 59.3%, and a cutoff score of 16.6%. The area under the curve (AUC) was higher in EZH2 (0.87) than BCL2 (0.56) (►Fig. 1A). Intergroup comparison of group II versus group IV, EZH2 showed a sensitivity of 90.6%, specificity of 90%, and a cutoff score of 55.2%, whereas BCL2 showed a sensitivity value of 81.3%, specificity of 90% with a cutoff score of 40.65%. The AUC depicted was more for EZH2 (0.99) than BCL2 (0.81) (►Fig. 1B). In group III versus group IV, EZH2 recorded a sensitivity of 75%, specificity of 70.4% with a cutoff score of 74.4%. BCL2 showed a sensitivity of 71.9%, specificity of 74.1%, and a cutoff score of 62.3%. The AUC value was observed to be higher for EZH2 (0.78) than BCL2 (0.74) (►Fig. 1C).

Histopathological Parameters in OVH and OVC

Intermediate to strong juxtaepithelial lymphocytic response was seen in OVC whereas OVH displayed weak response. There was a significant difference observed in keratin plugging ($p = 0.004$), juxtaepithelial lymphocytic response ($p = 0.000$), and frank endophytic growth ($p = 0.003$) (►Table 3).

Table 1 Clinicopathologic parameter distribution in study groups

Demographics		Group I	Group II	Group III	Group IV	Total	*p*-Value
Habit	Smoking	0	1 (1.2%)	10 (12.6%)	11 (13.9%)	22	0.006
	Tobacco	0	2 (2.5%)	3 (3.7%)	6 (7.5%)	11	
	Smoking and tobacco	0	3 (3.7%)	5 (6.3%)	3 (3.7%)	11	
	Smoking with alcohol	0	1 (1.2%)	0	1 (1.2%)	2	
	Smoking, tobacco, and alcohol	0	1 (1.2%)	0	0	1	
Site	Buccal mucosa	0	2 (2.5%)	14 (17.7%)	12 (15.1%)	28	0.000
	Floor of mouth	0	0	0	2 (2.5%)	2	
	Lip	0	2 (2.5%)	2 (2.5%)	3 (3.7%)	7	
	Palate	0	0	0	3 (3.7%)	3	
	Retromolar area	0	0	1 (1.2%)	4 (5.06%)	5	
	Tongue	0	2 (2.5%)	1 (1.2%)	1 (1.2%)	4	
	Maxillary alveolus	0	0	0	4 (5.06%)	4	
	Mandibular alveolus	0	0	4 (5.06%)	3 (3.7%)	7	
	Retrocommissural region	0	2 (2.5%)	5 (6.3%)	0	7	
	Gingiva	10 (12.6%)	0	0	0	10	
	Skin	0	2 (2.5%)	0	0	2	
Clinical presentation	Swelling	0	2 (2.5%)	1 (1.2%)	5 (6.3%)	8	0.000
	Ulcerative	0	3 (3.7%)	5 (6.3%)	5 (6.3%)	13	
	Ulceroproliferative	0	0	4 (5.06%)	21 (26.5%)	25	
	Proliferative growth	0	4 (5.06%)	13 (16.4%)	1 (1.2%)	18	
	Whitish patch	0	1 (1.2%)	4 (5.06%)	0	5	

Abbreviations: OSCC, oral squamous cell carcinoma; OVC, oral verrucous carcinoma; OVH, oral verrucous hyperplasia.
Note: Kruskal–Wallis test *p*-value: > 0.05, nonsignificant; < 0.05, significant; < 0.005, very significant. Group I – normal oral mucosa, group II – OVH, group III – OVC, group IV – OSCC.

Table 2 EZH2 and BCL2 expression in various study groups

	Group I	Group II	Group III	Group IV	*p*-Value
EZH2 score (mean ± SD)	11.06. ± 10.32	37.14 ± 14.07	63.14 ± 18.93	78.66 ± 24.62	0.000
BCL2 score (mean ± SD)	11.16 ± 11.03	23.24 ± 15.78	33.82 ± 31.34	63.70 ± 34.13	0.000

Abbreviations: OSCC, oral squamous cell carcinoma; OVC, oral verrucous carcinoma; OVH, oral verrucous hyperplasia; SD, standard deviation.
Note: Kruskal–Wallis test *p*-value: > 0.05, nonsignificant; < 0.05, significant; < 0.005, very significant. Group I – normal oral mucosa, group II – OVH, group III – OVC, group IV – OSCC.

Discussion

In the current study, there was a progressive incline in EZH2 LI from normal oral mucosa to OSCC similar to Sihavong et al.[16] Other studies conducted by Kidani et al and Cao et al also showed an increased EZH2 LI in OSCC than their premalignant counterparts.[15,25] However, the mean LI of EZH2 in OSCC in our study was higher than that of Kidani et al and Sihavong et al (50.7, 75.05), whereas our study depicted lower LI of EZH2 in normal oral mucosa than that of Sihavong et al and Kidani et al (31.36, 19.4).[15,16] This variation might be due to variation in the immunohistochemical staining method. In normal mucosa, EZH2 positive cells were primarily focused in the basal cell layer (►Fig. 2A). In group II, EZH2 positive cells were observed in the basal and the parabasal cell layer (►Fig. 2B), while in group III EZH2 positive cells extended from the basal cells to the upper part of spinous cell layer or in some cases surpassed it (►Fig. 2C). Similar pattern of expression was seen in groups I, II, and III by Sihavong et al and Kidani et al.[15,16] Studies conducted with K_i-67 in group II and III exhibit similar expression pattern.[16] The basal cells possess an innate ability to divide and undergo differentiation move superficially and are ultimately sloughed off the surface.[26] The close resemblance of EZH2 and K_i-67 expression pattern advocates the role of EZH2 in cell proliferation and differentiation of the oral epithelium and further stipulates the role of EZH2 as an oncogene in oral epithelial malignancies.[15,16,27] The expression in group IV was more in the peripheral tumor cells which is considered as the proliferative area of the lesion further corroborating the role of EZH2 in cell proliferation (►Fig. 2D).[16] Furthermore, the increase in EZH2 expression from group I to group IV attest

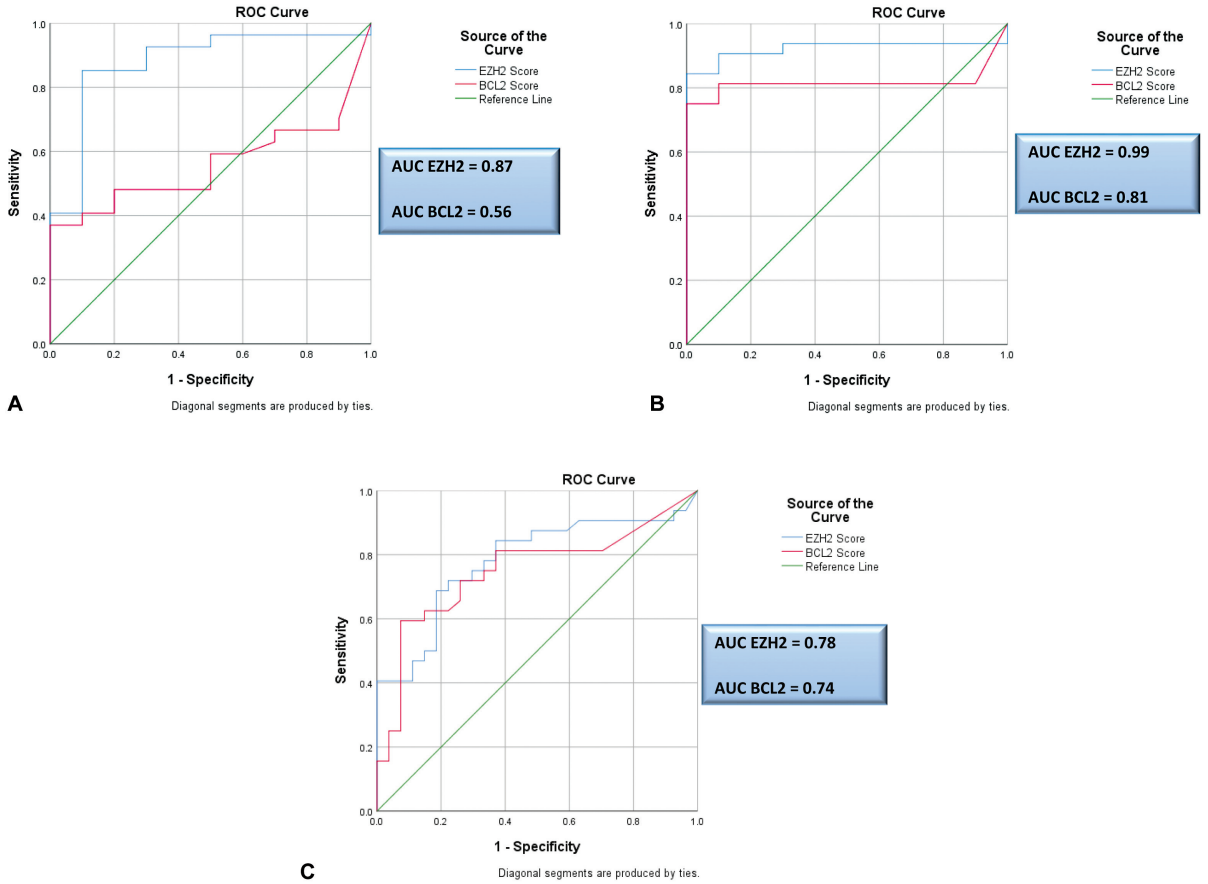

Fig. 1 Receiver operating characteristic (ROC) curve representing diagnostic efficacy between groups: (**A**) Graph I (group II vs. III); (**B**) graph II (group II vs. IV); (**C**) graph III (group III vs. IV).

Table 3 Association of six histopathological parameters in OVH and OVC

	Group II ($n = 10$)	Group III ($n = 27$)	*p*-Value
Surface projection	9 (90%)	26 (96.29%)	0.452
Keratin plugging	5 (50%)	24 (88.8%)	0.004
Atypia	5 (50%)	9 (33.3%)	0.353
Basilar hyperplasia	5 (40%)	12 (44.4%)	0.776
Juxtaepithelial lymphocytic response	10 (100%)	27 (100%)	0.000
Frank endophytic growth	3 (30%)	25 (92.5%)	0.000

Abbreviations: OSCC, oral squamous cell carcinoma; OVC, oral verrucous carcinoma; OVH, oral verrucous hyperplasia.
Note: Chi-square test *p*-value: > 0.05, nonsignificant; < 0.05, significant; < 0.005, very significant. Group I – normal oral mucosa, group II – OVH, group III – OVC, group IV – OSCC.

that EZH2 is involved in disease progression from premalignant lesions to frank malignancy.[16]

Apart from discrepancies in cellular proliferation, any disparity within the apoptotic pathway contributes to the

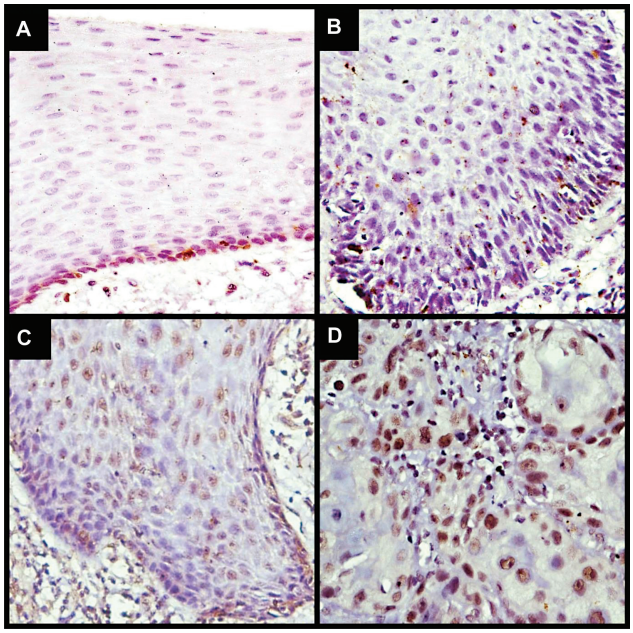

Fig. 2 Photomicrograph showing EZH2 expression in: (**A**) Normal oral mucosa (40 ×). (**B**) Oral verrucous hyperplasia (40 ×). (**C**) Oral verrucous carcinoma (40 ×). (**D**) Oral squamous cell carcinoma (40 ×).

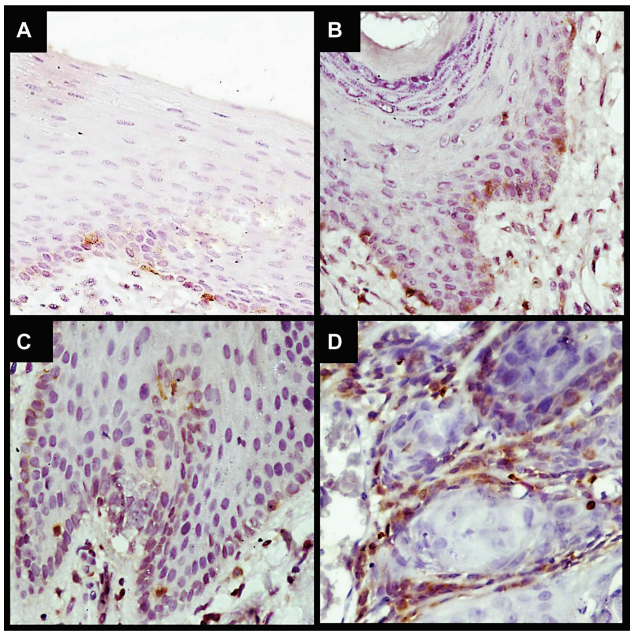

Fig. 3 Photomicrograph showing BCL2 expression in: (**A**) Normal oral mucosa (40 ×). (**B**) Oral verrucous hyperplasia (40 ×). (**C**) Oral verrucous carcinoma (40 ×). (**D**) Oral squamous cell carcinoma (40 ×).

immortalization of replicating cells, consecutively leading to genetic damage which ordinarily might instigate cell death.[28] Recent studies in lymphoma, cholangiocarcinoma, and OSCC cell lines have demonstrated concurrent decline in cellular apoptosis along with overexpression of EZH2.[1,29,30] BCL2 is a prime molecule involved in the apoptotic pathway which seeks to maintain the mitochondrial membrane integrity.[31] Aligning with the expression of EZH2 in our study, BCL2 also exhibited a gradual increase from group I to group IV. We observed very limited and infrequent BCL2 expression in the basal layer of normal tissue similar to the results obtained by Jairajpuri et al, Sudha and Hemavathy, and McAlinden RL et al (►**Fig. 3A**).[12,32,33] The expression pattern of BCL2 in group II was variable which was predominantly confined to the basal and parabasal layer of the epithelium (►**Fig. 3B**). For group III, the expression was mild and diffuse, which sometimes extended beyond the parabasal into the spinous cell layer (►**Fig. 3C**). Deng et al and Jairajpuri et al also observed similar expression pattern in group II and III; however, Thennevan et al suggested very limited expression in verrucous lesions.[12,17] Corresponding with the previous studies we concur that a proportional rise in the BCL2 expression from group I to III reflects its part in disease progression by increasing the survival rate of neoplastic cells and allowing clones of the neoplastic cells to proliferate and differentiate. In group IV, BCL2 immunoexpression was exaggerated and primarily confined to peripheral cells of the tumor islands similar to studies conducted by Juneja et al and Sudha and Hemavathy (►**Fig. 3D**).[22,32] However, few researchers have observed a decline in BCL2 expression in group IV which might suggest a major role of BCL2 during early carcinogenesis and in the later stages the established tumors render it redundant.[34]

The present study is a novel attempt to correlate the expression of EZH2, an epigenetic marker with BCL2, an antiapoptotic marker in oral verrucous lesions. Several authors have illustrated overlapping pathways of EZH2 and BCL2 in certain lymphoid malignancies which have significant treatment implications.[17,18] In cholangiocarcinoma cells EZH2 inactivates p16 and p27 which further suppresses apoptosis.[29] However, no such pathways have been explored in oral lesions. Although both EZH2 and BCL2 displayed analogous expression among the study groups, there was no significant correlation obtained. Kidani et al also showed no correlation between EZH2 expression and apoptotic index in oral epithelial and dysplasia and OSCC.[15] This suggests that EZH2 and BCL2 are independent of each other in oral verrucous lesions.

Various molecular biomarkers including p53, K_i-67, PCNA, cyclin D1, and EZH2 have been explored in distinguishing group II and III.[4,16] In the present study, diagnostic test analysis was conducted where EZH2 LI showed a sensitivity and specificity of 100 and 85.2%, respectively, which would be helpful in differentiating the same groups. Also, BCL2 showed a far lesser sensitivity and specificity of 50 and 59.3%, respectively. EZH2 has been previously utilized to differentiate cellular leiomyoma and well-differentiated leiomyosarcoma with a sensitivity of 91.3% and specificity of 100%.[35] Regardless, further studies are encouraged with a larger sample size to corroborate our data.

In attempt of strengthening the histopathological differentiation of group II and III, we observed the presence of certain histopathological parameters in our study. The clinical presentation of both lesions is almost always a raised proliferative or a verrucous growth which substantiates the occurrence of surface projections in the histopathology as well (►**Fig. 4C**).[6,36] Surface projection revealed a nonsignificant difference ($p = 0.452$) in group II and III. The characteristic keratin plugging which is considered as a key feature in group III yielded a significant difference ($p = 0.004$) which was in accordance with the data provided by Patil et al (►**Fig. 4A**).[10] Cytological atypia is often debated as a feature in verrucous lesions.[10] Our study exhibited 50% cases of group II and 33.3% group III cases positive for atypia which was far lesser than the results obtained by Jairajpuri et al and Thomas and Barrett who observed a 69.2 and 66% positivity, respectively, for atypia in group II[12,37] (►**Fig. 4B**). Patil et al revealed presence of atypia in 20% group III cases which is lesser than our study.[10] This might be due to the uneven sample distribution pattern. The association of basilar hyperplasia in both groups showed a nonsignificant difference ($p = 0.776$) (►**Fig. 4A**). Juxtaepithelial lymphocytic response was a distinctive feature observed in our study which yielded a 100% positivity for both verrucous entities (►**Fig. 4C**). We observed intermediate to strong lymphocytic response in group III cases whereas group II mainly exhibited a weak response with a significant difference ($p = 0.000$). Patil et al observed that 51.77% of total verrucous cases displayed a subepithelial lymphocytic response with a significant difference ($p < 0.05$).[10] The strong association of both these entities with this parameter justifies it as an

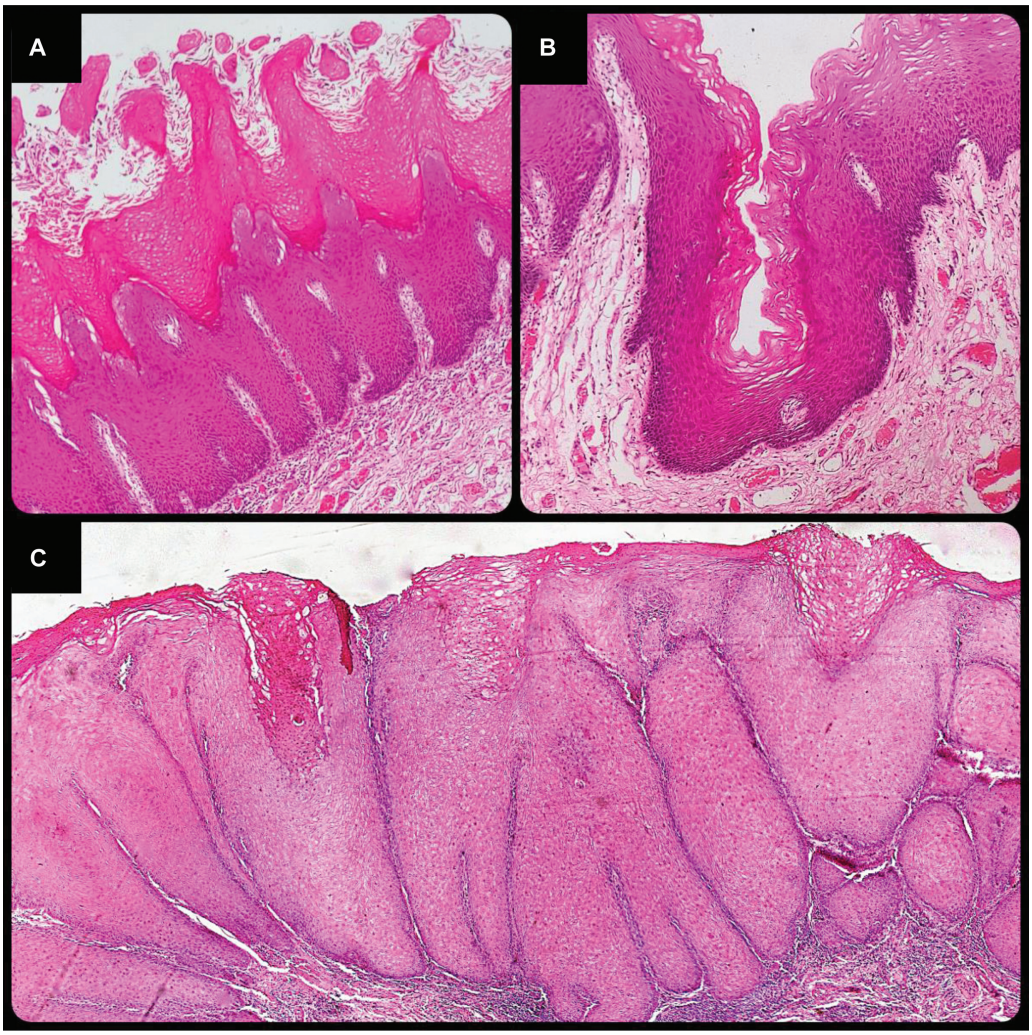

Fig. 4 Hematoxylin and eosin-stained section of oral verrucous hyperplasia (OVH) and oral verrucous carcinoma (OVC) showing: (**A**) Keratin plugging and basilar hyperplasia (20 ×). (**B**) Atypia and frank endophytic growth (20 ×). (**C**) Surface projection and juxtaepithelial lymphocytic response (10 ×).

indicator which could be incorporated as a prime histological differentiator. Note that 92.5% of group III cases and 33.3% group II cases displayed distinct frank endophytic growth with a significant difference ($p = 0.000$). Although absence of frank endophytic growth is a diagnostic criterion for verrucous hyperplasia, two cases from skin and one oral cavity showed signs of endophytic growth in focal areas (►**Fig. 4B**). This could point to a discrepancy in the characteristic histological picture between verrucous hyperplasia of oral cavity and cutaneous origin. Combining the results of all the histological parameters put forward by us clearly suggest that juxtaepithelial lymphocytic response serves as a consistent finding in multiple studies and could be considered as an important diagnostic criterion to distinguish OVH and OVC. Our data clearly depicts the inconsistencies in the currently followed histopathological diagnostic measures to affirm OVH and OVC and encourage the role of accessory immunohistochemical techniques. However, due to the inadequate sample size distribution there still exists a lacunae for more concrete results.

To summarize, identification and investigation of EZH2 and BCL2 in normal mucosa, OVH, OVC, and OSCC and their correlation assisted in distinguishing the verrucous lesions and provided a better understanding of the individual mechanisms. An epigenetic marker EZH2 complemented with a histological parameter of juxtaepithelial lymphocytic response could demarcate OVH and OVC. Since there is a scarcity is utilizing EZH2 as a routine immunohistochemical marker, it is pertinent to encourage additional research regarding its implementation. Collaborative efforts by utilizing immunohistochemical methods, histopathological parameters, and a wider consistent sample size is vital to provide acuity in diagnosing these entities which would further enhance treatment approaches.

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Conflict of Interest
None declared.

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