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Neutrophil-Lymphocyte Ratio: Adjunct Prognostic Biomarker in Oral Squamous Cell Carcinoma (OSCC) – A Study in Delhi NCR

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ABSTRACT

Background: Inflammation is now recognized as the seventh hallmark of cancer. Neutrophil-lymphocyte ratio holds promise as an adjunct biomarker in various cancers.
Materials and methods: 32 biopsy-confirmed OSCC patients and 32 healthy controls were enrolled in this prospective case-control study and venous blood samples were obtained and CBC reports were thus used for analysis of various haematological parameters such as NLR, using t-test, and Mann-Whitney U test using STATA.
Results: The median NLR was found to be raised in OSCC patients as compared to healthy controls and the difference was found to be statistically significant ((Median:3.83(2.31-5.56) vs. 2.14(1.80-2.66), p=0.0006)
Conclusions : NLR can be used as a useful tool for clinicians as an adjunct biomarker for the prognostication and potentially help in the diagnosis of OSCC in screening. Future studies should include a larger sample size and should be longitudinal in nature as well as should explore differences in NLR in benign, premalignant and malignant cases.

Key words: Neutrophil-lymphocyte ratio, biomarker, oral squamous cell carcinoma, cancer, hematological

INTRODUCTION

India has a high oral cancer disease burden, with estimated 130,000 cases diagnosed in 2020 alone. The prevalent practice of betel quid chewing, is a major driving force behind this.[1,2]

Inflammation is now described as "the seventh hallmark" of cancer and plays a crucial role in all phases of tumour development.[3] There is evidence that inflammation contributes paradoxically to tumour growth and metastasis. It has been observed that the presence of large amounts of neutrophils and macrophages in the tumour microenvironment is associated with worse outcomes.[4]

Peripheral neutrophilia may facilitate tumour growth and progression by various

proposed mechanisms such as releasing tumour growth promoting factors, prostaglandin E2 and proteases, destroying the extracellular matrix and regulating macrophage activity.[5]

Neutrophilia affects regulatory T-cells and inhibits activity of lymphocytes and natural killer cells. While lymphopenia on its own can hamper cell-mediated immunity and suppression of cancer cells.[6]

Neutrophil to lymphocyte ratio (NLR), is calculated simply as the ratio of neutrophil to lymphocyte count. It is a dependable, cheap and cost-effective marker of inflammation and can be evaluated from routine blood tests i.e. Complete blood count (CBC), as compared to molecular biomarkers which require invasive tissue specimens.[7] NLR is helpful in assessing the dynamic state of systemic inflammation and stress.[8] Neutrophil-to-lymphocyte ratio can be used as a prognosticator of survival, recurrence, lymph node status, and margin status in OSCC. Many meta-analyses have examined and helped establish the prognostic value of NLR in various solid tumours and found that an NLR above 3.0 (IQR 2.5-5.0) is significant and associated with poorer outcomes in various cancers.[9] However, the role of NLR in the diagnosis of OSCC remains to be studied in depth.[10] There is also controversy regarding the cut-offs for NLR in healthy individuals versus oral cancer patients.

As an easily measurable yet under-utilized and consequential parameter, its role in OSCC should be further explored and established. NLR can serve as a useful adjunct prognostic tool in OSCC and help improve patient outcomes and guide physicians in tackling this devastating illness. Thereby, the present study was conducted to compare the NLR values in biopsy confirmed OSCC cases and healthy controls.

MATERIALS AND METHODS

This was a hospital-based, prospective case-control study. It was conducted in the Pathology Department, in collaboration with the department of otorhinolaryngology (E.N.T) of a tertiary-care hospital in Delhi-NCR, India. The study was conducted from December 2023 to February 2024, for a total duration of 2 months. The study population comprised histo-pathologically confirmed cases of OSCC (oral squamous cell carcinoma) and an equal number of age and gender matched healthy controls visiting a tertiary-care hospital Delhi-NCR. Under our inclusion criteria, all consenting biopsy-proven patients of oral cavity cancer were included in the study, while those suffering from acute and chronic inflammatory or other immune-modulating conditions, auto-immune disorders, any haematological disorder (except anemia), chronic renal insufficiency, and corticosteroid or prolonged antibiotic therapy as well as those unable to consent were excluded. A total of 32 cases and 32 controls were enrolled in the study, due to logistical limitations and time-sensitive nature of the study. Data was collected from the histopathology reports and CBC reports of the cases as well as the controls and entered in a Microsoft Excel spreadsheet and simultaneously checked for any inadvertent errors. Venous blood samples of the patients were obtained by following aseptic conditions and precautions from the median cubital vein in potassium EDTA vacutainers for the estimation of various hematological indices. Samples were tested within 1 hour of collection to minimize variations. Complete blood count estimation was done using a 5 part hematology analyzer (Model:Sysmex XN 1000). NLR was calculated simply as the ratio of neutrophil to lymphocyte count, as discussed above. Results were doubly checked, so as to minimise errors.

Quantitative data (micro-factors) were cleaned and analysed using STATA version 17.0. Two group t-test, Shapiro-francia test and Mann Whitney U test were used for the analysis of the various parameters. Ethical approval was taken from the Institutional ethics committee, before the commencement of the study. Written informed consent was taken from the

participants after apprising them about the study. Only participants who voluntarily gave consent were enrolled in the present study.

RESULTS

The present study was conducted in 32 patients of OSCC and an equal number of age and gender matched controls with the baseline characteristics of the cases depicted in Table 1. In the present study, there were predominantly male oral cancer cases (81.2%) as compared to females (18.8%). Most patients belonged to the age group of 41-50 years (37.5%), closely followed by 51-60 years (34.3%), >60 years (15.6%), 31-40 years (9.3%), with 21-30 years (3.1%) being the least common age of presentation.

Based on the site of the lesion, tongue (34.3%) was the most common site affected, followed by buccal mucosa (28.1%), oral cavity and oropharynx (15.6%), soft palate (9.3%), tonsillar fossa (9.3%), while hard palate (3.1%) was the least common.

On classifying the cases according to Broder's classification of squamous cell carcinoma, most cases were of moderately-differentiated squamous cell carcinoma (75%), followed by well-differentiated (15.6%) and poorly-differentiated squamous cell carcinoma (9.3%).

To determine the normality of the distribution of various parameters, Shapiro-Francia test was performed for both cases and controls, with parameters having significant results ($p < 0.05$) being deemed as non-normal in distribution.

Table 1: Distribution of cases by clinical profile (N= 32)

Clinical Profile	Frequency (n)	Percentage (%)
GENDER		
Male	26	81.2
Female	6	18.8
AGE (years)		
21-30	1	3.1
31-40	3	9.3
41-50	12	37.5
51-60	11	34.3
>60	5	15.6
SITE		
Tongue (Base+Lateral border)	11	34.3
Buccal mucosa	9	28.1
Soft Palate	3	9.3
Hard Palate	1	3.1
Oral cavity	5	15.6
Tonsillar fossa	3	9.3
HISTOLOGY		
Poorly differentiated	3	9.3
Moderately differentiated	24	75
Well differentiated	5	15.6

Neutrophils (%), lymphocytes (%), monocytes (%), MCHC, PDW, P-LCR, absolute lymphocyte count were normally distributed and hence subjected to analysis by using independent t-test while other parameters were non-normal in distribution and thus analyzed by Mann-Whitney U test (Wilcoxon rank sum analysis).

For the various normally distributed parameters, two group t-test analysis was performed in which mean values of various hematological parameters were compared between cases and controls, with significant p values (<0.05) observed in neutrophils (%) (0.0001), lymphocytes (%) (0.0003), PDW (platelet distribution width) (0.0324), as summarised in Table 2. While other parameters such as MCHC, P-LCR, and absolute lymphocyte count did not have a statistically significant difference.

Table 2: Comparison of cases and controls by various normally-distributed hematological parameters using two group t- test (N= 64)

Parameter	CASES (n= 32) Mean value (S.D)	CONTROLS (n= 32) Mean value (S.D)	p VALUE
Neutrophils (%)	69.63 (10.37)	60.55 (6.16)	0.0001
Lymphocytes (%)	20.48 (17.18)	27.87 (5.83)	0.0003
Monocytes (%)	6.86 (2.56)	8.02 (1.69)	0.0388
MCHC	31.39 (1.36)	31.05 (1.19)	0.3076
PDW	15.39 (3.57)	17.80 (3.44)	0.0324
P-LCR	39.61 (11.32)	45.05 (8.48	0.0818
Absolute Lymphocyte count	1665.20 (723.69)	1971.62 (651.58)	0.08

Table 3: Comparison of cases and controls by various non-normally distributed hematological parameters using Mann-Whitney U test (Wilcoxon rank-sum analysis) (N= 64)

Parameter	CASES (n= 32) Median value (I.Q.R)	CONTROLS (n= 32) Median value (I.Q.R)	p VALUE
Hb	12.6 (11.8-13.6)	13.2(11.85-14.2)	0.3288
TLC	8145 (7010-9340)	7115(5625-8800)	0.0688
Eosinophils	2.35(1-3.6)	2.95(1.9-4.7)	0.2310
Basophils	0 (0-0.2)	0.4(0-0.6)	0.0106
Immature Granulocytes	0.4 (0.3-0.7)	0.4(0.2-0.4)	0.2127
Absolute Eosinophil count	0.135(0.08-0.32)	0.41(0.19-195.7)	0.0043
Platelet count	2.46(1.81-2.85)	1.855 (1.49-2.46)	0.0297
RBC count	4.49(3.81-5.08)	4.73(4.46-4.90)	0.2235
PCV	40.3 (36.2-44.3)	42.6 (39.65-45)	0.1685
MCV	92.25 (86.7-94.8)	91.35(88.85-93)	0.6040
MCH	29.2 (27.55-30.3)	28.25(27.65-29.4)	0.2694
RDW-CV	14(12.9-15.2)	14.4(14.1-15.55)	0.2252
NLR (Neutrophil-Lymphocyte Ratio)	3.83(2.31-5.56)	2.14(1.80-2.66)	0.0006
Absolute Neutrophil count (per microliter)	5236.48(4287-6863.16)	4110.39(3.34312-5436.93)	0.0092

On comparison of the various non-normally distributed haematological parameters in Table 3, between cases and controls, using Mann-Whitney U test, p-values were found to be significant (<0.05), for NLR (Neutrophil-to-lymphocyte ratio) ((0.0006), absolute neutrophil count (0.0092), platelet count (0.0297), basophils (%) (0.0106), and absolute eosinophil count (0.0043). Hb, TLC, eosinophils (%), immature granulocytes (%), RBC count, PCV, MCV, MCH did not have a statistically significant difference between oral cancer cases and controls. A box-whisker plot was drawn to compare median values of NLR between cases and controls (three cases had high values of NLR that were treated as outliers) as shown in Figure 1.

1. Figure 1: Box-whisker plot comparing NLR (cases vs. controls) [0= Controls, 1= Cases]
2. Comparison of mean values of various haematological parameters using two group t-test between cases and controls.

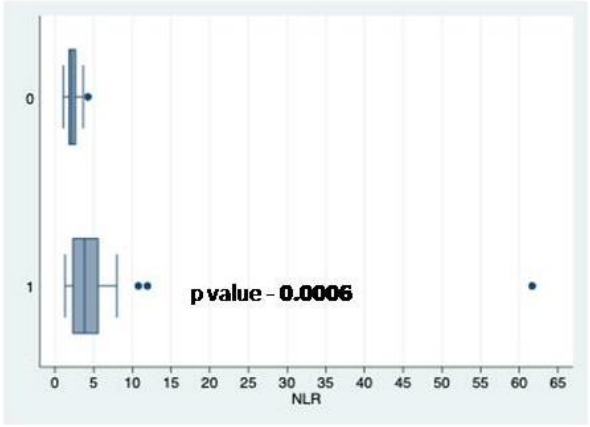

Figure 1

DISCUSSION

The present study was conducted to explore the role of NLR (Neutrophil-to-lymphocyte ratio in OSCC (oral squamous cell carcinoma), and for the comparison of NLR and various other haematological parameters obtainable from a routine CBC (complete blood count) report. There were 32 oral cancer patients and 32 healthy (age and gender matched) controls in the study. There were significantly more males than females (83.2% vs. 16.8%) suffering from oral cancer in this current study, similar to the studies conducted by Phulari et al.[11] as well as Khan SR et al.[12]This disparity can be explained by the simple explanation that the prevalence of various oral cancer risk factors like, smoking, betel quid chewing and alcohol consumption are all much higher in men than women.[13] Most patients belonged to the age group greater than 40 years, with 41-50 years (37.5%) being the most common. While tongue (34.3%) was the most common site of the lesion followed by buccal mucosa(28.1%), which was similar to that of previous studies, such as the study conducted by Tan et al.[14]

According to Broder's classification of SCC, in the present study population moderately differentiated SCC was the most common, followed by well differentiated SCC,and poorly differentiated SCC which was similar to the distribution in the study done by Rahaman et al.[15] However, this was different from the study done by Iype et al.[16] which suggested well differentiated SCC to be the most common, followed by MDSCC. These differences can be attributed to nominal differences in the study population.

A thorough comparison of NLR and various other haematological parameters was undertaken using appropriate statistical tests, between cases and controls, with statistical significance set at $p<0.05$.

In the current study, NLR was found to be raised among oral cancer cases than controls (Median:3.83(2.31-5.56) vs. 2.14(1.80-2.66), p=0.0006), which was similar to the results obtained by Phulari et al,[11] Düzlü, et al,[17] and Singh, et al.[10]It was also similar to the results obtained in LSCC (laryngeal squamous cell carcinoma) by Kum et al.[18] However it was different to the results obtained by Dutta et al, which showed no correlation between NLR and prognosis.[19]

Haematological parameters such as absolute neutrophil count, platelet count, neutrophils(%), lymphocytes(%), basophils (%), PDW and absolute eosinophil count also turned out to significantly different in OSCC patients and healthy individuals in this study population. Absolute neutrophil count was also significantly raised in cases as compared to controls as reported by Phulari S et al.[11] PDW was also found to be significantly different in a study by Hirahara et al. in patients with esophageal squamous cell carcinoma.[20]

While other parameters such as MCHC, P-LCR, absolute lymphocyte count, Hb, TLC, eosinophils (%), immature granulocytes (%), RBC count, PCV, MCV and MCH did not yield a statistically significant difference in this study. This was similar to Phulari S et al.[11] who also found no statistically significant difference in absolute lymphocyte count between oral cancer cases and controls.

NLR is a dynamic marker of inflammation that encapsulates both co-existing tumour-promoting and tumour-suppressing mechanisms, hence can be used as an indicator for prognosis. Its role in various solid tumours has been well established but there is a paucity of research in OSCC.

Future studies on NLR in OSCC should have a much larger sample size to help with better generalisability of the data, and can potentially include an additional study group consisting of patients with premalignant lesions, such as erythroplakia and leukoplakia to help identify cut-offs between healthy, premalignant and malignant cases. Longitudinal studies focusing on overall survival of OSCC cases can also be considered, as compared to the present study which was conducted for a short duration of time, to yield more insights into the prognostic value of NLR in OSCC.

CONCLUSION

It was found that NLR was significantly raised in OSCC cases, as compared to healthy controls along with other haematological parameters such as absolute neutrophil count, platelet count, neutrophils (%), lymphocytes (%), basophils (%), PDW and absolute eosinophil count which were also found to be statistically significant between OSCC patients and controls.

NLR can be used as a useful and cheap adjunct biomarker for OSCC, that can be obtained conveniently from CBC reports without the need of collecting any other blood or tissue samples.

Moreover, longitudinal and multi-centric studies with larger sample sizes are further needed to establish its prognostic value in OSCC, and establish cut-offs for its prognostic and potential diagnostic role as a screening tool.

Declaration by Authors

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