Original Article

Expression of CD 20 B-Lymphocyte in oral epithelial dysplasia and oral squamous cell carcinoma: A comparative immunohistochemistry study

Anela Thomas, Smitha T, Kavita Rao, Priya N S, Sheethal H S, Chitra S

Vokkalgara Sangha Dental College and Hospital, Bangalore, Karnataka, India

Abstract

Background: As the progressive trends in the field of immunotherapy, it is very favourable to reconsider the role played by B lymphocytes in the tumor microenvironment. Both the protumorogenic and antitumorogenic responses have to be evaluated to formulate an effective immunotherapeutic protocol.

Aim and Objective: The study was primarily conducted to assess the qualitative expression of B lymphocytes in pretumorogenic (oral epithelial dysplasia) and tumorogenic environment (oral squamous cell carcinoma). The differential immunohistochemical staining of CD 20 immune marker was assessed in about 60 cases that included 30 cases of oral epithelial dysplasia and 30 cases of oral squamous cell carcinoma.

Results: The study found significant correlation between CD 20 IHC immune expression and histopathological diagnosis along with significant correlation between the subject's age group and histopathological diagnosis.

Conclusion: Modulating the immune response in a precancerous state can be highly beneficial in implementing better immunotherapeutic strategies to treat or prevent malignancy at an early stage.

Keywords: CD 20 (cluster differentiate 20), IHC (immunohistochemistry), OED (oral epithelial dysplasia), OSCC (oral squamous cell carcinoma)

Address for correspondence: Dr. Anela Thomas, #1560, Shivalinaya, First Floor, 10th Cross Kumaraswamy Layout, Opposite Sagar Hospital Emergency Gate, Banashankari, Bengaluru - 560 078, Karnataka, India.
E-mail: docchitra22@gmail.com

Submitted: 02-Jan-2023, **Revised:** 04-Jan-2023, **Accepted:** 15-Mar-2023, **Published:** 13-Jul-2023

BACKGROUND

Head and neck squamous cell carcinoma (HNSCC) accounts for more than 90% of all head and neck cancers, with approximately 650,000 cases diagnosed each year worldwide.[1] Despite aggressive multimodal strategies to treat HNSCC using combinations of surgery, radiotherapy, and chemotherapy, the 5-year overall survival of carcinogen-related HNSCC is only 40%-50%. The concept of *"immunosurveillance"* is the ability of the body to recognize self and nonself. Both natural and acquired immunity have undoubtedly proven their role in check and balance system against tumor progression.[2] In the rapidly evolving field of immunotherapy, identification of biomarkers to predict the immune response can make such a therapy one of the clinically effective treatments of oral squamous cell carcinoma (OSCC). There are many parameters/biomarkers and methods that have been introduced during the last three decades for the assessment of immune response and cluster differentiate 20 (CD 20) is one among them.

Access this article online	
Quick Response Code:	Website: www.jomfp.in
	DOI: 10.4103/jomfp.jomfp_2_23

How to cite this article: Thomas A, Smitha T, Rao K, Priya NS, Sheethal HS, Chitra S. Expression of CD 20 B-Lymphocyte in oral epithelial dysplasia and oral squamous cell carcinoma: A comparative immunohistochemistry study. J Oral Maxillofac Pathol 2023;27:323-7.

The focus of the present study is to determine how the premalignant microenvironment is different from the tumor microenvironment; this might be modulating the immune response during oral carcinogenesis. Thereby, the present study was done to find out presence and distribution of B-lymphocytes in tumoral or peritumoral areas with routine H&E stain and detect B-lymphocytes with CD 20 immunohistochemistry (IHC) staining method.

MATERIALS AND METHODS

The study included archival samples of histopathological diagnosed confirmed cases of oral epithelial dysplasia (OED) (n = 30) and OSCC (n = 30) retrieved from the Department of Oral and Maxillofacial Pathology, V.S Dental College and Hospital, Bangalore and Department of General Pathology, KIMS Hospital, Bangalore. It included demographic data (age, sex), habit history, and type of biopsy technique (incisional or excisional) along with histopathological grading of OSCC and OED were collected and tabulated. (Ethics committee approval was obtained in date of 19-11-2019, KIMS ethical committee).

The 70 cases were studied which included OED cases (n = 30), OSCC (n = 30), breast carcinoma cases (n = 5), and positive control and normal oral mucosa harvested during coronal flap surgery of impacted third molar cases (n = 30) as negative control. The inclusion criteria in this study included only histological diagnosed cases of OED and OSCC with inflammatory infiltrate. Cases of OED without inflammatory infiltrate were not included and histological diagnosed cases of primary intraosseous carcinomas, metastatic carcinomas of oral cavity, and other epithelial tumours were strictly excluded.

The formalin-fixed and paraffin-embedded tissue sections were stained immunohistochemically for the expression of CD 20+ B-lymphocyte using primary antibodies and the antigen-antibody complex was visualized using the DAB detection kit (k3368; Dako). The CD 20 positive cells were counted at the invasive front in OSCC cases and CD 20 positive cells in the OED cases were counted in the subepithelial connective tissue stroma.

A stretch of 10 consecutive high-power fields (40x magnification) at the area of maximum invasion and area with maximum density of inflammatory cells were chosen. For cell counting in mild risk and severe OED cases, a stretch of 10 consecutive high-power fields (40x magnification) at the subepithelial connective tissue stroma with maximum density of inflammatory cells are selected. Immunostaining for CD 20 was interpreted as negative (-); 5% or less of the epithelial cells were positive, weakly positive, or low-grade staining (+); 5% to less than 25% of the epithelial cells were positive, moderately positive, or intermediate-grade staining (++); 25% to less than 50% of the epithelial cells were positive and strongly positive or high-grade staining (+++); and 50% or more of the epithelial cells were positive.

RESULTS

The statistical analysis performed on various parameters of the present study includes age, sex, habit history of tobacco, type of biopsy, histopathological diagnosis, and IHC staining grade. Correlation tests were done to determine the significance between the two study groups of OSCC and OED. All statistical procedures were performed using Statistical Package for Social Sciences (SPSS) 20.0. All quantitative variables like age and gender were expressed in mean and standard deviation. Qualitative variables were expressed in percentages especially of staining of CD 20 IHC marker. Chi-square test was used for association between variables and correlation between different groups. Probability value ($P < .05$) was considered statistically significant.

Table 1 shows IHC grading of CD 20 distribution between the range of 80% (high) and 8.3% (low), with an intermediate grade of about 11.7.

Table 2 whereas presents the correlation between expression of CD 20 IHC expression and histopathological diagnosis. It has about 70% high-grade expression in well differentiated squamous cell carcinoma (WDSCC), 20% intermediate, and 10% low. The moderately differentiated squamous cell carcinoma (MDSCC) and CD 20 IHC expression is about 80% high and 20% with intermediate

Table 1: Shows the type of IHC grading were 80.0% of the subjects high grading, 11.7% intermediate, and 8.3% with low grade

	n	%
High	48	80.0
Intermediate	7	11.7
Low	5	8.3

Table 2: Shows the correlation between expression of CD 20 IHC expression and histopathological diagnosis

	High *n* (%)	Intermediate *n* (%)	Low *n* (%)	*P*
WDSCC	7 (70)	2 (20)	1 (10)	
MDSCC	8 (80)	2 (20)	0	.03
PDSSC	10 (100)	0	0	
Mild ED	6 (60)	1 (10)	3 (30)	
Moderate ED	8 (80)	1 (10)	1 (10)	
Severe ED	9 (90)	1 (10)	0	

grade. However, the correlation established between poorly differentiated carcinoma (PDSCC) is about 100% with high-grade expression of CD 20 IHC marker. Whereas in cases of OED in mild epithelial dysplasia is about 60% with high grade, 10% is intermediate, and about 30% as low grade. In moderate cases of epithelial dysplasia, there is about 80% high-grade IHC, 10% as intermediate IHC grading, and 10% low grade. However, in cases of severe epithelial dysplasia there is about 90% correlation with high grade and 10% correlation with intermediate IHC grading.

Table 3 demonstrates association between age and H/P diagnosis that there is a significant association between age and their histopathological diagnosis with significant *P* value of. 01. This is statistically highly significant as the *P* value was less than. 05.

The H&E-stained sections observed under the light microscope shows CD 20 expression in OSCC and OED. The prominent areas assessed were peritumoral areas, tumoral areas, and inflammatory cells in the subepithelial connective tissue.

DISCUSSION

OSCC consists of more than 90% of oral cancer. The average incidence of new cases of oral cancer worldwide is around 354,864 (2% of all cancers). Among them, 227,906 (64.22%) cases are reported from Asia, where India accounted for 107,424 (30.27%) incidences as per 2018 data.[3]

The application of T cell as an immunotherapeutic tool has already gained acceptance especially in the cases of HNSCC. The role of humoral response, especially the role played by B lymphocytes is still considered controversial for their protumorogenic and antitumorogenic role in tumor microenvironment.

Mature B lymphocytes constitute about 10%-20% of circulating peripheral lymphocyte population. The function of B lymphocytes in humoral immune system includes producing antibodies, secreting cytokines, acting as antigen presenting cells, and as regulatory molecules. B cells recognize antigens through the B cell receptors complex on their cell membrane and evokes an antigen-antibody interaction.[4] B lymphocytes identify tumor-associated antigens and secrete specific antitumor antibodies as an immune reaction against tumor progression.

Table 3: Shows the correlation between different age groups and their histopathological diagnosis. This is highly significant as the *P* value was less than .01

	WDSCC *n* (%)	MDSCC *n* (%)	PDSSC *n* (%)	Mild ED *n* (%)	Moderate ED *n* (%)	Severe ED *n* (%)	*P*
25-35	1 (12.5)	0	1 (12.5)	4 (50.0)	1 (12.5)	1 (12.5)	
36-45	3 (16.7)	3 (16.7)	4 (22.2)	1 (5.6)	4 (22.2)	2 (11.1)	.01*
46-55	0	5 (35.7)	3 (21.4)	3 (21.4)	3 (21.4)	0	
56-65	3 (33.3)	1 (11.1)	0	1 (11.1)	0	4 (44.4)	
>65	3 (27.3)	1 (9.1)	2 (18.2)	1 (9.1)	2 (18.2)	3 (27.3)	

*: *P* value is highly significant. Chi-square test; *P* value < .05 is statistically significant

The present study highlights the importance of age group, as there is an increase in rise of oral cancer cases in younger individuals. The mean age group that as the maximum subjects is from 36-45 years, that was about 30%. The results of the study are similar to Abdulla R *et al*. (2018)[5] study conducted across South India between 1996 and 2012. The present study had similar results to the study conducted by Shruthi Singh *et al*. (2020),[6] on prevalence of OSCC and OED.

The risk of malignant tumors increases with age.[7-13] The present study shows a correlation with age and histopathological diagnosis, that is consistent with the concept of immunosenescence with age progression. Studies conducted by various author like on immune system and age like Kline GH *et al*. (1999),[14] Caruso C *et al*. (2013),[15] and Cepeda S *et al*. (2018)[16] also proves the same especially age changes related to B lymphocytes.

The present study is at a preliminary attempt to show the immune response especially B lymphocytes in epithelial dysplasia and OSCC. The role of B lymphocytes is critically expressed in pretumor environment or premalignancy state. The present study on the cases of epithelial dysplasia shows similar study results as Ellis and Auclair.[17] (1996) done in HLA/DR-positive cells. The increase in immune cell infiltration with progression of oral epithelium from hyperkeratosis to dysplasia and carcinoma is noted by G Gannot *et al*. (2002).[18] The study done on human tongue tissues (n = 53) diagnosed as hyperkeratosis (11 cases), mild dysplasia (nine cases), moderate and severe dysplasia (14 cases), and squamous cell carcinoma (19 cases) The results revealed that in the tongue lesions, the changes in the epithelium from normal appearance to transformed were accompanied by a corresponding increase in the infiltration of CD4, CD8, CD14, CD19+20, and HLA/DR-positive cells. The most significant change was an increase in B lymphocytes in tongue lesions and was in accordance with the transformation level.

The humoral response in the tumor microenvironment especially lymphocytic infiltration into the tumor microenvironment (TME) is generally considered to

represent host immunity against tumors. The CD 20 being a surface immune marker that acts as an independent prognostic marker in early-stage oral-tongue cancer, which emphasizes the biological impact of clustered B-cells and supports their critical role in antitumor responses in OSCC. Tsou *P et al.* (2016),[19] Lao X M *et al.* (2016),[20] and Wirsing A M *et al.* (2018)[21] study results suggested the antitumor response of B lymphocytes in the tumor microenvironment. This includes secretion of antibodies, presentation of tumor antigen to adjacent T-cells, and production of immune-potentiating cytokines, such as IFNγ and IL12.[22-26] On the other hand, B-cells may mediate a protumorogenic effect through induction of neovascularization, becoming regulatory B-cells (Bregs). This was suggested from the results of the study Zhou X *et al.* (2016).[27] The other studies conducted by de Visser KE *et al.* (2005)[28] and Ammirante *et al.* (2010)[29] suggested the production of immune-suppressive cytokines, such as IL10, IL-35, and TGFβ would promote the protumorogenic response in the tumor microenvironment.

The results presented illustrated in the present study shows an increased expression of CD 20 B lymphocyte in advanced stages of OSCC. In PDSCC there was 100% expression of CD 20 IHC marker that suggested an increased humoral response of B lymphocytes in the tumor microenvironment, subsequently associated as in MDSCC and WDSCC. The study results were consistent with findings of C. Phanthunane *et al.* (2021)[30] study, using multiplex *in situ* immunofluorescence and computational image analyses of 138 patients with T1-T2 primary oral-tongue squamous cell carcinoma, observed a high density of CD 20 cells that clustered together in the IM-S regions. The study concluded that the so-called CD 20 Cluster Score acts as a strong independent prognostic factor in early-stage oral-tongue cancer. However, the study results of Wouters MCA *et al.* (2018)[31] and Gentles AJ *et al.* (2015)[32] contradicting pointing out that tumor infiltrated B-cells and B-cells–associated genes expressed in cancers tend to give a negative prognosis or even no effect on patients' survival.

The CD 20 immune marker provides adequate evidence of B lymphocytic infiltration into the peritumoral, tumoral, and connective tissue of dysplastic lesions. The study ascertained the expression of B lymphocytes in the tumor microenvironment through the differential staining of CD 20 IHC marker.

The future perspectives to validate the results of the present study can include to assess the B lymphocytes by quantitative measures to signify the B cell population and composition. Additionally, larger sample size and advanced molecular techniques could also aid in the study.

CONCLUSION

This study assessed the clinical parameters and the expression of CD 20 in OSCC (n = 30) and OED (n = 30) cases using IHC. This study revealed that there is significant variation in expression of CD 20 B lymphocytes in both malignant and potentially malignant cases. Age as a clinical parameter of the present study correlated with the histopathological diagnosis of OSCC and OED cases. The association with age and immune response was ascertained and found similar to previous studies done in the same field. The probable humoral immune response through B lymphocytes is expressed both in pretumor and tumor microenvironment. However, the dual roles played by B lymphocytes are to be critically analyzed and assessed through advanced research modalities. Possibilities of B-lymphocyte associated with immune therapy can be fairly considered as a treatment option or as an adjuvant therapeutic aid in future.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Primers 2020;6:92.
2. Ribatti D. The concept of immune surveillance against tumors. The first theories. Oncotarget 2017;8:7175-80.
3. Cancer Genome Atlas N. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature 2015;517:576-82.
4. Downs-Canner SM, Meier J, Vincent BG, Serody JS. B Cell function in the tumor microenvironment. Annu Rev Immunol 2022;40:169-93.
5. Abdulla R, Adyanthaya S, Kini P, Mohanty V, D'Souza N, Subbannayya Y. Clinicopathological analysis of oral squamous cell carcinoma among the younger age group in coastal Karnataka, India: A retrospective study. J Oral Maxillofac Pathol 2018;22:180-7.
6. Singh S, Singh J, Chandra S, Samadi FM. Prevalence of oral cancer and oral epithelial dysplasia among North Indian population: A retrospective institutional study. J Oral Maxillofac Pathol 2020;24:87.
7. Cho YA, Yoon HJ, Lee JI, Hong SP, Hong SD. Relationship between the expressions of PD-L1 and tumor-infiltrating lymphocytes in oral squamous cell carcinoma. Oral Oncol 2011;47:1148-53.
8. Mincer HH, Coleman SA, Hopkins KP, Tenn M. Observations of the clinical characterstics showing histological epithelial dysplasia. Oral Surg 1972;33:390-4.
9. Pawelec G. Hallmarks of human "immunosenescence": Adaptation or dysregulation? Immun Ageing 2012;9:15.
10. Caruso C, Accardi G, Virruso C, Candore G. Sex, gender and immunosenescence: A key to understand the diferent lifespan between men and women? Immun Ageing 2013;10:20.

11. Turner JE. Is immunosenescence influenced by our lifetime "dose" of exercise? Biogerontology 2016;17:581-602.
12. Accardi G, Caruso C. Immune-inflammatory responses in the elderly: An update. Immun Ageing 2018;15:11. doi: 10.1186/s12979-018-0117-8.
13. Aiello A, Accardi G, Candore G, Caruso C, Colomba C, Di Bona D, *et al*. Role of immunogenetics in the outcome of HCMV infection: Implications for ageing. Int J Mol Sci 2019;20:685.
14. Kline GH, Hayden TA, Klinman NR. B cell maintenance in aged mice refects both increased B cell longevity and decreased B cell generation. J Immunol 1999;162:3342-9.
15. Caruso C, Accardi G, Virruso C, Candore G. Sex, gender and immunosenescence: A key to understand the diferent lifespan between men and women? Immun Ageing 2013;10:20. doi: 10.1186/1742-4933-10-20.
16. Cepeda S, Cantu C, Orozco S, Xiao Y, Brown Z, Semwal MK, *et al*. Age-associated decline in thymic B cell expression of aire and aire-dependent self-antigens. Cell Rep 2018;22:1276-87.
17. Ellis GL, Auclair PL. Benign epithelial neoplasms in tumors of the salivary glands. Washington, DC: Armed Forces Institute of Pathology; 1996. p. 468.
18. Gannot G, Gannot I, Vered H, Buchner A, Keisari Y. Increase in immune cell infiltration with progression of oral epithelium from hyperkeratosis to dysplasia and carcinoma. British journal of cancer. 2002;86:1444-8.
19. Tsou P, Katayama H, Ostrin EJ, Hanash SM. The emerging role of b cells in tumor immunity. Cancer Res 2016;76:5597-601.
20. Lao XM, Liang YJ, Su YX, Zhang SE, Zhou XI, Liao GQ. Distribution and significance of interstitial fibrosis and stroma-infiltrating B cells in tongue squamous cell carcinoma. Oncol Lett 2016;11:2027-34.
21. Wirsing AM, Ervik IK, Seppola M, Uhlin-Hansen L, Steigen SE, Hadler-Olsen E. Presence of high-endothelial venules correlates with a favorable immune microenvironment in oral squamous cell carcinoma. Mod Pathol. 2018;31:910-22.
22. Hamanaka Y, Suehiro Y, Fukui M, Shikichi K, Imai K, Hinoda Y. Circulating anti-MUC1 IgG antibodies as a favorable prognostic factor for pancreatic cancer. Int J Cancer 2003;103:97-100.
23. Kurtenkov O, Klaamas K, Mensdorff-Pouilly S, Miljukhina L, Shljapnikova L, Chuzmarov V. Humoral immune response to MUC1 and to the Thomsen-Friedenreich (TF) glycotope in patients with gastric cancer: Relation to survival. Acta Oncol 2007;46:316-23.
24. Hirasawa Y, Kohno N, Yokoyama A, Kondo K, Hiwada K, Miyake M. Natural autoantibody to MUC1 is a prognostic indicator for non-small cell lung cancer. Am J Respir Crit Care Med 2000;161:589-94.
25. Bruno TC, Ebner PJ, Moore BL, Squalls OG, Waugh KA, Eruslanov EB, *et al*. Antigen-presenting intratumoral b cells affect cd4(+) til phenotypes in non-small cell lung cancer patients. Cancer Immunol Res 2017;5:898-907.
26. Deola S, Panelli MC, Maric D, Selleri S, Dmitrieva NI, Voss CY, *et al*. Helper B cells promote cytotoxic T cell survival and proliferation independently of antigen presentation through CD27/CD70 interactions. J Immunol 2008;180:1362-72.
27. Zhou X, Su YX, Lao XM, Liang YJ, Liao GQ. CD19(+) IL-10(+) regulatory B cells affect survival of tongue squamous cell carcinoma patients and induce resting CD4(+) T cells to CD4(+) Foxp3 (+) regulatory T cells. Oral Oncol 2016;53:27-35.
28. de Visser KE, Korets LV, Coussens LM. De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. Cancer Cell 2005;7:411-23.
29. Ammirante M, Luo J-L, Grivennikov S, Nedospasov S, Karin M. B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. Nature 2010;464:302-5.
30. Phanthunane C, Wijers R, de Herdt M, Langeveld TP, Koljenovic S, Dasgupta S, *et al*. B-cell clusters at the invasive margin associate with longer survival in early-stage oral-tongue cancer patients. Oncoimmunology 2021;10:1882743. doi: 10.1080/2162402X.2021.1882743.
31. Wouters MCA, Nelson BH. Prognostic significance of tumor-infiltrating b cells and plasma cells in human cancer. Clin Cancer Res 2018;24:6125-35.
32. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, *et al*. The prognostic landscape of genes and infiltrating immune cells across human cancers. Nat Med 2015;21:938-45.