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IMMUNOEXPRESSION AND CORRELATION OF CYTOTOXIC T LYMPHOCYTES IN ORAL SQUAMOUS CELL CARCINOMA WITH LYMPH NODE STATUS: A RETROSPECTIVE STUDY

Oncology

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ABSTRACT

Background & Objectives: In OSCC, the presence of regional lymph node metastasis at presentation is the most significant adverse prognostic factor and a major determinant of poor survival. Tumor-infiltrating lymphocytes (TILs) often infiltrate solid malignant tumours and extensive lymphocyte infiltration has been related with a more favourable prognosis in patients with various cancers. OSCC often contain large mononuclear cell infiltrates, comprised mainly of T cells, which could reflect an in situ immune reaction against the malignant OSCC cells. The aim of this retrospective study was to evaluate the expression of Cytotoxic T lymphocyte in OSCC using immunohistochemical marker CD8+(CTLs) and correlate these findings with the status of lymphnode.

Methods: The study was conducted on tissue sections obtained from histopathologically diagnosed cases of OSCC (n=30) retrieved from the archives of Department of Oral and Maxillofacial Pathology. The sample consisted of cases showing lymph node metastasis (n=15) and those without pathologic lymph node involvement (n=15). The sections were evaluated by using immunohistochemical staining technique with marker CD8 for Cytotoxic T lymphocytes. The mean immunoexpression of Cytotoxic T lymphocyte was evaluated and correlated with lymphnode status.

Results: A statistically significant increase in the count of CTLs (CD8+) was observed in lymph node negative pN(-) as compared to lymph node positive cases pN(+) of OSCC.

Interpretation & Conclusion: CTLs (CD8+) are involved in modulating the immune response and can contribute to the dissemination or control of metastatic neoplastic cells. It can be considered that T-cell mediated adaptive immunity plays a key role in anti-tumour immunity.

KEYWORDS

Cytotoxic T lymphocyte, CD8, Immune response, Lymph node metastasis, OSCC.

INTRODUCTION:

The term 'Oral cancer' is used to describe any malignancy that arises from the oral cavity comprising of lip, tongue, buccal mucosa and oropharynx[1]There are an estimated half-a-million of cases of cancer of the oral cavity and pharynx occurring annually, and a quarter-of-a-million deaths[2]. India accounts for more than one-fourth of world's burden.[3] The high mortality rate may be due to the fact that oral carcinoma cells easily invade into territorial tissues and metastasize to the cervical lymph nodes. Hence it is necessary to know the mechanisms and factors involved for cancer progression, for it to be a metastatic disease. The process of metastasis in regional lymph nodes begins with invasive growth of tumor cells and its detachment from the primary lesion. This is followed by lymphogenous transport of cancer cells that results in lodgement and proliferation of cancer cells in and around lymph nodes.[4]

Cancer can be considered with regard to a step-wise development functionally grouped into three phases: initiation, promotion, and progression. Initiation is characterized by genomic changes within the "cancer cell," such as point mutations, gene deletion and amplification and chromosomal rearrangements leading to irreversible cellular changes. The concept that tumor development is the result of processes involving both the cancer cells themselves and non-cancer cells[5] consisting of tumour microenvironment which contains innate immune cells (including macrophages, neutrophils, mast cells, myeloid-derived suppressor cells, dendritic cells, and natural killer cells) an adaptive immune cells (T and B lymphocytes) and their surrounding stroma which consists of fibroblasts, endothelial cells, pericytes, and mesenchymal cells. These diverse cells communicate with each other by means of direct contact or cytokine and chemokine production and act in autocrine and paracrine manners to control and shape tumor growth. It is the expression of various immune mediators and modulators as well as the activation state of different cell types in the tumor microenvironment that dictate in which direction the balance is tipped and whether tumor-promoting inflammation or antitumor immunity will ensue. In established tumours, this balance is profoundly tilted toward protumor inflammation. However, it is safe to assume that tumor promoting inflammation and antitumor immunity coexist at different points along the path of tumor progression.[6]

Inflammatory elements like Activated memory- and cytotoxic tumor infiltrating T-lymphocytes (TILs) are considered to be manifestations of a specific host immune reaction against cancer cells, related to the cytotoxic activity and the production of growth modulating cytokines of TIL.[7]Tumor-infiltrating lymphocytes (TIL) were found to correlate with improved prognosis in several types of cancer. The presence of TIL is considered a reflection of the immune response to the tumor.[8] Hence TIL plays an important role in anti tumour immune response. Each T cell is genetically programmed to recognize a specific cell bound antigen by means of an antigen specific T cell receptor (TCR).[9] This complex helps in activation of T lymphocytes and release cytokines thereby mediating immune response.

T-lymphocytes can be T- helper cells and Cytotoxic T- cells. T- helper cells serves as a major regulator of all immune functions by forming lymphokines which act on other cells of immune system hence having no direct role.[10]Cytotoxic T lymphocytes(CTLs) formed from naïve T cells release cytotoxic granules, rich in perforin and granzyme B (GB) which cause apoptosis of neoplastic cells in lymph nodes and at tumour site.[11,12] Thereby CTLs (CD8+) are involved in modulating the immune response and can contribute to the dissemination or control of metastatic neoplastic cells.[13,14]A lower density of activated CTLs were seen in metastatic when compared to non metastatic lymph nodes in cases of OSCC.[11]

Thus, the aim of the present retrospective study is to evaluate the expression of Cytotoxic T lymphocyte in OSCC using immunohistochemical marker CD8+(CTLs) and correlate these findings with the status of lymphnode.

MATERIALS AND METHODS:

The present study was carried out on tissue sections obtained from histopathologically diagnosed cases of OSCC (n=30) treated with neck dissection, retrieved from the archives of Department of Oral and Maxillofacial Pathology,MGM dental College and Hospital. The study was carried out for a duration of 2 years from 2014-2016. Our sample consisted of cases showing lymph node metastasis (n=15) (Figure 1) and those without pathologic lymph node involvement (n=15) (Figure 2).Recurrent cases of OSCC were excluded from the study. Sections from tumor proper were subjected to immunohistochemical staining technique with marker CD8 for Cytotoxic T lymphocytes.[15] Five randomly selected high power fields in the tumour microenvironment of OSCC were chosen and mean number of Cytotoxic T lymphocytes were calculated.[16] (Figure 3) The analysis was performed using statistical tests such as descriptive test, independent student's't' test. A significance level of .05 was applied to decide the statistical significance of the hypothesis being tested.

RESULTS:

On immunohistochemical evaluation of the mean range of the immunoexpression of CTLs in all the selected cases (n=30) ranged from 131.4 -221. Descriptive analysis showed the mean count of CTLs (CD8+) in pN(+) cases (n=15) was 158.32 (SD=24.66) whereas in pN(-) cases(n=15), it was 177.96 (SD=19.99).(Table I) An increase in the count of CTLs (CD8+) was observed in lymph node negative pN(-) as compared to lymph node positive cases pN(+) of OSCC.(Graph 1) Independent student's't' test revealed a statistically significant difference in the mean value of CTLs(CD8+) (p= 0.023) when correlated with lymphnode status.(Table II)

DISCUSSION:

The present study was carried out on tissue sections obtained from histopathologically diagnosed cases of OSCC (n=30) retrieved from the archives of Department of Oral and Maxillofacial Pathology. Our sample consisted of cases showing lymph node metastasis (n=15) and those without pathologic lymph node involvement (n=15).In our study, we evaluated and correlated the mean immunoexpression of CTLs (CD8+) with pathologic lymphnode status in OSCC. We found that the mean count of CTLs (CD8+) in pN(+) cases (n=15) was 158.32 (SD=24.66) whereas in pN(-) cases(n=15), it was 177.96 (SD=19.99) and this difference was statistically significant (p= 0.023). Our result was in accordance with **Gonsalves A et al[11],** who in a study on cervical lymph nodes in OSCC, reported that CD8+ T cells were significantly reduced in lymph node positive patients. **Snyderman CH et al[17],** in his study on HNSCC reported that decreased number of CTLs(CD8+) were associated with greater incidence of cervical lymphnode metastasis. **Dos antos Pereira J et al[18]** in OSCC reported higher number of CTLs(CD8+) were significantly associated in nonmetastatic tumours. **Balermpas et al[19],** in HNSCC, demonstrated that the high levels of CTLs (CD8+) were a good prognostic factor. **Kolzer et al[20]**, in his study on endoscopic biopsies in colorectal carcinoma showed that intratumoural infiltration of CTLs (CD8+) were significantly associated with lymph node status while stromal CTLs (CD8+) were insignificant. **Jung IK[21] et al** in endometrial adenocarcinoma demonstrated that high number of CTLs (CD8+) were significantly associated with low grade, early stage, less myometrial invasion and absence of lymphnode metastasis. Similarly, **Ropponen et al[22], Naito et al[23]** depicted that there is a strong evidence for an association between a high number of CTLs (CD8+) and lower tumour grade, presence of peritumoural lymphocytes, better survival rate and absence of lymph node metastases in cases of colorectal cancer. **Chiba et al[24], Koch et al[25] and Baker et al[26]** stated similar findings of CTLs (CD8+) in colorectal cancer. **Zancope et al[27]** in his study on oral squamous cell carcinoma showed insignificant difference between CTL(CD8+) count and lymphnode status.

It has been considered that T-cell mediated adaptive immunity plays a key role in anti-tumour immunity. Adaptive immunity prevents the development of tumours and inhibits tumour progression.[28] Inflammatory elements like Activated, memory- and cytotoxic tumor infiltrating T-lymphocytes (TILs) are considered to be expression of a specific host immune reaction against cancer cells, related to the cytotoxic activity and the production of growth modulating cytokines(Interferon Interleukin 2, Tumour Necrosis Factor-alpha) of TIL.[7] Each T cell is genetically programmed to recognize a specific cell bound antigen by means of an antigen specific T cell receptor (TCR).[9]This complex helps in activation of T lymphocytes and release cytokines (Interferon , Interleukin 2, Tumour Necrosis Factor-alpha) thereby mediating immune response. T-cell function (particularly Cytotoxic T lymphocytes) in a tumor microenvironment can be modulated through two mechanisms: first being mediated by direct exocytosis of perforin and granzyme containing granules and second, FasR/ FasL apoptotic pathway.[29] Cooperation between secreted granzymes and perforin has been shown to cause typical cytotoxic lymphocyte-induced lysis and DNA fragmentation.[30] FasL-bearing cytotoxic T cells promote apoptosis in FasR-bearing target cells. In the Fas-mediated pathway, there is engagement of cytotoxic lymphocyte membrane ligand (FasL) with an apoptosis-inducing target cell surface receptor (FasR) which triggers apoptosis of the target cell.[31,32] The functional expression of FasL on tumor cells is a mechanism of tumor escape from immunological detection of TILs that has been proposed by several groups.[33,34,35]

According to **Lee HW et al[36]**, high densities of immune cells related to adaptive immunity, that is, total T cells (TIL density), CTLs, and memory T cells, are correlated with the absence of lymph node metastasis. He suspected that the prognostic role of TIL is mainly contributed to decreased metastatic potential. He anticipated the following possible mechanisms that results in reduction of metastatic potential by TILs. First, clones with metastatic potential commonly express larger amounts of aberrantly expressed proteins, including proteins that contribute to metastasis, which may act as tumour-associated antigens. As a result, these clones are more prone to be destroyed by in situ immune reactions. Second, a high number of TIL indicates a healthy immune system, and therefore, immune reaction occurring in lymph node may also exert a proper function against tumour cells that have been drained into lymph nodes in patients with high TIL densities. Third, tumour burden of metastatic foci in lymph node is less than those of primary foci, and thus, metastatic foci are more likely to be susceptible to complete destruction by immune reaction.

In our study we elucidated the immunoexpression of Cytotoxic T Lymphocyte and their role in lymphnode metastasis in OSCC. Moreover, our results suggest that adaptive immunity may act to prevent tumour progression. Our findings can help predict clinical outcome and define patient subgroups with an unfavourable prognosis in OSCC.

CONCLUSION:

Immunoexpression of Cytotoxic T lymphocytes(CD8+) was reduced significantly in OSCC cases with lymph node metastasis pN(+) than in those without lymph node metastasis pN(-). This shows that reduced number of CD8+T cells in tumour may have a predictive value in determining the metastatic potential of OSCC. However further studies with larger sample size needs to be undertaken to authenticate our findings.

CONFLICT OF INTEREST: Nil

TABLE I: Comparative evaluation of the mean immunoexpression of Cytotoxic T lymphocyte(CD8+) with lymphnode status(pN) in OSCC cases.

Lymph node status(pN)	No. of cases (n)	No. of (CD8+) cells In 5 HPF (Mean)
pN+ve	15	158.32
pN-ve	15	177.96

TABLE I: evaluates the mean immunoexpression of Cytotoxic T lymphocyte(CD8+) with lymphnode status(pN) in OSCC cases. pN(+) cases (n=15) showed mean value of CTLs(CD8+) : 158.32 while pN(-) cases (n=15) had mean value of CTLs(CD8+) : 177.96

Table II: Statistical correlation of the mean immunoexpression of Cytotoxic T lymphocyte(CD8+) with lymphnode status(pN) in OSCC cases.

I) Descriptive Statistics:

	Lymph node			
	Positive		Negative	
	Mean	Standard Deviation	Mean	Standard Deviation
CD8+	158.32	24.66	177.96	19.99

ii) Independent t-test results:

	t-test value	df	p-value	Mean Difference
CD8+	-2.396	28	.023	-19.64000

*The p value is significant at the 0.05 level.

Table II illustrates the **descriptive statistics** correlating mean immunoexpression of Cytotoxic T lymphocytes (Cd8+) with lymphnode status(pN) in OSCC cases. The statistical test used was **independent student's't' test**. There was a statistically significant difference in the mean immunoexpression of CTLs(CD8+) (p value=0.023) between pN+ and pN- cases of OSCC.

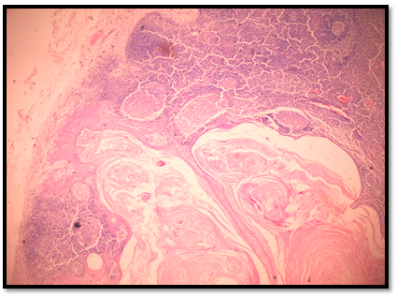


Figure 1: Photomicrograph of H & E stained soft tissue section showing pN(+) lymphnode. [Hematoxylin and Eosin, 40x]

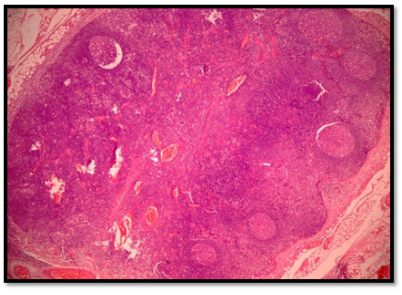


Figure 2: Photomicrograph of H & E stained soft tissue section showing pN(-) lymphnode. [Hematoxylin and Eosin, 40x]

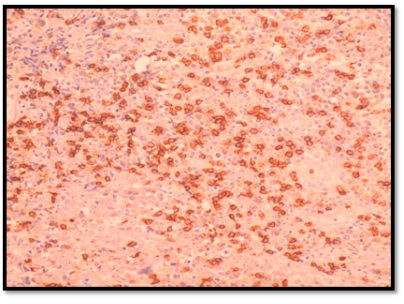
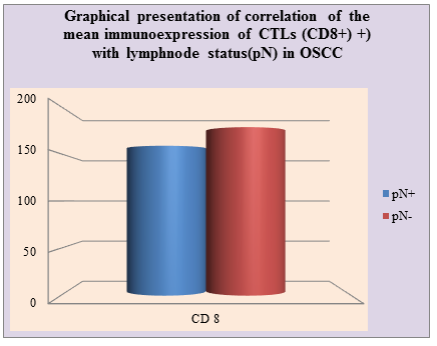


Figure 3: Photomicrograph of immunohistochemically stained soft tissue section of OSCC in primary tumour showing Cytotoxic T-lymphocytes(CD8+). [Immunohistochemical Stain, 400x]



Graph 1: correlates the mean immunoexpression of CTLs (Cd8+) with lymphnode status(pN) in OSCC cases. An increase in the count of CTLs(CD8+) was observed in lymph node negative pN(-) as compared to lymph node positive cases pN(+) of OSCC.

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