Acta Scientific Dental Sciences

Volume 1 Issue 2 July 2017

Research Article

Inflammation Associated Changes at Tumor Site and in Circulation in Node Negative Oral Squamous Cell Carcinoma

Shaikh Shayma[1], Raiya Birva[2] and Vora Hemangini[3]*

[1]*M.Sc. Student, Cancer biology Department, Gujarat Cancer and Research Institute, Ahmedabad, India*

[2]*Research assistant, Immunohematology lab, Cancer biology Department, Gujarat Cancer and Research Institute, Ahmedabad, India*

[3]*Associate professor, Cancer biology Department, Gujarat Cancer and Research Institute, Ahmedabad, India*

***Corresponding Author:** Hemangini H vora, Cancer biology Department, The Gujarat Cancer and Research Institute, Ahmedabad, Gujarat, India.

Received: June 22, 2017; **Published:** July 13, 2017

Abstract

Aim: To study inflammation associated changes in circulation as well as at tumor site of OSCC.

Method: A total of 87 patients of OSCC were enrolled in this study. WBC differential count data were collected from available hemogram reports of patients and compared with 25 age-matched Healthy Controls. Immunohistochemically localization of TGF-β, NFkB, and CD3 were evaluated on FFPE tissues on Ventana Benchmark XT automated immunostainer. These markers were correlated with clinicopathological parameter and disease-free survival.

Results: In comparison to Healthy Controls, OSCC patients had significantly increased percentage of Neutrophils ($p = 0.012$) and decreased percentage of Lymphocytes ($p = 0.001$) along with elevated Neutrophil to Lymphocyte Ratio ($p = 0.001$). In relation to clinicopathological variables, increased Neutrophils and NLR along with decreased Lymphocytes was significantly associated with large tumor size. However, with respect to DFS no significant association was found. Further, tumor infiltrating CD3+ T cells was observed in tumor core, tumor stroma and tumor margin, no significant association was found between CD3+ T-cells and clinicopathological variables. With respect to DFS a trend of increased incidence of disease relapse was observed in patients having low infiltration of CD3+ T-cells as compared to the patients with high infiltration of CD3+ T-cells. In tumor tissue, nuclear expression of NFκB was observed in 86% of patients. In correlation to clinicopathological variables, a significant high expression of NFκB was observed in patients with large tumor size ($p = 0.006$) and advanced disease stage ($p = 0.003$). Further a trend of increased expression of NFκB was observed in well differentiated tumor cells.

With respect to DFS, a significant increase incidence of relapse with reduced DFS was observed in patients with positive expression of NFκB ($p = 0.017$). Further, 96% of patients showed TGFβ expression in squamous epithelium. In relation to clinicopathological parameter significantly increased expression of TGFβ was observed with moderately differentiated tumor cells ($p = 0.001$). With respect to DFS a trend of increase incidence of relapse with reduced DFS was observed in patients with positive expression of TGF β as compared to patients with negative TGF β expression.

Conclusion: The present study observed Lymphopenia and Neutrophilia with high NLR in OSCC patients and its association with disease aggressiveness. Peripheral Neutrophilia create proinflammatory environment which may promote TGFβ and NFκB expression in tumor microenvironment. Moreover, High expression of TGF β and NFκB as well as reduced infiltration of CD3+ T cells associated with poor disease-free survival in OSCC patients.

Keywords: Inflammation; Tumor Infiltrating T Cells

Abbreviations

DFS: Disease Free Survival; NFκB: Nuclear Factor Kappa B; NLR: Neutrophil to Lymphocyte Ratio; OSCC: Oral Squamous Cell Carcinoma; TGFβ: Tumor Growth Factor beta

Introduction

Oral cancer is highly prevalent among men, annually over 3,00,000 new cases of oral cancer are diagnosed all over the world. Asian countries including India has higher incidence than Western countries, which is attributed to the habit of chewing tobacco and betel quid. At Gujarat Cancer and Research Institute, a regional cancer centre, oral cancer constitutes approximately 18% of total cancers in male and the major histologic subtype is Oral Squamous Cell Carcinoma (OSCC). About 50% oral cancer patients reveal lymph metastasis at the time of diagnosis and they had poor disease specific survival. For clinically N0 oral cancer clinician use Wait-and -See policy for further treatment because they were considered as early stage patients, however some of these patients has disease specific survival less than 1 year, therefore study of clinically N0 oral cancer patients is required for risk identification.

Chronic inflammatory response plays an important role in the development and progression of OSCC. Hanahan and Weinberg [1] proposed that the tumor microenvironment is infiltrated by innate and adaptive immune system cells specially T lymphocytes that enable tumors to mimic inflammatory conditions seen in normal tissues. Moreover, early inflammation releases proinflammatory cytokines and growth factors like TGFβ, EGF, PDGF, HGF, oncostatin M and VEGF [2] into tumor microenvironment, release of such cytokines is mainly mediated through the activation of transcription factor NFκB, which in turn leads to the induction of genes involved in proliferation, survival, angiogenesis and metastasis. Also, increased release of pro inflammatory cytokines induces a systematic inflammatory response in circulation. There are several lines of evidence to date suggesting that the total white blood cell count as well as its components, such as neutrophils, lymphocytes, and the neutrophil-to-lymphocyte ratio (NLR) can predict survival in a variety of malignancies, including lung, breast, esophageal, gastric, colorectal, hepatic and pancreatic cancers [3-10].

Giving this background, in this study an attempt was made to understand the inflammation associated changes in circulation as well as at tumor site of node negative OSCC.

Materials and Methods

Patients: In this retrospective study, total of 87 patients of node negative with Oral Squamous Cell Carcinoma (OSCC) diagnosed in the year of 2013 - 2014 who were undergone for surgery followed by radiotherapy and/ or chemotherapy were included whereas the patients with Lymph node involvement were excluded. Preoperative WBC differential count at diagnosis and clinicopathological data such as age, gender, habit, tumor site, tumor size, disease stage and histological grade were recorded from available hospital records. Formalin Fixed Paraffin embedded tissue blocks of 87 OSCC patients were collected for immunohistochemistry analysis. Patients provided the informed consent to use their sample for the study. This study was approved by the Institutional Scientific Review Board and Ethics Committee.

Immunohistochemical Localization:

Immunohistochemical localization of CD3 + T-cell, NFκB and TGF β were evaluated on formalin fixed paraffin embedded tissue blocks containing primary tumor evaluated by Hematoxylin and Eosin (H & E) staining, on Ventana Benchmark XT autoimmunostainer using Ventana reagents (Ventana, USA). The commercially available antibodies used were anti-CD3 antibody (Clone F7.2.38, Dako), anti- NFκB (Clone P105/p50, abcam), anti- TGFβ antibody (TB21, Gene Tex). The tissue blocks were obtained from archives of department of Pathology of institute. 3-4 µm thin sections were cut on microtome (Leica, Germany) and taken on to 3-Aminopropyltriethoxysilane (APES) coated slides. Briefly, the protocol includes following steps of deparafinization using EZ solution, antigen retrival for 60 minutes using retrival solution CC1, and incubation with ultra-view DAB Inhibitor for 4 minutes, 100 µl of anti-CD3 antibody at 370C for 32 minutes, anti- NFκB and anti- TGFβ antibody at 370C for 48 minutes respectively, ultra-view HRP Multimer for 8 minutes, counterstained with hematoxylin for 8 minutes and mounted with DPX.

Scoring

For NFκB and TGF β, the immunoreactivity scored as negative for (0, no immunoreactivity), 1+ (< 10% cells stained), equivocal 2+ (10 - 40% cells stained) and 3+ (≥ 40% cells stained).

For tumor infiltrating CD3+ T-cells, number of infiltrated cells in three different area of tumor core (CT) and tumor margin (TM) was calculated and the mean value was used. Based on the percentile rank patients were divided in to three group Low, Intermediate and High infiltration.

Statistical Analysis:

Statistical analysis was performed by using SPSS software version 20. Comparison of Neutrophil, Lymphocyte, Neutrophil to Lymphocyte Ratio (NLR) and Monocyte to Lymphocyte Ratio (MLR) was done by using Student's t-Test. For clinicopathological correlation regression and chi-square analysis was done and for Disease Free Survival analysis, univariate was done by using Kaplan Meier curve. Values were considered as statistically significant if it was ≤ 0.05.

Results and Discussion

Comparison of circulating WBC count between OSCC patients and Healthy Controls:

In comparison to Healthy Controls, OSCC patients had significantly increased mean ± SD value of Neutrophils (58.8 ± 6.6 v/s 63.50 ± 8.3, p = 0.012), Absolute Neutrophil Count (4 ± 1.2 v/s 5.3 ± 1.7, p = 0.001), Neutrophil to Lymphocyte Ratio (2.06 ± 0.69 v/s 2.8 ± 1.2, p = 0.004) and Monocyte to Lymphocyte Ratio (0.17 ± 0.09 v/s 0.31 ± 0.13, p = 0.001) along with decreased mean ± SD value of Lymphocytes (29.9 ± 6.4 v/s 24.3± 6.04, p = 0.001) and Absolute Lymphocyte Count (1.9 ± 0.51 v/s 2.0 ± 0.6) (Table 1).

		Mean ± SD	p value
Neutrophils	HC	58.8 ± 6.6	0.012
	OSCC	63.50 ± 8.3	
Lymphcytes	HC	29.9 ± 6.4	0.001
	OSCC	24.9 ± 6.1	
NLR	HC	2.06 ± 0.69	0.004
	OSCC	2.8 ± 1.2	
MLR	HC	0.17 ± 0.09	0.001
	OSCC	1.31 0.13	

Table 1: Comparison of Circulating WBC Count between OSCC Patients and Healthy Controls.

Correlation of circulating WBC with clinicopathological variables:

The patients were grouped into low count and high count using Median value as cut off and correlated with clinicopathological variables such as age, gender, habit, tumor site, tumor size, stage and grade.

When Neutrophils were correlated with clinicopathological variables a significant high incidence of increased percentage of neutrophils were observed in patients with T3 tumor size (04/05, 80%, p=0.003) as compared to the patients with T2 (20/27, 74%), T4 (12/23, 52%) and T1 (09/32, 28%) tumor size; Stage III patients (03/04, 75%, p = 0.02) as compared to stage II (20/29, 69%), stage IV (12/23, 52%) and stage I (10/31, 32%) patients. Further a trend of increased percentage of neutrophil was observed in a patient with Grade I (17/26, 65%) as compared to the patients with Grade II (26/56, 46%) and Grade III tumors (02/05, 40%) tumors. However, no such correlation was observed with other clinicopathological variables like age, gender, habit and tumor site (Table 2).

Characteristics		N	Neutrophils Median - 64.6		Lymphocytes Median - 24.4		NLR Median - 2.6	
			Low N (%)	High N (%)	Low N (%)	High N (%)	Low N (%)	High N (%)
Age	< 45	43	24 (56)	19 (44)	17 (40)	26 (60)	24 (56)	19 (44)
	≥ 45	44	18 (41)	26 (59)	25 (57)	19 (43)	16 (36)	28 (64)
Gender	Male	65	31 (48)	34 (52)	31 (48)	34 (52)	29 (45)	36 (55)
	Female	22	11 (50)	11 (50)	11 (50)	11 (50)	11 (50)	11 (50)
Habit	With Habit	75	36 (48)	39 (52)	35 (47)	40 (53)	35 (47)	40 (53)
	Without Habit	12	06 (50)	06 (50)	07 (58)	05 (42)	05 (42)	07 (58)
Tumor site	Tongue	37	18 (49)	19 (51)	20 (54)	17 (46)	17 (46)	20 (54)
	Buccal Mucosa	50	24 (48)	26 (52)	22 (44)	28 (56)	23 (46)	27 (54)
Tumor Size	T1	32	23 (72)	09(28)*	08(25)***	24 (75)	22 (69)	10(31)****
	T2	27	07 (26)	20(74)*	18(67)***	09 (33)	06 (22)	21(78)****
	T3	05	01 (20)	04(80)*	03(60)***	02 (40)	01 (20)	04(80)****
	T4	23	11 (48)	12(52)*	13(57)***	10(44)	11 (48)	12(52)****
Stage	Stage I	31	21 (68)	10(32)**	10 (32)	21 (68)	19 (61)	13 (39)
	Stage II	29	09 (31)	20(69)**	17 (59)	12 (41)	09 (31)	20 (69)
	Stage III	04	01 (25)	03(75)**	02 (50)	02 (50)	01 (25)	03 (75)
	Stage IV	23	11 (48)	12(52)**	13 (57)	10 (43)	11 (48)	12 (52)
Histological Grade	G1	26	09 (35)	17 (65)	13 (50)	13 (50)	09 (35)	17 (65)
	G2	56	30 (54)	26 (46)	27 (48)	29 (52)	28 (50)	28 (50)
	G3	05	03 (60)	02 (40)	02 (40)	03 (60)	03 (60)	02 (40)

Table 2: Correlation of Circulating WBC with Clinicopathological Variables.

*= X 2 =14.1, r = 0.18, p = 0.003
** = X 2 = 9.03, r = 0.13, p = 0.02
***= X 2 =11.5, r = - 0.23, p = 0.01
**** = X 2 = 14.2, r = 0.14, p = 0.003

When Lymphocytes were correlated with clinicopathological variables a significant high incidence of decreased percentage of lymphocytes were observed in a patient with T2 tumor size (18/27, 67%, p = 0.01) as compared to patients with T3 (03/05, 60%), T4 (13/23, 57%) and T1 tumor size (08/32, 25%). However, no such correlation was observed with other clinicopathological variables like age, gender, habit, tumor site and grade (Table 2).

Further, Neutrophil to Lymphocyte ratio (NLR) was correlated with clinicopathological variables where, a significant high incidence of increased percentage of NLR was observed in a patient with T3 tumor size (04/05, 80%, p = 0.003) as compared to patients with T2 (21/27, 78%), T4 (12/23, 52%) and T1 tumor size (10/32, 31%). A trend of increased percentage of NLR was observed in patients with ≥ 45 years of age (28/44, 64%) as compared to patients with < 45 years of age (19/43, 44%); Grade I tumors (17/26, 65%) as compared to Grade II (28/56, 50%) and Grade III tumors (02/05, 40%). However, no such correlation was observed with other clinicopathological variables like gender, habit, tumor site and stage (Table 2).

In univariate analysis, with respect to Neutrophils, Lymphocytes and NLR no significant association was found with disease free survival (DFS) (Table 4).

Correlation of tumor infiltrating CD3 + T-cells with clinicopathological variable:

In correlation with clinicopathological variables like age, gender, habit, tumor site, tumor size, stage and grade no significant association was found with any parameter. (Table 3). However,

with respect to DFS, a trend of increased incidence of disease relapse was observed in patients having low infiltration of CD3+ T-cells (12/38, 32%) as compared to the patients with intermediate (5/25, 20%) and high infiltration of CD3+ T-cells (3/24, 12%). Moreover, patients with having low CD3+ and intermediate T cells infiltration had recurrence within first two years of treatment (p = 0.012) (Table 4, Figure 1.1 - 1.3, Graph 1).

Characteristics		N	Infiltration of CD3+ T-cells			TGFβ Expression		Nuclear NFκB Expression	
			Low N (%)	Inter-mediate N (%)	High N (%)	Positive N (%)	Negative N (%)	Negative N (%)	Positive N (%)
Age	<45	43	18 (42)	12 (28)	13 (30)	03 (07)	40 (93)	07 (16)	36 (84)
	≥45	44	20 (46)	13 (29)	11 (25)	01 (02)	43 (98)	05 (11)	39 (89)
Gender	Male	65	30 (46)	17 (26)	18 (28)	03 (5)	62 (95)	08 (12)	57 (88)
	Female	22	08 (36)	08 (36)	06 (28)	01 (5)	21 (95)	04 (18)	18 (82)
Habit	With Habit	75	31 (42)	22 (29)	22 (29)	04 (05)	71 (95)	12 (16)	63 (84)
	Without Habit	12	07 (58)	03 (25)	02 (17)	00 (00)	12 (100)	00 (00)	12(100)
Tumor site	Tongue	37	16 (43)	08 (22)	13 (35)	01 (03)	36 (97)	07 (19)	30 (81)
	Buccal Mucosa	50	22 (44)	17 (34)	11 (22)	03 (06)	47 (94)	05 (10)	45 (90)
Tumor Size	T1	32	14 (44)	06 (19)	12 (37)	02 (06)	30 (94)	01 (3)	31 (97)**
	T2	27	14 (52)	07 (26)	06 (22)	02 (07)	25 (93)	03 (11)	24 (89)**
	T3	05	03 (60)	01 (20)	01 (20)	00 (00)	05 (100)	00 (00)	05(100)**
	T4	23	07 (30)	11 (48)	05 (22)	00 (00)	23 (100)	08 (35)	15(65)**
Stage	Stage I	31	16 (52)	06 (19)	09 (29)	02 (07)	29 (93)	00 (00)	31(100)
	Stage II	29	12 (41)	08 (28)	09 (31)	02 (07)	27 (93)	04 (14)	25 (86)***
	Stage III	04	03 (75)	00 (00)	01 (25)	00 (00)	04 (100)	00 (00)	04(100)***
	Stage IV	23	07 (30)	11 (48)	05 (22)	00 (00)	23 (100)	08 (35)	15(65)***
Histological Grade	G1	26	12 (46)	05 (19)	09 (35)	01 (04)	25 (96)*	03 (12)	23 (88)
	G2	56	23 (41)	19 (34)	14 (25)	01 (02)	55 (98)*	08 (14)	48 (87)
	G3	05	03 (60)	01 (20)	01 (20)	02 (40)	03 (60)*	01 (20)	04 (80)

Table 3: Correlation of CD3+ T-Cells, Tgfβ and Nfκb with Clinicopathological Variable.

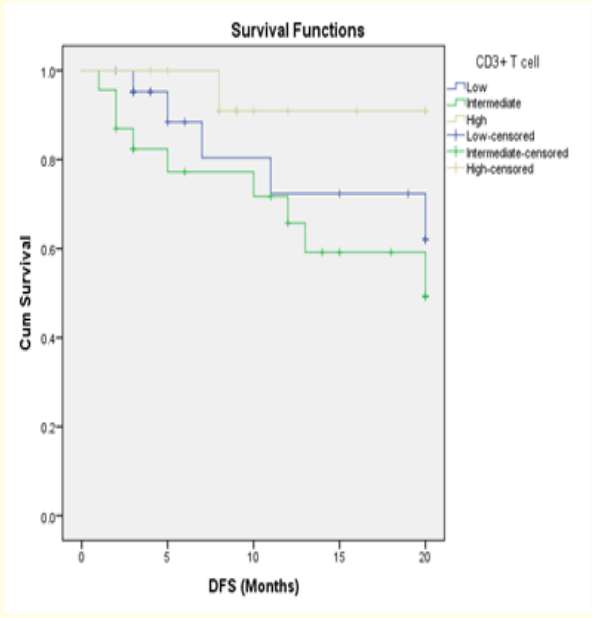
* = X 2 = 15.33, r = -0.198, p = 0.001

**= X 2 =12.6, r = - 0.34, p = 0.006

***= X 2 =14.1, r = - 0.37, p = 0.003

Neutrophils	N	Relapsed N (%)	Remission N (%)	Log rank	df	p
Low	42	13 (31)	29 (69)			
High	45	07 (16)	38 (84)	0.37	1	0.54
Lymphoytes						
Low	42	09 (21)	33 (79)			
High	45	11 (24)	34 (76)	0.41	1	0.84
NLR						
Low	40	11 (27)	29 (73)			
High	47	09 (19)	38 (81)	0.42	1	0.51
MLR						
Low	41	13 (31)	28 (69)			
High	46	07 (15)	39 (85)	2.15	1	0.14
CD3+ T-cells						
Low	38	12 (32)	26 (68)			
Intermedate	25	05 (20)	20 (80)			
High	24	03 (12)	21 (88)	3.33	2	0.18
TGFβ						
Negative	03	01 (33)	02 (67)			
Positive	84	19 (23)	65 (77)	0.87	1	0.35
NFκB						
Negative	12	05 (42)	07 (58)			
Positive	75	15 (20)	60 (80)	5.7	1	0.02*

Table 4: Univariate Analysis for Disease Free Survival.



Graph 1: Kaplan-Meier plot of CD3+ T cells for Disease Free Survival (DFS).

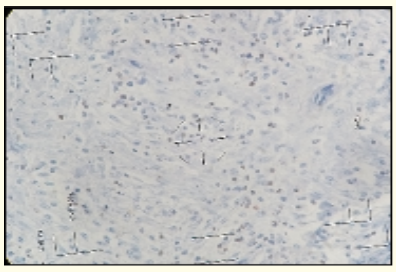
Correlation of NFκB expression with clinicopathological variable:

In tumor tissue, nuclear expression of NFκB was observed in 86% (75/87) of patients while 14% (12/87) of patients were negative for NFκB expression. In correlation to clinicopathological variables, a significant high expression of NFκB was observed in patients with T3 tumor size (05/05, 100%, p = 0.006) as compared to the patients with T1 (31/32, 91%), T2 (24/27, 89%) and T4 (15/23, 65%) tumor size; stage III disease (04/04, 100%, p = 0.003) as compared to the patients with stage I (31/31,100%), stage II (25/29, 86%) and stage IV disease (15/23, 65%). Further a trend of increased expression of NFκB was observed in grade I tumors (23/26, 88%) as compared to grade II (48/56, 87%) and grade III (04/05, 80%) tumors (Table 3). With respect to DFS, a significant increase incidence of relapse with reduced DFS was ob-

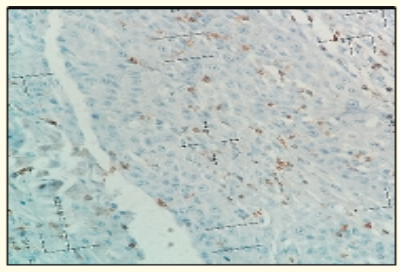
served in patients with positive expression of NFκB (15/75, 20%, p=0.017) as compared to patients with negative expression of NFκB (05/12, 42%) (Table 4, Figure 1.4).

Correlation of TGF β expression with clinicopathological variable:

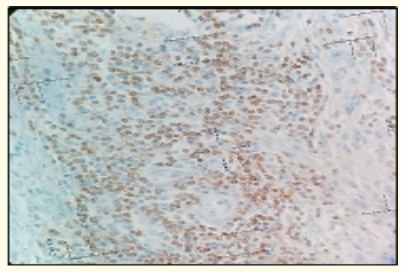
In tumor tissue, expression of TGFβ was observed in 96% (84/87) of patients while 04% (03/87) of patients were negative for TGFβ expression. In correlation to clinicopathological variables, a significant high expression of TGFβ was observed in patients with grade II tumors (55/56, 98%, p = 0.001) as compared to the patients with grade I (25/26, 96%) and grade III (03/05, 60%) tumors. However, no such correlation was observed with other Clinicopathological variables like age, gender, habit, tumor site, tumor size and stage (Table 3). With respect to DFS, a trend of increase incidence of relapse with reduced DFS was observed in patients with positive expression of TGF β (23%, 19/84) as compared to patients with negative TGF β (33%, 01/03) expression (Table 4, Figure 1.5).



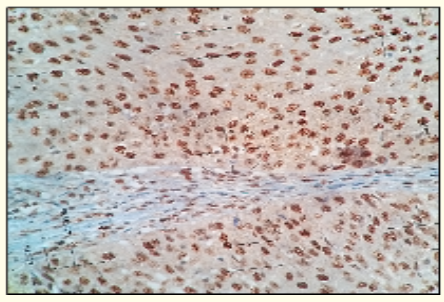
1.1: Negative Staining



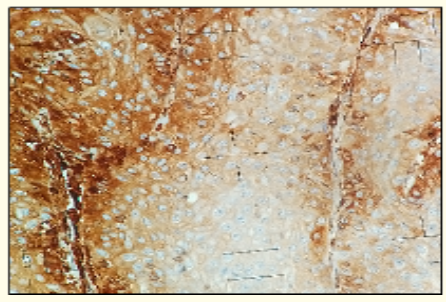
1.2: Stromal Infiltration of CD3+ T-cells



1.3: Tumor Core and Margin along with Stromal Infiltration of CD3+ T-Cells



1.4: Nuclear Staining of NFκB



1.5: Positive Staining for TGF β in Tumor Tissue

Figure 1: Immunohistochemical Localization of CD3+ T-cells, NFκB and TGFβ.

Discussion

Inflammation, in the form of local and systemic inflammatory responses, is a key factor for oral carcinogenesis. Evidences suggest that the systemic inflammatory response is associated with alterations in circulating white blood cells, specifically the presence of Neutrophilia with a relative Lymphopenia.

Among various characteristics of the systemic inflammatory response, Neutrophil to Lymphocyte (NLR) is an easily measured, reproducible, and inexpensive marker of subclinical inflammation. The prognostic role of NLR has been documented in multiple cancers, disease settings and treatments including malignancies of the colon [11], ovaries [12], urothelium [13] and pancreas [14]. To date, over 60 studies have examined the clinical utility of the NLR to predict patient's outcome in a variety of cancers [15]. In present study, Neutrophils and Lymphocytes counts and its ratio along with monocyte to lymphocyte ratio in peripheral blood of OSCC patients were evaluated and compared with healthy controls. We observed significantly increased Neutrophils and decreased Lymphocytes count with high NLR in OSCC patients as compared to healthy controls suggesting systemic inflammatory response in OSCC patients.

High NLR may reflect increased inflammation and/or decreased immune reaction in these patients.

In relation to clinicopathological variables, it was reported that increased Neutrophils, high NLR and decreased Lymphocytes were significantly associated with large tumor size and older age. Similar to our findings, other study group had also observed association of increased NLR with advancing age, larger tumours and stage of disease in Head and Neck Carcinoma patients [16] and in Breast cancer patients [15].

Increased Neutrophil in advance stage tumor may associate with higher proportions of immature cells and altered functional status which ultimately promotes tumor growth. However, with respect to DFS, no significant association was found. Similar to our study, Christos., *et al*. [17]. found, neither the baseline neutrophil count nor the lymphocyte count had a statistically significant impact on Disease Specific Survival. However, SalehRachidi., *et al.* [18] found that patients in highest tertile of neutrophil counts and those in the lowest textile of lymphocytes experienced shorter survival than rest of the population.

It has been suggested that in tumor microenvironment tumors produce chemotactic factors that actively recruit mononuclear cells mainly lymphocytes and macrophages to tumor sites [2,19] this immune cell is not distributed randomly, but seems to be organized in more or less dense infiltrates in the centre of the tumoral zone (CT), at invasive margine (IM) of tumoral nests and in lymphoid islets adjacent to the tumor. Whether this recruitment is orchestrated by the tumor or is generated by surrounding tissue cells in response to the tumor is currently an unanswered question. In our study, 43% of patients having low, 29% of patients had intermediate and 28% patients had high CD3+ T-cells infiltration. In relation to clinicopathological variable, no significant association was found. With respect to DFS, patients with low infiltration of CD3+ T- cells had poorer clinical outcome as compared to intermediate and high infiltration. These results suggest that T cell mediated adaptive immunity play important role in anti tumor immunity and prevents tumor recurrence. Similar to our findings majority of the study shows the association of T cell infiltration with favourable prognosis in variety of cancer like gastric, breast and Head and neck carcinoma. Moreover, it can be used as predictive marker for recurrence. However, subtyping of these tumors infiltrating T cells is important.

Chronic inflammation also associated with NFκB activation which leads to secretion of proinflammatory cytokines which in turn leads to the induction of genes involved in proliferation, survival, angiogenesis and metastasis [20]. In present study, a significant high expression of NFκB was observed in patients with larger tumor size and advanced disease stage. Numerous cytokines such

as IL-1, TNF and IL-6 are regulated by NF-kB have shown to be growth factors for tumor cells [21]. With respect DFS, a trend of increased incidence of relapse with reduced DFS was observed in patients with NFκB expression.

Inflammatory condition also promotes growth factor stimulation. TGFβ (Tumor Growth Factor beta) is a one of the most studied growth factor which involve in transition from antitumor to protumor phenotype of Neutrophils [22]. The evidence supporting TGF-β as a potent regulator of the tumor microenvironment comes from studies with specific deletion of TGFBR2 in a variety of epithelial cells, including mammary, pancreatic, intestinal, colon, and head-and-neck squamous cell carcinoma [2] in which deletion of TGFBR2 in these epithelial cells results in increased tumor progression and metastasis. However, over-expression of TGFβ1 in head and neck epithelia results in inflammation, angiogenesis and epithelial hyperproliferation [23]. In our study, significantly higher cytoplasmic expression of TGF β in moderately differentiated tumors was reported. With respect to DFS, a trend of increased incidence of relapse with reduced DFS was observed in patients with TGF β expression [24].

Conclusion

The present study observed Lymphopenia and Neutrophilia with high NLR in circulation of OSCC patients. Within tumor micro environment, low CD3+ T cell infiltration, high expression of NFκB and TGF β associated with poor disease-free survival in OSCC patients. Thus, inflammatory molecules are used to identify high risk node negative oral cancer patients. In our future study, we analyse T cells phenotype on larger cohort of patients to establish immune score system, which may be incorporated with conventional risk predicting staging system.

Acknowledgements

I would like to express my sincere gratitude to my mentor Dr. Hemangini H Vora for her continuous support and guidance as well as for her patience; motivation and enthusiasm that made the whole study period a wonderful learning experience. I am deeply thankful to Dr. Birva N Raiya, Research Assistant, Immunohematology Division for assisting me in my work throughout the study period. I am deeply indebted to God and my parents Mr. Amiruddin G Shaikh and Mrs. Kherunnisha A Shaikh.

Conflict of Interest

There is no conflict of interest.

Bibliography

1. Cavallo Federica., *et al.* "2011: the immune hallmarks of cancer". *Cancer Immunology, Immunotherapy* 60.3 (2011): 319-326.

2. Fondevila C., *et al.* "p53 and VEGF expression are independent predictors of tumour recurrence and survival following curative resection of gastric cancer". *British Journal of Cancer* 90.1 (2004): 206-215.

3. Sharaiha Reem Z. "Elevated preoperative neutrophil: lymphocyte ratio as a predictor of postoperative disease recurrence in esophageal cancer". *Annals of surgical oncology* 18.12 (2011): 3362-3369.

4. Chua W., *et al.* "Neutrophil/lymphocyte ratio predicts chemotherapy outcomes in patients with advanced colorectal cancer". *British Journal of Cancer* 104.8 (2011): 1288-1295.

5. Teramukai Satoshi., *et al.* "Pretreatment neutrophil count as an independent prognostic factor in advanced non-small-cell lung cancer: an analysis of Japan Multinational Trial Organisation LC00-03". *European Journal of Cancer* 45.11 (2009): 1950-1958.

6. Halazun Karim J., *et al.* "Negative impact of neutrophil-lymphocyte ratio on outcome after liver transplantation for hepatocellular carcinoma". *Annals of Surgery* 250.1 (2009): 141-151.

7. Bhatti Imran., *et al.* "Preoperative hematologic markers as independent predictors of prognosis in resected pancreatic ductal adenocarcinoma: neutrophil-lymphocyte versus platelet-lymphocyte ratio". *The American Journal of Surgery* 200.2 (2010): 197-203.

8. Ding Pei-Rong., *et al.* "Elevated preoperative neutrophil to lymphocyte ratio predicts risk of recurrence following curative resection for stage IIA colon cancer". International journal of colorectal disease 25.12 (2010): 1427-1433.

9. Ray-Coquard Isabelle., *et al.* "Lymphopenia as a prognostic factor for overall survival in advanced carcinomas, sarcomas, and lymphomas". *Cancer Research* 69.13 (2009): 5383-5391.

10. Wang De-shen., *et al.* "Comparison of the prognostic values of various inflammation based factors in patients with pancreatic cancer". *Medical Oncology* 29.5 (2012): 3092-3100.

11. Mallappa S., *et al.* "Preoperative neutrophil to lymphocyte ratio> 5 is a prognostic factor for recurrent colorectal cancer". *Colorectal Disease* 15.3 (2013): 323-328.

12. Cho HanByoul., *et al.* "Pre-treatment neutrophil to lymphocyte ratio is elevated in epithelial ovarian cancer and predicts survival after treatment". *Cancer Immunology, Immunotherapy* 58.1 (2009): 15-23.

13. Gondo Tatsuo., *et al.* "Prognostic value of neutrophil-to-lymphocyte ratio and establishment of novel preoperative risk stratification model in bladder cancer patients treated with radical cystectomy". *Urology* 79.5 (2012): 1085-1091.

14. An Xin., *et al.* "Elevated neutrophil to lymphocyte ratio predicts survival in advanced pancreatic cancer". *Biomarkers* 15.6 (2010): 516-522.

15. Guthrie Graeme JK., *et al.* "The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer". *Critical Reviews in Oncology/Hematology* 88.1 (2013): 218-230.

16. Trellakis Sokratis., *et al.* "Polymorphonuclear granulocytes in human head and neck cancer: enhanced inflammatory activity, modulation by cancer cells and expansion in advanced disease". *International Journal of Cancer* 129.9 (2011): 2183-2193.

17. Christos

18. SalehRachidi, BS *et al.* "Neutrophil-to-lymphocyte ratio and overall survival in all sites of head and neck squamous cell carcinoma". *Head and Neck* 38 (2016): E1068-E1074.

19. Balkwill Fran. "Cancer and the chemokine network". Nature Reviews Cancer 4.7 (2004): 540-550.

20. Granot Zvi and Jadwiga Jablonska. "Distinct functions of neutrophil in cancer and its regulation". *Mediators of Inflammation* (2015).

21. Pahl Heike L. "Activators and target genes of Rel/NF-κB transcription factors". *Oncogene* 18.49 (1999): 6853-6866.

22. Lu Shi-Long., *et al.* "Overexpression of transforming growth factor β1 in head and neck epithelia results in inflammation, angiogenesis, and epithelial hyperproliferation". *Cancer Research* 64.13 (2004): 4405-4410.

23. Cassatella Marco A. "The production of cytokines by polymorphonuclear neutrophils". *Immunology Today* 16.1 (1995): 21-26.

24. Balkwill Frances R and Alberto Mantovani. "Cancer-related inflammation: common themes and therapeutic opportunities". *Seminars in Cancer Biology* 22.1 (2012).

Volume 1 Issue 2 July 2017