Original Article

Emerging insights of NK cells immunosurveillance in histomorphologic prognostic indicators of oral squamous cell carcinoma

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Abstract

Background: IFN-gamma and natural killer (NK) cells have been considered the most effective cells in the combat of cancer, contributing to better prognosis and longer survival. The aim of the study was to analyze and correlate the CD 57 immunopositive NK cell-mediated Interferon-γ pathway in regulating immune mechanisms in Oral Squamous Cell Carcinoma.

Materials and Methodology: The study sample was composed of a total of 40 cases of histopathologically confirmed cases of Oral Squamous cell carcinoma (OSCC). Clinical data such as age, gender, habit history, signs and symptoms, and TNM staging were obtained for each case. The biopsy specimens of the cases obtained were fixed with 10% neutral buffered formalin and processed and embedded in paraffin wax. 3-4 μ thick sections were taken for hematoxylin and eosin staining and immunohistochemistry procedure. A saliva sample was collected from each patient and stored at 20 degree Celsius for estimation of salivary interferon-gamma levels using the sandwich ELISA technique.

Results: CD 57 NK cells quantitative assessment was significantly associated with tumor budding, cell nest size, the pattern of invasion, lymphocytic host response, NK cell morphology, Depth of invasion, and Tumor thickness. The ratio of CD 57 immunopositive NK cells to salivary IFN-γ levels showed a significant association with histopathological grades, tumor size, and lymph node status.

Conclusion: Adoptive cellular transfer therapy with NK cells has been advocated in both experimental models and clinical trials in treating hematopoietic malignancies. The strategy is based on reviving the patient innate immune surveillance and control of tumor invasion by the infusion of activated NK cells. The IFN-gamma and NK cell infiltration in oral squamous cell carcinoma might show a distinctive tumor microenvironment with a favorable local cytotoxic immune response against neoplastic cells.

Keywords: CD 57, IFN-gamma, intratumoral influence, oral squamous cell carcinoma, peritumoral

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INTRODUCTION

Conceptual progress in the last decade has added emerging hallmarks of potential generalization in carcinogenesis focusing on evading immune destruction. In addition to cancer cells, tumors exhibit another dimension of complexity: they contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the "tumor microenvironment." With the advent of better markers for accurately identifying the distinct cell types of the immune system, it is now clear that virtually every neoplastic lesion contains immune cells present at densities ranging from subtle infiltrations detectable only with cell type-specific antibodies to gross inflammations that are apparent even by standard histochemical staining techniques.[1]

Historically, such immune responses were largely thought to reflect an attempt by the immune system to eradicate tumors, and indeed, there is increasing evidence for antitumoral responses to many tumor types with an attendant pressure on the tumor to evade immune destruction.[2]

The inflammatory cells in a tumor microenvironment operate in conflicting ways: both tumor-antagonizing and tumor-promoting leukocytes can be found, in various proportions, in most if not all neoplastic lesions. NK cells participate in the innate immune response and play an important role in the defense against viral infections as well as in tumor surveillance and are also involved in shaping adaptive immune responses through their production of cytokines. NK cells produce several cytokines and chemokines that coordinate innate and adaptive immune responses and are the major sources of IFN-γ in the first hours of infection before antigen-specific T-cell production of IFN-γ. They express many different receptors that can either trigger activation or mediate inhibition of their effector's functions.[3]

The counterintuitive existence of both tumor-promoting and tumor-antagonizing immune cells can be rationalized by invoking the diverse roles of the immune system: On the one hand, the immune system specifically detects and targets infectious agents with the adaptive immune response, and on the other, the innate immune system is involved in clearing dead cells and cellular debris.

There is an established fact that CD8+ cells in the peritumoral microenvironment positively affect the survival of OCSCC patients as they contribute to a more effective cytotoxic immune response against oral neoplastic cells.[4] The important challenge is to decipher the contribution of distinct NK cell subsets to natural immune surveillance as well as to the response to therapeutic interventions by immunomodulatory drugs and therapeutic antibodies.

Thus, the following study is intended to evaluate and correlate the expression of CD57 immunopositive NK cells and salivary IFN-γ levels and explain their role in the progression of Oral Squamous cell carcinoma cases which may pave the way for guided differentiation of specific subsets of NK cells for adoptive transfer. Immunomodulation based on the interaction of NK cells with tumor cells could be useful in developing potential immunotherapy targeted at making the tumor cells less vulnerable to immune suppression in Oral Squamous cell carcinoma cases.

MATERIALS AND METHOD

Patients and tissue samples

The study was conducted in the Department Of Oral and Maxillofacial Pathology and Microbiology, I.T.S Dental College, Muradnagar, Ghaziabad, on archival tissue samples which were submitted for histopathological evaluation. The samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax to obtain 3 μ sections for the immunohistochemistry procedure. Study samples consisted of total of 40 cases, which included ten cases of the normal oral mucosa (controls) and 30 cases of OSCC. Ethical approval from the institutional review board was obtained for this study. Clinical data such as age, gender, habit history, signs and symptoms, and TNM staging were obtained for each case.

Grading of OSCC

A few other histomorphological criteria were taken into consideration like Tumour budding in the Study group (Acc to Wang C *et al*.)[5]-Type 1 (score 0), Type 2 (Score 0-2), Type 3 (>3). Cell nest size in Study group. (Acc to Parekh D. *et al*.)[6]- Type 1 (Large and intermediate nests) and Type 2 (Small nests or single cells) and Pattern of invasion in the study group (Parekh D *et al*.)[6]- POI 1, 2, 3 as Nonaggressive and POI 4, 5 as Aggressive. Depth of Invasion in the study group. (Parekh D. *et al*.)[6] Depth of invasion- Type 1 as less than 4 mm and Type 2 as more than 4 mm. Tumor Thickness (Po wing Yuen *et al*.)[7]- Type 1 up to 3 mm, Type 2 as 3-9 mm, Type 3 as >9 mm.

Saliva sample

A saliva sample was collected from each patient (5 ml) and stored at 20 degree Celsius for estimation of salivary interferon-gamma levels using the sandwich ELISA technique.

Immunohistochemistry with CD57

Three-micrometer-thick sections from archival formalin-fixed paraffin-embedded tissues were placed on poly-L-lysine-coated slides for immunohistochemistry. CD57 expression was analyzed by immunohistochemical examination with an antibody. For each antibody, the deparaffinized tissue sections were placed in 10 mmol/L citrate buffer, pH 6.0, and heated to cycles of 95°C and 98°C for 13 minutes. Immunohistochemical staining for these proteins was performed by the avidin-biotin complex procedure with a streptavidin-biotin complex peroxidase kit. Primary antibody-monoclonal anti-CD57 antibody (Biogenex Ind Pvt Ltd, Fremont, CA, USA clone number-, catalog number- AM3140817) along with secondary antibody-poly HRP secondary detection system (Biogenex Ind Pvt ltd) was used. For CD57, the tonsil served as the positive control. Positive staining for CD57 was identified as granular cytoplasmic brown color. All the immunostained slides were viewed under the light microscope.

Quantitative analysis

The density and the percentage of NK cells in the stroma near the invasive front of oral SCC (peritumoral) were determined. In addition, the number of NK cells within the cancer nests (intratumoral) region was also evaluated.[8] Five Randomly selected fields with a minimum of 20 cells per field at 400X magnification were selected so that a minimum of 100 cells were evaluated per case and the percentage of positive tumor cells over total number of neoplastic cells present in the same area was scored.

Statistical analysis

The resulting data were analyzed using SPSS software version 24. Data has been expressed as mean and standard deviation. Differences between the different variables were analyzed using the ANOVAs test, Pearson's Chi-square test, and Mann Whitney test was also calculated.

RESULTS

The mean score of NK cells in the intratumoral subset of tumor islands decreased from a number of tumor buds score 0 (3.06 ± 1.77) to tumor bud score0-2 (1.56 ± 1.18) to tumor bud score >3 (0.65 ± 0.25). In the peritumoral stromal region, the mean quantitative score of NK cells decreased from no. of tumor buds score 0 (5.61 ± 3.05) to 0-2 (3.07 ± 2.30) to >3 (0.89 ± 0.53). Similarly, the mean score decreased in the intratumoral subset of tumor islands in cell nest size from large and intermediate nests (2.75 ± 1.76) to small nests or single cells (1.31 ± 1.26). In the peritumoral stromal region, the mean quantitative score decreased from large and intermediate nests (2.35 ± 1.50) to small nests or single cells (1.32 ± 1.23). The mean score of NK cells decreased in the intratumoral subset of tumor islands in the pattern of invasion from Type 1 (3.22 ± 2.07) to Type 2 (2.44 ± 1.46) to Type 3 (0.96 ±0.55) to Type 4 (0.50 ± 0.14) but increased in Type 5 (0.52 ± 0.09). In the peritumoral stromal region, the mean quantitative score of NK cells decreased from Type 1 (2.81 ± 1.90) to Type 2 (2.30 ± 1.04) to Type 3 (0.73 ± 0.66) Type 4 (0.25 ± 0.07) but increased in Type 5 (0.67 ± 0.09). The mean score of NK cells decreased in the intratumoral subset of tumor islands in lymphocytic host response from mild (2.48 ± 1.45) to moderate (2.02 ± 1.73) to dense (1.39 ± 1.84). In the peritumoral stromal region, the mean quantitative score of NK cells decreased from mild (1.16 ± 1.53) to moderate (1.89 ± 1.41) to dense (1.3 ± 1.33). The mean quantitative score of the small round was (1.75 ± 1.40) and the large round was (2.03 ± 1.60) in the peritumoral stromal region. The overall total mean count of NK cells of the small round was (3.73 ± 2.70) and of the large round was (4.17 ± 3.55). The mean values were statistically significant for all the histopathological parameters ($p \leq 0.05$). [Figures 1-3].

However, mean salivary Interferon-γ levels with histopathological prognostic indicators in a study group with tumor budding, cell nest size, the pattern of invasion, lymphocytic host response, morphology, depth of invasion, and tumor thickness showed no correlation. [Table 1 and Figure 4].

On correlating the ratio of CD57 positive NK cells to Salivary Interferon-γ levels with histopathological prognostic indicators in the study group, the mean score in the subset of tumor islands correlated with significant values. [Table 2].

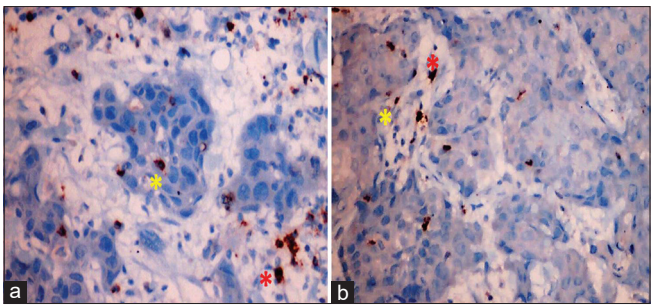


Figure 1: (a) CD57 positive NK cells intratumoral (marked with yellow asterisk) and peritumoral (marked with red asterisk) region of tumor cells in Moderately differentiated OSCC (IHC, 40x magnification) (b) CD57 immunoexpression in small (marked with yellow asterisk) and large (marked with red asterisk) granular cytoplasmic subset of NK cells in moderately differentiated OSCC (IHC, 40x magnification)

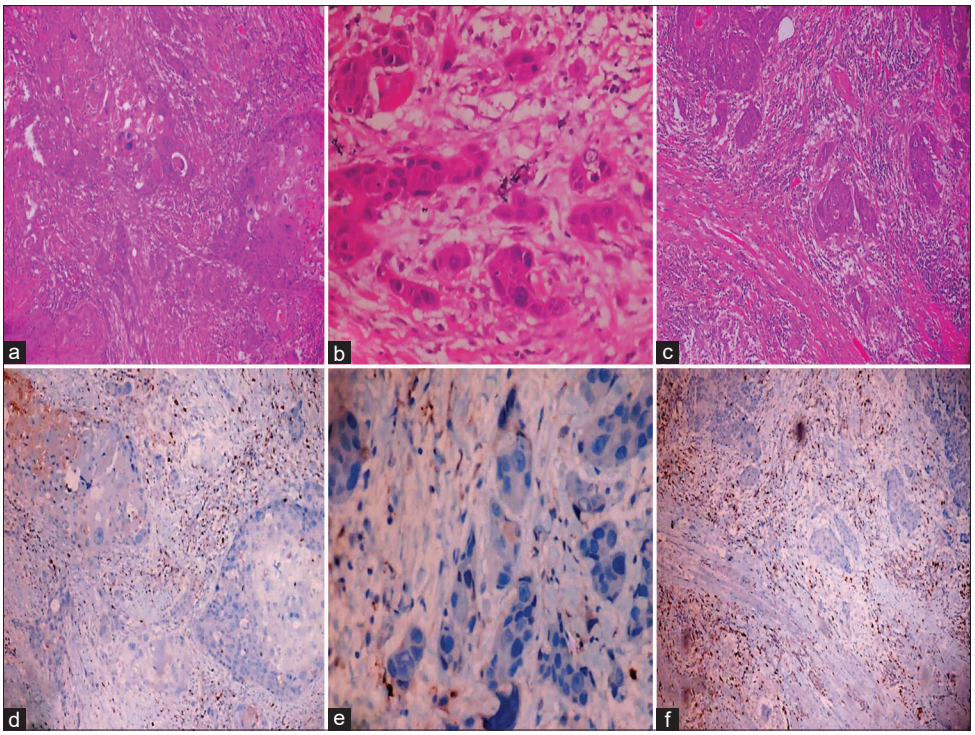


Figure 2: (a) Tumor cells arranged in large and intermediate size nests in well differentiated OSCC (H&E, 10x magnification) (b) Small cords and buds of tumor nests in moderately differentiated OSCC (H&E, 40x magnification) (c) Band of lymphoid infiltration surrounding infiltrating tumor islands suggestive of dense lymphocytic host response (H&E, 10x magnification) (d) Increased immunoexpression of CD57 positive NK cells in peritumoral subset of large cell nests in well differentiated OSCC (IHC, 10x magnification) (e) Few CD 57 positive NK cells surrounding the small buds of tumor nests in moderately differentiated OSCC (IHC, 40x magnification) (f) Strong immunoexpression of CD57 positive peritumoral population of NK cells in a background of dense lymphocytic host response (IHC, 10 x magnification)

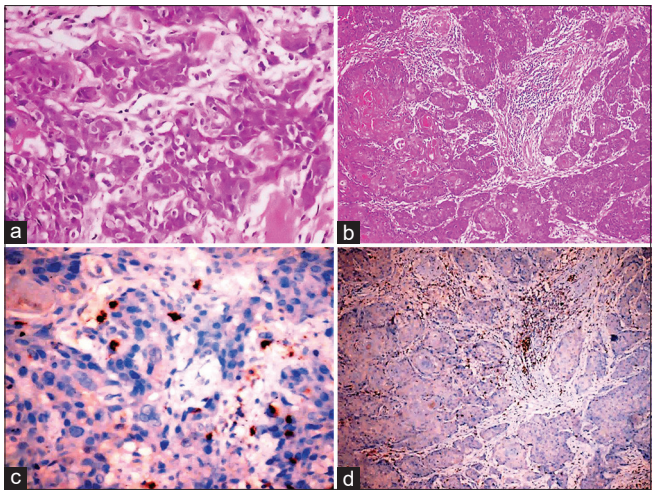


Figure 3: (a) Aggressive pattern (POI 4) of infiltrating tumor nests (H&E,10x magnification) (b) Non-Aggressive pattern (POI 1) of tumor islands (H&E, 10x magnification) (c) Intratumoral immunoexpression of CD57 positive NK cells in aggressive pattern (POI 4) of OSCC (IHC, 40x magnification) (d) Increased immunoexpression of peritumoral subset of CD 57 positive NK cells in non-aggressive (POI 1) pattern of OSCC (IHC, inset 40x magnification)

The mean score in the intratumoral subset of tumor islands in histopathological grades increased from well-differentiated OSCC (0.1310 ± 0.0997) to moderately differentiated OSCC (0.1540 ± 0.1511) but decreased in poorly differentiated OSCC (0.3202 ± 0.3731). In the peritumoral subset of tumor islands, the mean quantitative score increased from well-differentiated OSCC (0.1148 ± 0.1005) to moderately differentiated OSCC (01706 ± 0.2027) to poorly differentiated OSCC (0.3806 ± 0.3055) whereas the total mean score of total NK cells/IFNγ decreased from well-differentiated OSCC (1.34 ± 1.25) to moderately differentiated OSCC (0.625 ± 013) with a slight increase in poorly differentiated OSCC (0.625 ± 013). Although the mean values were statistically not significant ($p > 0.05$). [Graph 1].

There was a positive correlation of lymphocytic host response with total quantitative CD 57 positive NK cells in the subset of tumor islands. The coefficient of determination is given ($R^2 = 0.602$) and Y is a line of regression given equally to (0.807x + 0.035) which shows lymphocytic host response is 60% determined by total quantitative CD 57 positive NK cells. [Graph 2].

DISCUSSION

Immunotherapy has revolutionized cancer treatment, with checkpoint blockade therapy showing remarkable efficacy in several cancer subtypes.[9] NK cells participate

Table 1: Correlation of histomorphometric parameters with CD 57 positive NK cells and salivary Interferon-γ levels

Parameters	CD57 positive NK cells			*P*	Salivary Interferon-γ levels (Mean±SD)	*P*
	Intratumoral	Peritumoral	Total			
Tumour budding						
0	3.06±1.77	2.54±1.53	5.61±3.05	0.00	6.88±6.61	0.318
0-2	1.56±1.18	1.51±1.32	3.07±2.30		1.533±33.54	
>3	0.65±0.25	0.89±0.53	1.54±0.74		3.08±2.97	
Cell nest size						
Large and intermediate nests	2.75±1.76	2.35±1.50	5.10±3.02	0.05	6.38±6.43	0.452
Small nests or single cells	1.31±1.26	1.32±1.23	2.63±2.33	0.022	1.11±27.35	
Pattern of invasion						
Type 1	3.22±2.07	2.81±1.90	6.03±3.79	0.001	6.80±4.68	0.042
Type 2	2.44±1.46	2.30±1.04	4.74±2.17		5.88±6.79	
Type 3	0.96±0.55	0.73±0.66	1.70±0.96		2.11±42.6	
Type 4	0.50±0.14	0.25±0.07	0.75±0.070		2.93±3.35	
Type 5	0.52±0.09	0.67±0.09	1.2±0.16		3.72±3.37	
Lymphocytic Host response						
Mild	2.48±1.45	1.16±1.53	4.64±2.75	0.002	1.57±30.29	0.190
Moderate	2.02±1.73	1.89±1.41	3.91±3.03		4.88±2.96	
Dense	1.39±1.84	1.3±1.33	2.69±2.98		3.2±2.32	
Morphology						
Small Round	1.9±1.50	1.75±1.40	3.73±2.70	0.01	2.10±1.59	0.16
Large round	2.14±2.09	2.03±1.60	4.17±3.55		3.19±2.60	
DOI						
Less than 4 mm	2.96±1.96	1.87±1.001	3.01±2.08	0.003	1.704±25.48	0.470
More than 4 mm	4.71±1.01	3.01±2.01	5.02±1.67		6.108±6.78	
Tumour Thickness						
Upto 3 mm	14.39±33.742	3.019±2.02	2.08±1.38	0.006	4.82±3.51	0.340
3 to 9 mm	6.93±6.50	2.129±1.01	1.02±0.028		1.36±28.04	
More than 9 mm	5.08 ±	1.209±3.89	3.09±0.82		2.59±2.41	

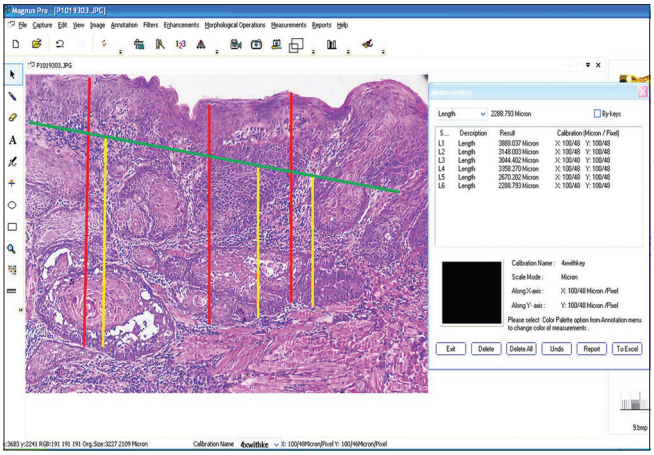


Figure 4: Measurement of depth of invasion, tumor thickness and basement membrane level using Morphometric analysis (Magnus Pro software)

in the innate immune response and play an important role in the defense against viral infections as well as in tumor surveillance and are also involved in shaping adaptive immune responses through their production of cytokines.[4]

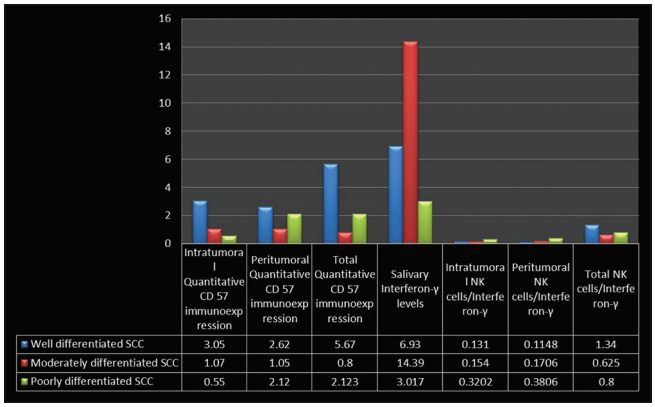
Regulation of IFN-γ production in NK cells shares many aspects with T cells, including the signaling pathways and transcription factors (TFs) required for efficient transcription. Thus, the following study was arrived at to evaluate and correlate the expression of CD57 immunopositive NK cells and salivary IFN-γ levels and explain their role in the progression of Oral Squamous cell carcinoma cases.

The overall total mean count decreased from well-differentiated OSCC (5.67 ± 2.87) to poorly differentiated OSCC (2.123 ± 0.65). Our results are in accordance with Katou *et al*.[10] who have shown that intratumoral CD8+ T lymphocytes and NK cells are phenotypically inactivated related to those present in the stroma of SCC, characterizing, thus, a possible mechanism of the tumoral cells to escape immune recognition. From these results, it can be postulated that the tumoral infiltration of the CD8+ and NK cells is considered a key factor in anti-tumor immunity. Cytokines promote the survival, proliferation, differentiation, and activation of lymphocytes.[11]

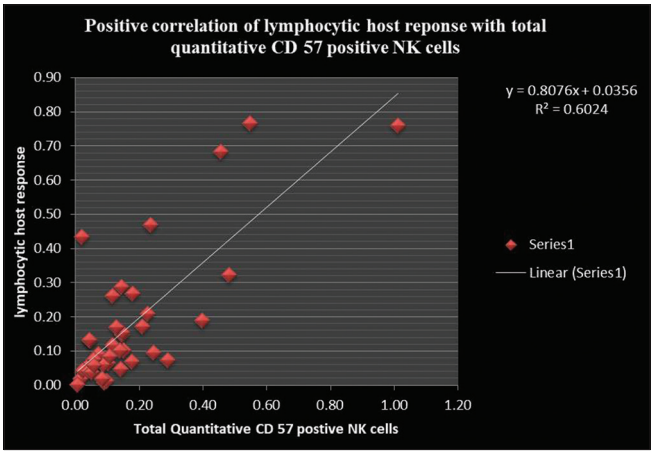
In the present study, the results demonstrated the correlation of quantitative assessment of CD57-positive NK cells with the site in the study group. Zancope *et al*.[8] evaluated the population of CD8+ and NK cells, by immunohistochemistry, in samples of oral cavity Squamous cell carcinoma (OCSCC). They observed that the number of peritumoral and intratumoral CD8+ and NK cells was significantly higher in LSCC when compared with control, pre-malignant lesions, and OCSCC. A higher proportion of peritumoral CD8+ cells demonstrated a correlation

Table 2: Correlation of histomorphometric parameters with total, peritumoral and intratumoral CD 57 positive NK cells to salivary Interferon-γ levels ratio

Parameters	(Mean±SD)		
	Intratumoral NK cells/Interferon-γ	Peritumoral NK cells/Interferon-γ	Total NK cells/Interferon-γ
Tumour budding			
0	0.45±1.68	2.08±1.89	8.06±7.98
0-2	2.55±2.05	3.78±2.09	3.76±1.65
>3	3.05±1.98	4.78±3.02	1.67±1.09
P	0.00	0.004	0.001
Cell nest size			
Large and intermediate nests	1.56±2.04	1.07±1.67	2.07±2.67
Small nests or single cells	6.05±3.04	0.87±0.02	3.76±4.09
P	0.05	0.02	0.006
Pattern of invasion			
Type 1	1.05±2.04	0.67±0.56	4.08±2.89
Type 2	2.05±2.90	1.29±1.78	2.06±3.80
Type 3	3.05±1.89	1.78±0.93	1.56±1.67
Type 4	5.65±1.98	2.78±0.93	0.42±0.87
Type 5	6.09±3.04	1.08±8.08	2.09±3.67
P	0.62	0.03	0.05
Lymphocytic Host response			
Dense	1.08±6.89	3.06±1.67	1.08±6.78
Moderate	9.67±3.056	2.09±3.07	2.56±3.85
Mild	3.08±2.45	1.07±1.05	7.04±2.86
P	0.02	0.04	0.01
Morphology			
Small Round	2.05±1.08	1.25±2.76	1.06±0.65
Large Round	3.108±0.98	7.07±9.76	2.56±9.05
P	0.06	0.21	1.05
DOI			
Less than 4 mm	2.05±1.54	8.65±3.05	0.06±2.67
More than 4 mm	3.06±1.34	2.96±3.08	1.45±4.89
P	0.000	0.001	0.000
Tumor thickness			
Upto 3 mm	6.09±2.98	2.07±2.78	2.76±1.67
3 to 9 mm	7.86±1.098	1.09±1.56	1.08±8.56
More than 9 mm	9.00±5.89	3.09±3.08	2.45±3.08
P	0.002	0.003	0.001



Graph 1: Distribution of NK cell population, IFN-γ and their ratio in study cases



Graph 2: Correlation of CD57 positive NK cells and lymphocytic host response in the study groups

with a lower neoplastic proliferative index. Santos *et al*.[12] demonstrated that the intratumoral density of CD57+ cells was lower in OSCCs presenting a poor prognosis. On the other hand, an increase in the proportion of peripheral populations of CD57+ CD4+ and CD57+ CD8+ T-cells was significantly associated with the advanced clinical stage, and therefore may act as effector cells for OSCC progression, exerting influence on the systemic immunity of patients with OSCC.[13]

One of the earliest studies on tumor prognostication in oral SCC is by Bryne *et al*.[14] who modified Broder's grading based on keratinization, nuclear atypia, and mitotic

count. The histological risk assessment model proposed by Brandwein-Gensler *et al*.[4] is based on a five-tiered WPOI, the presence of PNI in small or large nerves (>1 mm), and a three-tiered Lymphocyte Host Response grading.[4]

A novel grading system using tumor budding activity and cell nest size (CNS) at the invasive front has recently been validated for oral SCC Boxberg *et al*.(2017)[15] for risk stratifying head and neck SCCs. Tumor buds are small cell nests (≤5 cell clusters) or single cells, which dissociate/bud into the surrounding stroma. Though the clinical significance of Tumor Bud is well established in sites like the colon and incorporated into routine reporting datasets, it has not been adequately evaluated for oral SCCs.[16]

In our study presence of CNS of ≤5 cells at the tumor edge, significantly correlated with prognosis with a statistically significant *P* value = 0.05. Tumor budding is a measure of the cellular discohesion of the tumor and represents epithelial-mesenchymal transition.[5]

Angadi *et al*.[17]reported the presence of Tumor Buds in 89% of the 75 cases of oral squamous carcinoma reviewed by them, with 45.3% showing high-intensity budding (>10 buds/20 × HPF). Brandwein-Gensler M *et al*.[4] showed that aggressive patterns of invasion (WPOI-4 and 5) were significantly associated with poorer OSCC and positive lymph nodes, in comparison to nonaggressive ones (groups 1-3) in their cohort. The presence or absence of WPOI-5 is now incorporated in the CAP minimum dataset for reporting in oral SCCs. Similar to the study by Björkström *et al*.[18] NK in our study all patterns of invasion showed clinical significance with *P* value <0.05. Tumor interface characteristics of the pattern of invasion and the degree of LHR help to identify poor biology early-stage tumors, which can fail traditional therapy, even when adequately excised, and should be considered for more aggressive adjuvant treatment.

Tumor-infiltrating natural killer (NK) cells (TINKs) are crucial immune cells in tumor defense and might be related to tumor prognosis. However, the results were discrepant among different studies also to the best of our knowledge our study is one of its kind in which as 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 a generation of antitumor cytotoxic T-lymphocytes through the 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 up-regulates the expression of MHC Class I and MHC Class II molecule.

We also correlated the ratio of CD57 positive NK cells to Salivary Interferon-γ levels with tumor size (T), Lymph node status (N), and TNM Stage in the study group, and the results showed the mean score in the subset of tumor islands in tumor size increases from T1 (0.172 ± 0.152) to T2 (0.194 ± 0.278) but decreased in T3 (0.141 ± 0.139). The buccal mucosa is the most common site for OSCC in southeastern Asia, due to habits of areca nut- and tobacco chewing.[19] In accordance with above-mentioned data, we also evaluated the correlation of the ratio of CD57 positive NK cells to Salivary Interferon-γ levels with the site in the study group. Our present results depicted the mean score in the intratumoral subset of tumor islands was more in buccal mucosa with the highest value of (0.416 ± 0.186) followed by palate, tongue, and alveolar ridge as per in the peritumoral subset of tumor islands the mean score was also more in buccal mucosa with the highest value with (0.160 ± 0.193) followed by the tongue, alveolar ridge, and palate. Only the total NK cells to Interferon gamma ratio was statistically significant. In accordance with our results Zancope *et al*.[8] suggested that this cell population, in this region, may contribute to a more effective cytotoxic immune response against oral neoplastic cells.

Lymphocytic host response (LHR) in the Risk Model is histologically quantified as the density of lymphocytes at the tumor interface.[12] It is classified as strong, intermediate, or weak, and is inversely associated with the risk of decreased time to disease progression. Zancope and colleagues *et al*.[8] also found that greater interface CD8 T cells were associated with longer mean survival. Katou *et al*.[10] demonstrated differences in the activation states between intratumoral and interface lymphocytes associated with oral Squamous cell carcinoma. Further studies are highly encouraged to explore the regulatory mechanisms of the tumor-infiltrating processes of NK cells and the enhancement methods of the anti-tumor effects. In addition, targeting the function state of NK cells might be a promising treatment strategy for solid tumors, such as IL-15-mediated CD56 activation. However, great care is needed when extrapolating the prognostic value into treatment potential. Notably, discrepant results were achieved in the current study, which might decrease the evidence grade to some extent. The inconsistent results might be attributed to a limited number of included studies in certain analysis.

CONCLUSION

Understanding immune surveillance and mechanisms of neoplastic cell's interact with the tumor stromal microenvironment forms an important link between

understanding the biological behavior and cellular activity of tumor cells. The evaluation of quantitative results of NK cells needs to be correlated with survival plots with long-term-follow-ups in a cohort of patients with locoregional spread and lymph node involvement would be the most suitable approach to estimate the prognostic value of these markers in clinical settings.

Adoptive cellular transfer therapy with NK cells has been advocated in both experimental models and clinical trials in treating hematopoietic malignancies. The strategy is based on reviving the patient innate immune surveillance and control of tumor invasion by the infusion of activated NK cells.

Therapeutic antibodies, Chemical antigen receptors, or bispecific proteins can all retarget NK cells precisely to tumor cells. Therapeutic antibody blockade of the immune checkpoints of NK cells has been suggested to overcome the immunosuppressive signals delivered to NK cells.

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Conflicts of interest

There are no conflicts of interest.

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