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IMMUNOEXPRESSION AND CORRELATION OF TUMOUR INFILTRATING B LYMPHOCYTES, IN ORAL SQUAMOUS CELL CARCINOMA WITH LYMPH NODE STATUS-A RETROSPECTIVE STUDY

Oral Pathology

Dr. Amit U. Bhandarwar*	Resident, MDS, Department of Oral Pathology and Microbiology, MGM Dental College & Hospital, Kamothe,Navi Mumbai *Corresponding Author
Dr. Shilpa Patel	Professor & Head, MDS, Department of Oral Pathology and Microbiology, MGM Dental College & Hospital, Kamothe, Navi Mumbai.
Dr. Jigna Pathak	Professor, MDS, Department of Oral Pathology and Microbiology, MGM Dental College & Hospital, Kamothe, Navi Mumbai
Dr. Niharika Swain	Reader, MDS, Department of Oral Pathology and Microbiology, MGM DentalCollege & Hospital, Kamothe, Navi Mumbai
Dr. Vibhuti S. Mhatre	Resident, MDS, Department of Oral Pathology and Microbiology, MGM Dental College & Hospital, Kamothe, Navi Mumbai
Dr. Satyajit Tekade	Professor & Head, MDS, Department of Oral Pathology and Microbiology, Modern Dental College & Research Center, Indore

ABSTRACT

Objectives: To evaluate and correlate the immunoexpression of (TIBCs) Tumour Infiltrating B cells (CD20+) in Oral Squamous Cell Carcinoma with lymph node status**.**

Material And Methods: The 30 paraffin blocks of histopathologically diagnosed cases of OSCC treated with neck dissection which was retrieved from the archives of the Department of Oral Pathology and Microbiology. The sample size of (n=15) cases showing lymph node metastasis pN(+) & (n=15) without lymph node involvement pN(-). We evaluated the expression of TIBCs using antibodies specific for B cell, in OSCC and correlated the same with lymph node status.

Results: On statistically evaluating and correlating the mean immunoexpression of TIBCs (CD20+) with pathologic lymph node status in tumour front area of primary tumour, we found that the mean count of TIBCs (CD20+) in pN(+) cases was 21.92 whereas in pN(-) cases it was 22.51 (p value : 0.902) by **Mann Whitney Test.**

Conclusion: It is important to understand the diverse role of B cells & its immune response in cancer, yielding a novel role as a predictive & prognostic marker which impacts the therapeutic approaches.

KEYWORDS

B lymphocytes, TIBCs, OSCC

INTRODUCTION:

Oral cancer is a serious and growing problem in many parts of the World.[1] Carcinogenesis is a multistep process which mainly comprises of cellular or molecular changes in host cells as well as alteration in interaction between transformed cells and host defence system.[2] In addition to this, host defence system play a vital role in either promotion or regression during the process of carcinogenesis.[2] Immune cells that infiltrate tumors engage in an extensive and dynamic crosstalk with cancer cells. [3]. B cells play decisive roles in immunosuppression and in regulating antitumour response and in carcinogenesis.[4][5] The potential contribution of B cells in modulating the immune response to tumour development is less investigated. Thus the aim of this study was to evaluate morphological distribution & prognostic impact of B cell lymphocytes in correlation with lymph node metastasis, as in OSCC loco-regional lymph node metastasis is the most significant adverse prognostic marker and major determinant of poor survival rate.

MATERIALS AND METHODS

This study was conducted on 30 paraffin blocks of histopathologically diagnosed cases of OSCC treated with neck dissection which were retrieved from the archives of the Department of Oral Pathology and Microbiology. The study was carried out for a duration of 2 years from 2015-2017. Out of 30 samples, (n=15) cases showing without lymph node metastasis (pN-) **[figure** 1] & (n=15) with lymph node involvement (pN+) **[figure** 2**].** The recurrent cases of OSCC were excluded from the study. Sections from the tumor proper were subjected to IHC staining technique with the CD20 marker for B lymphocytes. Evaluation of the Tumour infiltrating B lymphocytes (TIBCs) in tumor front area, correlated with lymph node status, of OSCC cases was done. **[Figure** 3, **figure** 4**].**

Method of analysis: Five randomly selected high power fields (HPF) 400X magnification, in the tumour front area of primary tumor were chosen and mean number of these B cells was calculated. **Mann-Whitney Test** was used to compare the mean immunoexpression of TIBCs (Cd20+), with the lymph node status. A significance level of 0 .05 was applied to decide the statistical significance of the hypothesis being tested.

Fig. 1 Section of pN(-) lymphnode. [H & E, 40x]

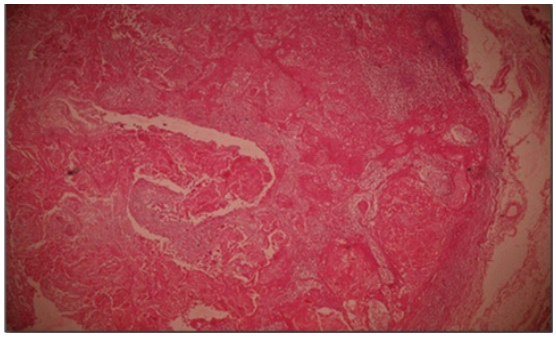

Fig. 2 Section of pN(+) lymphnode.[H & E, 40x]

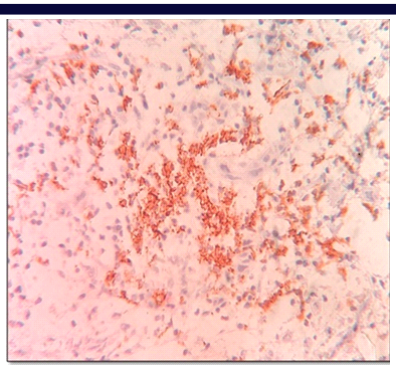

Fig. 3 Primary tumor with high expression of TIB - lymphocytes (Cd20+) in tumour front area. [IHC stain 40 x]

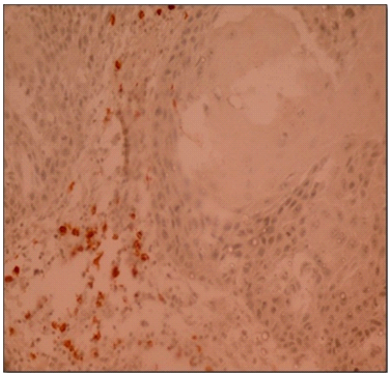

Fig. 4 Primary tumor with low expression of TIB-lymphocytes (Cd20+) in tumour front area. [IHC stain, 40 x]

RESULTS

On statistically evaluation and correlation of the mean immunoexpression of TIBCs (Cd20+) with pathologic lymph node status in tumor front area of primary tumor, we found that the mean immunoexpression of the Tumour Infiltrating B lymphocytes(CD20+) ranged from 9.8 to 52.8 .The mean count of TIBCs (CD20+) in pN(+) cases (n=15) was 21.92 (SD= 9.45) whereas in pN(-) cases (n=15) it was 22.51 (SD= 1.41) **(Table 1).** An increase in the count of TIBCs (CD20+) was observed in lymph node negative pN(-) as compared to lymph node positive cases pN(+) of OSCC.**(Fig. 5 Bar Graph 1). Mann Whitney Test** revealed a statistically non significant difference in the mean value of TIBCs (CD20+) (p: 0.902). **[Table 1a].**

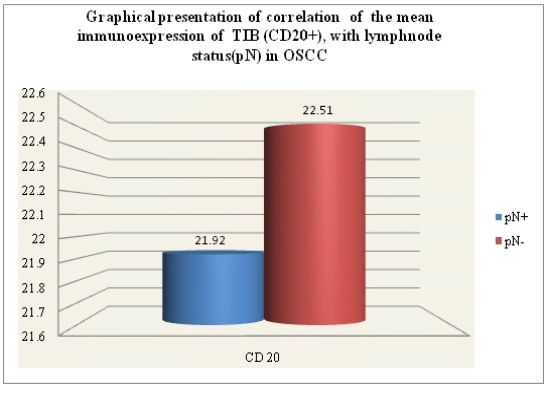

Fig.5 Bar graph 1: An increase count of TIB (CD20+), was observed in L.N. negative pN(-) = 22.51 than L.N. positive cases pN (+) =21.92 .

TABLE 1:

Lymph node Status (pN)	No. of cases (n)	No. of (CD20+)cells In 5 HPF (Mean)
pN+	15	21.92
pN-	15	22.51

Comparative evaluation of the mean immunoexpression of TIB lymphocytes (Cd20+), in tumour front area of primary tumour with lymph node status (pN) in OSCC cases

Table 1a: Statistical correlation of the mean immunoexpression of TIB lymphocytes (Cd20+), with lymph node status (pN) in OSCC cases.

	Lymph node				Mann-Whitney Test (p value)
	Positive		Negative		
	Median	Standard Deviation	Median	Standard Deviation	.902
CD20+	19.00	9.45	15.60	1.41	

*The p value is significant at the 0.05 level.

DISCUSSION

An attempt was made in the present retrospective study to immunohistochemically evaluate the expression of Tumour infiltrating B lymphocytes (TIBCs) in OSCC and to correlate the same with lymph node status. The OSCC cases (n=30) were immunohistochemically evaluated for TIBCs using antibodies specific for B cell (CD20). Five randomly selected high power fields (HPF) in the tumour front area of primary tumor were chosen and mean number of these B cells was calculated. Descriptive statistical analysis was carried out. **Mann-Whitney Test** was used to compare the mean immunoexpression of TIBCs (CD20+) with the lymph node status.

On statistically evaluating and correlating the mean immunoexpression of TIBCs (Cd20+) with pathologic lymph node status in OSCC, we found that the mean count of TIBCs (CD20+) in pN(+) cases (n=15) was 21.92 (SD= 9.45) whereas in pN(-) cases (n=15) it was 22.51 (SD= 1.41) and this difference was statistically not significant(p: 0.902).

In accordance with our study **Mahmoud SM, Lee AH, et al,** 2012 investigated the density & localisation of B lymphocytes (Cd20+) in three different compartments i.e Intratumoral compartements , within the distant stroma and within the adjacent stroma of invasive breast carcinoma. Their results showed that higher number of CD20+ cells were found in the stroma away from the carcinoma(mean 12 cells)compared to intratumoral or adjacent stromal compartments (mean 1 cell). However they did not get the significant association with the lymph node status.[6] **Pretscher D, Distel LV et al, 2009** in their study, have seen infiltration of B cell lymphocytes in different compartments i.e. primary tumor, intraepithelial & peritumoral areas in metastatic lymph node and uninvolved lymph node.CD20+ cell infiltration was significantly increase in metastatic lymph node as compared to primary tumor(P<0.005). Patients with high number of peritumoral B lymphocytes had significantly better outcome. [7] **Ou Z, Wang Y, et al 2015 also found more number of B cell population at tumor site of** Bladder carcinoma (BCa) than normal surrounding tissue. These B cells significantly increase the BCa invasion & metastasis by modulation of the IL-8/Androgen receptor by the MMPs signals (Matrix Metalloproteinases signals – MMP1 & MMP2) supporting protumoral role of B cells in carcinogenesis. [8] **Podolsky MA, Bailey JT, et al 2017** who in their study showed that position of tumor-initiating cells within a stratified squamous epithelial tissue provoked distinct B- and CD4 T-cell interactions, which establish unique tumor microenvironments that regulate tumor development and response to +immunotherapy. In the mice models they found that B cells were largely present within the tumors, but not in the secondary lymphoid nodes.[9] **Woo JR, Liss MA, et al. 2014 in** their study showed non significant value in low & intermediate risk groups of PCa(Prostate Cancer).While they have seen significant increase in the number of B cell population in intratumoral area of high risk & recurrence cases.[10] This was in accordance with our study.

Quan N, Zhang Z et al, 1999 studied on 344 rats by injecting the tumor cells into them and saw the B lymphocyte involvement in experimental metastases. They observed rapid influx of the B cells into the lung after I.V. tumor cell injection into the rat, which approximately increased by 10 –fold after 6 hr. They found that percentage of lymphocytes in the lung, spleen and blood after tumor injection, showed increase number of B cells (P<0.05). They suggested that the B lymnphocytes are important in protection against tumor development in the rats and involvement of B lymphocytes in host defence against metastases.[11]

CONCLUSION

From the result of our study, it is apparent that the B lymphocytes are a part of humoral immune response in cancer. Thus, it is important to understand the diverse role of B cells & its immune response in cancer,

yielding a novel role as a predictive & prognostic marker which impacts the therapeutic approaches. Till date, few studies have evaluated dynamics of B cells in pre and post specific anti-cancer therapy. Therefore, there is an urge for further studies authenticating specifically the role of B lymphocytes in OSCC which needs to be undertaken for establishing it as a novel immunotherapeutic biomarker.

CONFLICT OF INTEREST: Nil

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