Evaluation of natural killer cell (CD57) as a prognostic marker in oral squamous cell carcinoma: An immunohistochemistry study

Rashmi Agarwal, Minal Chaudhary[1], Shruti Bohra[1], Shree Bajaj[1]

Department of Oral and Maxillofacial Pathology, Dr. B. R. Ambedkar Institute of Dental Sciences and Hospital, Patna, Bihar, [1]Department of Oral and Maxillofacial Pathology and Microbiology, Sharad Pawar Dental College, Wardha, Maharashtra, India

Abstract

Objectives: Natural killer (NK) cells are important effector lymphocytes. NK cells are considered to represent innate immune system. NK cells target and kill aberrant cells such as virally infected and tumorigenic cells. The purpose of this study was to assess the expression of CD57 in oral squamous cell carcinoma (OSCC) and to correlate the expression of CD57 with 3 years survival in patients with OSCC.

Materials and Methods: About 100 histopathologically diagnosed cases of OSCC of various grades were divided into two groups, i.e., Group I (dead patients) and Group II (live patients) from the archives of Department of Oral Pathology and Microbiology. CD57 was detected in these tissues by immunohistochemistry.

Result: The results were analyzed using Spearman's correlation coefficient and students unpaired t-test. The mean CD57 labeling index in Group II was significantly higher than that found in Group I ($P = 0.000$). There was a significant correlation ($P = 0.00$) in the mean CD57 levels between Groups I and II and prognosis of patient.

Conclusion: CD57 could be a good prognostic marker for OSCC patients.

Key Words: CD57, natural killer cell, oral cancer, oral squamous cell carcinoma

INTRODUCTION

Cancer is a hyperproliferative disorder.[1] Oral cancer ranks from 6th to 8th as the most common cancer in the world.[2] Cancer presently is the second most common cause of morbidity and mortality in the world after cardiovascular problems.[3] Cancers are heterogeneous cellular entities whose growth is dependent on reciprocal interactions between genetically altered cells and the microenvironment in which they reside. The microenvironment or more commonly referred as the tumor stroma is required for nutritional support and for the removal of waste products. It comprises of connective tissue, blood vessels, innate and adaptive immune cells, etc.[4] Innate immune cells include granulocytes, dendritic cells, macrophages, natural killer (NK) cells and mast cells.[5,6] NK cells play a critical role both in innate immunity and adaptive immunity through cytokine secretion or by direct interaction with dendritic cells.[7,8] NK cells provide resistance to pathogens and facilitate tumor immunosurveillance by two mechanisms which involve cytokine release interferon gamma (IFN-γ) and perforin-dependent

Access this article online

Quick Response Code:

Website:
www.jomfp.in

DOI:
10.4103/0973-029X.185933

How to cite this article: Agarwal R, Chaudhary M, Bohra S, Bajaj S. Evaluation of natural killer cell (CD57) as a prognostic marker in oral squamous cell carcinoma: An immunohistochemistry study. J Oral Maxillofac Pathol 2016;20:173-7.

target cell elimination.[7] Thus, this study was an attempt to analyze the presence of NK cells which is an integral part of tumor stroma and its effect on tumor progression.

MATERIALS AND METHODS

Sample collection

One hundred histologically diagnosed samples of oral squamous cell carcinoma (OSCC) of various grades were retrieved for a period of year 2007–2010 from the archives of Department of Oral Pathology and Microbiology. The protocol used was approved by the Institutional Ethical Committee. All the samples were subdivided into two groups, those who were dead: Group I and those who were alive: Group II. Each patient's relative was called every 6 month and was asked about the status of the patient. At the last follow-up, taken after approximately 3 years after surgery, 56 of 100 (56%) patients were alive and disease free, 5 (5%) patients were alive with recurrence of disease and 39 (39%) patients died of the disease.

Sample processing

The paraffin-embedded tissue section of 4 μm was obtained from archival tissues which were stained for routine hematoxylin and eosin and for the expression of CD57 by immunohistochemistry (IHC). Sections were hydrated with increasing grades of alcohol and brought to distilled water and treated with hydrogen peroxide (H_2O_2) to eliminate endogenous peroxidase activity. Then, antigen retrieval with tri-sodium citrate for CD 57 (Clone TB01, Lot # 00081714, primary antibody monoclonal mouse antihuman antibody - Dako) was carried out. The tissue was incubated sequentially with primary antibody CD57, which binds to specific tissue antigen on mononuclear inflammatory cells. Dako Envision system HRP, labeled polymer detection system and 3,3'-Diaminobenzidine substrate solution (DAB) was used that resulted in the formation of a colored precipitate at the tissue antigen binding sites. Visualization was aided by counter staining with hematoxylin.

CD57 labeling index evaluation

In each slide, 5 high power fields (HPF) were selected. In each HPF, cytoplasm of the cells stained with CD57, regardless of the intensity of the stain were counted as positive. The total number of positive cells was obtained by addition of total number of positive cells in each HPF. To obtain labeling index, a total number of positive cells were divided by 5. The sections stained with CD57 were examined under Leica DMLB2 (Leica Microscope) at 40x. The positive control was examined for the presence of the colored end product at the site of the target antigen (DAB chromogen brown end product). The presence of the colored end product was interpreted as positive staining.

The absence of the colored end products and nonspecific staining in negative control confirmed the specificity of the primary antibody. Cells were considered positive for CD57 if there was intracytoplasmic DAB staining (chromogen color). All lymphoproliferative cells whose cytoplasm stained brown were scored positive regardless of intensity of staining.

RESULTS AND OBSERVATION

CD57 LI: CD57 was expressed in OSCC in decreasing order from well differentiated to poorly differentiated SCC [Figures 1-3]. CD57 expressed predominantly in mononuclear inflammatory cells around the tumor islands of OSCC. However, few CD57 positive cells were scattered even away from the tumor island. CD57 was significantly higher in patients who were alive than those who were dead. On correlating CD57 labeling index with status of life (either dead or alive) of patients with OSCC using Spearman's correlation coefficient, P value was found to be significant ($P = 0.00$). When Pearson's linear correlation was used, then also P value was significant ($P \leq 0.0001$). Higher CD57 positive cells were present in normal lymph node than OSCC samples.

DISCUSSION

Carcinomas are malignant neoplasms derived from epithelial cells and represent the most common form of human cancer.[5] Oral cancer ranks from 6th to 8th as the most common cancer in the world.[2] Numerous studies have been conducted on various aspects of OSCC. These studies have focused mostly on the functions of oncogenes and tumor suppressor genes, and the signaling pathways regulating cell proliferation and/or cell death. However, such studies have informed us only about one aspect of tumor environment ignoring the fact that cancers are heterogeneous cellular entities whose growth is dependent on
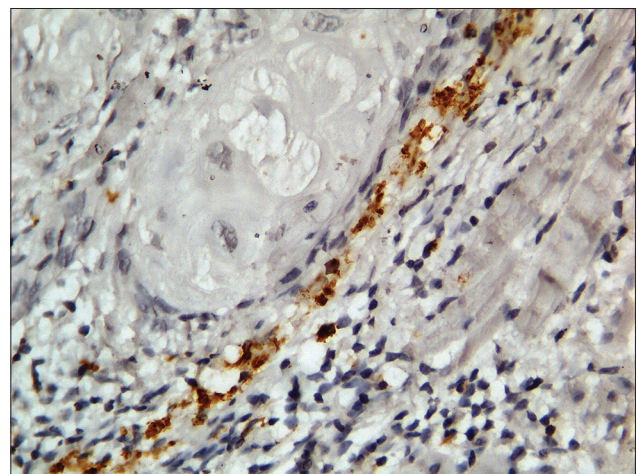
Figure 1: Well-differentiated squamous cell carcinoma showing CD57+ cells within mononuclear inflammatory cell infiltrate in the tumor stroma (IHC stain, ×400)
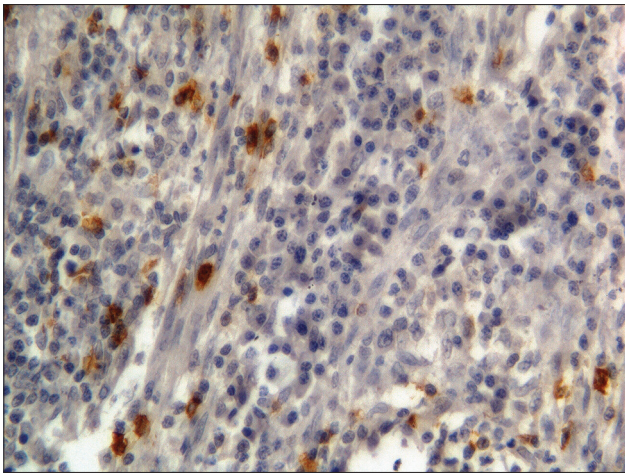
Figure 2: Moderately differentiated squamous cell carcinoma showing CD57+ cells within mononuclear inflammatory cell infiltrate in the tumor stroma (IHC stain, ×400)
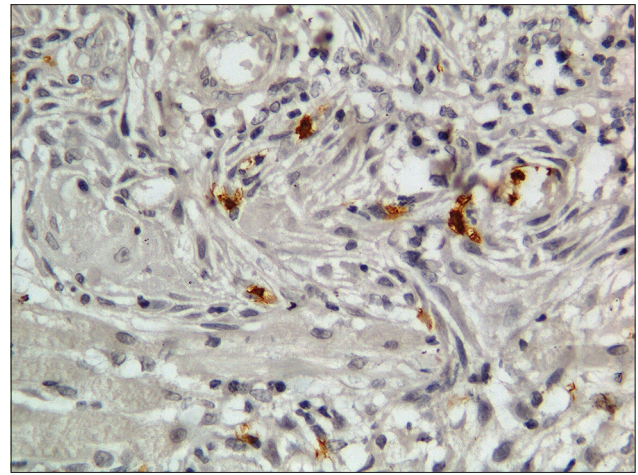
Figure 3: Poorly differentiated squamous cell carcinoma showing CD57+ cells within mononuclear inflammatory cell infiltrate in the tumor stroma (IHC stain, ×400)

reciprocal interactions between genetically altered cells and the microenvironment in which they reside. The microenvironment or more commonly referred as the tumor stroma is required for nutritional support and for the removal of waste products. The tumor stroma comprises connective tissue, blood vessels, innate and adaptive immune cells, etc.[4] Innate immune cells include granulocytes, dendritic cells, macrophages, NK cells and mast cells.[5,6] NK cells play a critical role both in innate immunity and adaptive immunity through cytokine secretion or by direct interaction with dendritic cells.[7,8] The key role of human immune system is to confront cells undergoing carcinogenesis. Immune system manipulation against the tumor cell is multifactorial. The immune defense against tumor cell is mediated initially by the innate immune cells, i.e., NK cells, NK T cells, cytokines and complement proteins, and later by adaptive immune system (B and T cells).[4] NK cells are one of the most important effector T-lymphocytes with an effective antitumor effect.[8,9] NK cells are bone marrow-derived lymphocytes comprising 10–20% of peripheral blood mononuclear cells.[10]

Cell surface expression of major histocompatibility complex (MHC) Class I is often downregulated by tumors and virally infected cells. This enables these cells to escape cytotoxic T-lymphocyte killing. NK cells can recognize and kill cells that have downregulated MHC Class I molecules.[11] The 3-year survival in patients with OSCC who were treated with surgical resection was about 56% patients, 5% patient survived but with recurrence and 39% patients were dead. The average 3 years survival rate was 56%. These survival rates were in concert with those documented by Forastiere *et al.* They documented that 35–55% of patients with head and neck squamous cell carcinoma remained disease free after 3 years post standard treatment.[12] Mortality in the patient with

OSCC followed up over a period of 3 years could be due to recurrence or delay in treatment. The presence of NK cells in OSCC patients was evaluated in this study with IHC. This is the first study in which a labeling index of NK cells in OSCC was calculated. Earlier studies were semi-quantitative and qualitative in which expression of CD57 was evaluated either in terms of positivity or negativity, intensity of staining or a definite number of cells/HPF. However, in the present study, CD57 labeling index was calculated, i.e., number of positive cells/HPF was evaluated. After extensive research of data, we found that this was the first documented instance where the CD57 labeling index was calculated. Ninety-four percent samples were positive for CD57 expression. The labeling index was less in dead patients, whereas the labeling index in patients who had survived was more. The mean labeling index was 3.67 and 10.67 in dead and alive patients, respectively. Due to lack of similar study in OSCC, we could not carry out any direct comparisons, and hence, we have made comparison with the results documented for other carcinomas. Ishigami *et al.* carried out a similar study on gastric carcinoma cases and showed that more number of NK cell infiltrate in patients showed better prognosis than those showing less NK cell infiltrate.[13] Villegas *et al.* also carried out similar study in lung squamous cell carcinoma concluding that patients with less NK cell infiltrate showed worse prognosis than those showing more NK cell infiltrate.[14] The positive correlation of CD57 with survival in tumor stroma may be due to the fact that NK cells play a major role in eliminating neoplastic cells. During the course of tumor establishment, cancer cells evolve strategies to evade this specific cytotoxic T-lymphocytic response. It has been well documented that in OSCC, there is either downregulation or lack of MHC Class I protein expression. Loss of MHC Class I molecule is an effective way of evading host immunosurveillance other than NK cells. This activates NK cells, as NK cells does

not require MHC Class I expression by the target cells.[11] NK cells possess NK receptors, i.e., activatory and inhibitory NK cell receptor (NKR). Killer inhibitory receptor molecules recognize MHC Class I and when they engage their ligand, NK cell-mediated lysis is inhibited. Therefore, a cell devoid of MHC Class I expression may be able to evade specific cytotoxic T-lymphocytes recognition but would remain a target for NK cells.[15] NKG2D ligand binds to NKG2D ligand-specific protein, transmits activation signal and induces immune effector cells to clear tumor cells.[16] Around 6% of samples showed negative staining for CD57, among them 3% were dead and 3% were alive. We could not offer any explanation for this lack of expression. This may be due to shortcomings at the time of processing.

The present study was aimed at the correlation of the expression of CD57 with 3 years survival in patients with OSCC. This study revealed that there was a significant correlation of CD57 labeling index with the status of life. As the CD57 labeling index increased, it was most likely that the patient was alive. The mean CD57 labeling index was much more in live patients as compared to the dead one. This study was in accordance with Liska *et al.* on colorectal carcinoma which documented that risk of overall short survival was 2.5 fold higher in patients with low tumor infiltration by CD57+ lymphocytes.[17] There are similar studies conducted on prostate carcinoma, colorectal carcinoma and lung carcinoma by Liu *et al.*, Coca *et al.* and Villegas *et al.* respectively, who also documented that increase in tumor infiltration by CD57+ lymphocytes results in a better prognosis.[14,18-20]

Evidence exists that growth and metastatic growth of tumor are dependent on their capacity to evade host immune surveillance and overcome host defense. All tumors express antigens, but in many instances, an inadequate immune response is seen.[21] The antitumor response by the immune inflammatory cells in the tumor microenvironment is usually imparted by cytotoxic T-lymphocytes and NK cells.[22]

The association of increase survival with increase in CD57 expression could be due to:

1. Cytotoxic T-lymphocytic response is MHC Class I restricted as their activation is dependent on MCH Class I expression on target cells. However, there are various studies in the literature which have stated reduction or absence of MHC Class I molecule expression by neoplastic cells. Tumors with downregulated classical MHC Class I expression allows them to escape cytotoxic T-lymphocytes immunosurveillance. Thus, NK cells play a major role as a cytotoxic cell for those tumor cells that have lost MHC Class I expression.[11,23]

2. NK cells are also known to regulate hematopoiesis and antibody production by B cells. Thereby increasing immunosurveillance.[24]

3. Early appearing, tumor infiltrating NK cells play a crucial role in the generation of antitumor T lymphocytes. There is a possibility that NK cells have an influence on generation of antitumor cytotoxic T-lymphocytes through production of IFN-γ. This cytokine environment is important for the development of antigen-specific CD4+ and CD8+ T-cells. Furthermore, it is well documented that IFN-γ upregulates the expression of MHC Class I and MHC Class II molecule. Thus, the upregulation of MHC Class II on macrophages and MHC Class I on tumor cell allow T-cells to recognize tumor-specific antigen.[24]

4. NK cells do not have TCRs on the cellular membrane, and their activation does not require MHC Class I expression by the target cells. NK cells express a surface receptor that is NKR that can be classified as inhibitory NKR and activatory NKR. Inhibitory NKRs are – KIR and CD94-NKG2A/B and activatory NKRs are NKG2D, DNAM-1 and natural cytotoxicity receptors.[25-27] These inhibitory KIR and CD94-NKG2A/B receptors are responsible for recognition of different alleles of MHC Class I molecules. Single NK cell comprises numerous KIR. The lack of even a single MHC Class I sensitize them to NK cell cytotoxicity. The activatory NKR triggers spontaneous and potent cytotoxicity.[25,11]

5. Evidence shows a link between tumorigenesis, the DNA damage response and the immune response. DNA damaging agents or DNA lesions associated with tumorigenesis activate the DNA damage response in damage cells. This response results in upregulation of NKG2D ligand which stimulates the NK cells to attack the diseased cells.[26]

6. NK cells express low affinity for immunoglobulin G receptor CD16 (activatory NKR), which enables them to recognize and kill target cells opsonized with antibodies by antibody dependent cell-mediated cytotoxicity.[9]

Despite all the ways in which the NK cell recognize target cell, secretory lysozyme exocytosis and perforin-dependent target cell elimination are required. This is divided into four stages:

a. Activating, lytic immunologic synapse forms at the point of contact with the target cell, resulting in rearrangement of the actin cytoskeleton

b. Microtubules organizing center of the NK cells and secretory lysosomes are polarized toward the lytic synapse

c. Secretory lysosomes dock with the plasma membrane at the lytic synapse, followed by

d. Release of cytotoxic content.[9,11]

CONCLUSION

Thus, we conclude that increase in the expression of CD57 in the tumor stroma of OSCC may serve as a good prognostic marker for the patients.

Financial support and sponsorship

Department of Oral and Maxillofacial Pathology and Microbiology, Sharad Pawar Dental College, Wardha.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest 2007;117:1175-83.
2. Marocchio LS, Lima J, Sperandio FF, Corrêa L, de Sousa SO. Oral squamous cell carcinoma: An analysis of 1,564 cases showing advances in early detection. J Oral Sci 2010;52:267-73.
3. Khan Z. An overview of oral cancer in indian subcontinent and recommendations to decrease its incidence. Webmed Central CANCER 2012. doi: 10.9754/journal.wmc. 2012.003626. Available from: http://www.webmedcentral.com/article_view/3626. [Last accessed on 2016 Jun 28].
4. Kufe DW, Pollock RE, Weichselbam RR, Bast RC, Gansler TS, Holland JF, *et al*. Holland-Frei Cancer Medicine. 6th ed. Hamilton (ON): BC Decker; 2003.
5. Bhowmick NA, Moses HL. Tumor-stroma interactions. Curr Opin Genet Dev 2005;15:97-101.
6. Tlsty TD, Coussens LM. Tumor stroma and regulation of cancer development. Annu Rev Pathol 2006;1:119-50.
7. Bryceson YT, Chiang SC, Darmanin S, Fauriat C, Schlums H, Theorell J, *et al*. Molecular mechanisms of natural killer cell activation. J Innate Immun 2011;3:216-26.
8. Lopez-Vergès S, Milush JM, Pandey S, York VA, Arakawa-Hoyt J, Pircher H, *et al*. CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16 NK-cell subset. Blood 2010;116:3865-74.
9. Topham NJ, Hewitt EW. Natural killer cell cytotoxicity: How do they pull the trigger? Immunology 2009;128:7-15.
10. Anderson SK. Biology of natural killer cells: What is the relationship between natural killer cells and cancer? Will an increased number and/or function of natural killer cells result in lower cancer incidence? J Nutr 2005;135:2910S.
11. Cruz I, Meijer CJ, Walboomers JM, Snijders PJ, Van der Waal I. Lack of MHC class I surface expression on neoplastic cells and poor activation of the secretory pathway of cytotoxic cells in oral squamous cell carcinomas. Br J Cancer 1999;81:881-9.
12. Forastiere AA, Goepfert H, Maor M, Pajak TF, Weber R, Morrison W, *et al*. Concurrent chemotherapy and radiotherapy for organ preservation in advanced laryngeal cancer. N Engl J Med 2003;349:2091-8.
13. Ishigami S, Natsugoe S, Tokuda K, Nakajo A, Che X, Iwashige H, *et al*. Prognostic value of intratumoral natural killer cells in gastric carcinoma. Cancer 2000;88:577-83.
14. Villegas FR, Coca S, Villarrubia VG, Jiménez R, Chillón MJ, Jareño J, *et al*. Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer. Lung Cancer 2002;35:23-8.
15. Grandis JR, Falkner DM, Melhem MF, Gooding WE, Drenning SD, Morel PA. Human leukocyte antigen class I allelic and haplotype loss in squamous cell carcinoma of the head and neck: Clinical and immunogenetic consequences. Clin Cancer Res 2000;6:2794-802.
16. Wang J, Li C, Yang D, Jian XC, Jiang CH. Clinico-pathological significance of MHC-I type chain-associated protein A expression in oral squamous cell carcinoma. Asian Pac J Cancer Prev 2012;13:715-8.
17. Liska V, Vycital O, Daum O, Novak P, Treska V, Bruha J, *et al*. Infiltration of colorectal carcinoma by S100+dendritic cells and CD57+lymphocytes as independent prognostic factors after radical surgical treatment. Anticancer Res 2012;32:2129-32.
18. Liu X, Zhan B, Tomoyoshi T, Masanori T. Immunohistochemical study of HNK-1 (Leu-7) antigen in prostate cancer and its clinical significance. Chin Med J (Engl) 1995;108:516-21.
19. Coca S, Perez-Piqueras J, Martinez D, Colmenarejo A, Saez MA, Vallejo C, *et al*. The prognostic significance of intratumoral natural killer cells in patients with colorectal carcinoma. Cancer 1997;79:2320-8.
20. Khan A, Baker SP, Patwardhan NA, Pullman JM. CD57 (Leu-7) expression is helpful in diagnosis of the follicular variant of papillary thyroid carcinoma. Virchows Arch 1998;432:427-32.
21. Foss FM. Immunologic mechanisms of antitumor activity. Semin Oncol 2002;29 3 Suppl 7:5-11.
22. Zancope E, Costa NL, Junqueira-Kipnis AP, Valadares MC, Silva TA, Leles CR, *et al*. Differential infiltration of CD8+and NK cells in lip and oral cavity squamous cell carcinoma. J Oral Pathol Med 2010;39:162-7.
23. Paul P, Rouas-Freiss N, Khalil-Daher I, Moreau P, Riteau B, Le Gal FA, *et al*. HLA-G expression in melanoma: A way for tumor cells to escape from immunosurveillance. Proc Natl Acad Sci U S A 1998;95:4510-5.
24. Kurosawa S, Harada M, Matsuzaki G, Shinomiya Y, Terao H, Kobayashi N, *et al*. Early-appearing tumour-infiltrating natural killer cells play a crucial role in the generation of anti-tumour T lymphocytes. Immunology 1995;85:338-46.
25. Vivier E, Ugolini S, Blaise D, Chabannon C, Brossay L. Targeting natural killer cells and natural killer T cells in cancer. Nat Rev 2012;12:239-52.
26. Zamai L, Ponti C, Mirandola P, Gobbi G, Papa S, Galeotti L, *et al*. NK cells and cancer. J Immunol 2007;178:4011-6.
27. Joyce MG, Sun PD. The structural basis of ligand recognition by natural killer cell receptors. J Biomed Biotechnol 2011;2011:203628.