Original Article

Diagnostic Role of Neutrophil-Lymphocyte Ratio in Oral Cavity Cancers: A Prospective Study

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Abstract

Introduction: Squamous cell carcinoma (SCC) of oral cavity and oropharyngeal region is considered as an aggressive malignant neoplasm, because of high morbidity and mortality. It commonly occurs in middle-aged males and older individuals.

Aims and Objectives: The purpose of the study was to analyze the relationship between preoperative levels of neutrophil-lymphocyte ratio (NLR) with clinicopathologic factors and prognosis in oral cavity SCC patients.

Materials and Methods: A total of 810 subjects were studied from January 2019 to December 2020 and 270 each were in three different groups – malignant, premalignant, and control group. Inclusion criteria were to be available with common blood count just before procedure. The hemogram parameters including NLR were compared between the groups.

Results and Conclusion: Lymphocyte count was found to be significantly decreased in oral cavity cancers (OCCs) compared with premalignant oral cavity lesions and control group. In contrast, NLR was significantly higher in OCCs and in oral SCC compared with premalignant oral cavity lesions and control group. The receiver operating characteristics curve analysis suggested of 2.75 as NLR cutoff value in predicting malignancy. Area under curve denotes 0.973, sensitivity - 91.11%, specificity - 91.48%. NLR was first shown to be significantly elevated in OCCs and in oral cavity SCC in this study. In our opinion, NLR may be helpful in identifying high-risk lesions of oral cavity harboring malignancy. It, therefore, has significant potential as a biomarker for risk stratification in oral cavity SCC.

Key words: Leukocyte, Neutrophil-lymphocyte ratio, Oral cavity cancer, Squamous cell carcinoma

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the most common head-and-neck cancers and is the 8th most common cancer worldwide.[1] In India, OSCC ranks number one in terms of incidence among men and third among women.[2] In majority of SCCs, the patient had a history of tobacco chewing and is preceded by potentially malignant lesions, such as leukoplakia, erythroplakia, and oral submucous

fibrosis (OSMF). The possible precancerous nature of submucous fibrosis was first mentioned by Paymaster in 1956 who described the development of slow-growing SCC in one-third of the cases with submucous fibrosis.[3]

Carcinoma development is an intricate complex mechanism and the multifactorial causation makes it more difficult to find specific prognostic and therapeutic biomarkers. Thus, the development of newer diagnostic and predictive approaches that are less invasive, economical, and amenable to repeated sampling is imperative.[4] Early detection of OSCC and OSMF transforming into malignancy can drastically improve the treatment outcomes and prognosis.

Attempts have been made at several diagnostic tests which can guide the difference between benign and malignant lesions, one such test being fluorescence

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visualization.[5] Another such technique is narrowband imaging. Such facilities are only available at higher centers where adequate equipment and the expertise are available.[6]

Recently, there is an increasing interest in more basic investigations like the neutrophil-lymphocyte ratio (NLR) as inflammatory responses play diverse roles at different stages of tumor development, including initiation, promotion, malignant conversion, invasion, and metastasis.[7]

SCC of oral cavity and oropharyngeal region is considered as an aggressive malignant neoplasm, because of high morbidity and mortality. Tumorigenesis and tumor development are related not only to the biological characteristics of tumors but also to elements of inflammation in the tumor microenvironment. Many inflammatory factors, such as interleukin-6 (IL-6), IL-1β, and tumor necrosis factor-α, are involved.[8]

NLR is one of the most studied and confirmed prognostic parameters in laryngeal analysis also. NLR is an inexpensive, reproducible, and widely available blood test, and could be a useful as inflammatory marker.[9] Several studies have suggested association between inflammation and cancer. Inflammation plays a dual role in progression and development of cancer. Tumors are infiltrated by leukocytes. Neutrophils help in growth and progression of malignancy, whereas lymphocytic response against tumor cells helps to control tumor growth and progression.[10] Lymphocytes and neutrophils seem to have reverse effects on cancer cell growth and progression.[11] Neutrophils are the most common cell type seen in the early stages of acute inflammation, whereas lymphocytes are enrolled in chronic infections. Systemic inflammation activated by cancer cells is known to be involved in tumor growth by promoting angiogenesis.[5,11]

An elevated NLR can guide the health care worker at a peripheral center to be more mindful of the possibility of a malignant lesion and thus be quick at referring the case for a biopsy. The present study aims to validate the use of this simple biochemical test for the diagnosis of this disease, namely, oral cavity malignancy which carries such a high rate of morbidity and mortality.

MATERIALS AND METHODS

Study Universe
Study was conducted in the outpatient department (OPD) patients attending the Department of Otorhinolaryngology and Head-Neck Surgery, SMS Medical College and Hospital, Jaipur.

Study Place
The present study was conducted on outdoor patients in the Department of Otorhinolaryngology and Head- Neck Surgery, SMS Medical College and Hospital, Jaipur.

Study Period
The study period was from January 2019 to December 2020.

Study Design
This was a cross-sectional.

Study Type
This was a hospital-based descriptive study.

Methodology
The study subjects were divided into three groups as follows:

Group I: 270 patients presenting with histopathologically confirmed SCC of oral cavity.

Histopathologically, oral cavity malignancies were divided on the basis of their degree of differentiation: (1) Well-differentiated SCC (WDSCC), (2) moderately differentiated SCC (MDSCC), and (3) poorly differentiated SCC (PDSCC).

Group II: 270 patients presenting with histologically confirmed pre-malignant lesions.

Group III: 270 control group comprised age- and sex-matched healthy subjects who had no complaint or history of any major illness in recent past and belonged to the similar socioeconomic group as head-and-neck cancer patients.

All the patients who were willing to undergo examinations, blood investigations, and biopsy and were histologically proved were included in the study population. Cases who presented with recurrence, acute, and chronic systemic infection and who underwent prior treatment such as surgery, chemotherapy, and radiation were excluded from the study group.

OBSERVATIONS AND RESULTS

Sample size was calculated at 80% study power and alfa error of 0.05, assuming standard deviation 1.3. For minimum detectable mean difference of NLR 0.35 in oral cavity cancers (OCCs), 262 patients were required in each group as sample size, which was further enhanced and rounded 270 patients in each group as final sample size. Blood sample was subjected to the assessment of common blood count, ALC, and NLR, in healthy

subjects, premalignant cases and malignant cases of OCCs.

In total 270 malignant cases (Group I), mean age was 48.19 years with standard deviation ±12.35 years, while in premalignant group (Group II), mean age was 44.99 years with standard deviation ±12.31. While in control group (Group III), mean age was 35.5 years with standard deviation ±10.56.

In Group I, 23.7% were female and 76.3% were male. This shows male predominance. While in Group II, out of 270 cases, 37% were female and 63% were male. While in Group III, out of 270 cases, 51.1% were female and 48.9% were male. In 270 malignant cases, 78.9% were tobacco chewer and 21.1% were non-chewer. While in group of premalignant, non-chewers were 45.6% and tobacco chewers were 54.4%. While in control group, non-chewers were 73% and tobacco chewers were 27%.

Mean NLR in malignant group was 3.56 ± 1.05, mean NLR in premalignant group was 2.21 ± 0.40 while mean NLR in control group was 1.98 ± 0.38. Mean NLR in MDSCC subtype was 3.69 ± 0.98, was 5.37 ± 1.44 in PDSCC, and was 3.14 ± 0.56 in WDSCC.

Mean NLR was found to be highest in T3 cases which was 4.51, followed by T2 cases 3.69, followed by T1 cases 3.11, and followed by T4 case which was 2.7. From the receiver operating characteristic (ROC) curve, a cutoff value of 2.75 was defined as pre-treatment NLR (sensitivity – 91.11%, specificity – 91.48%, and corresponding area under curve – 0.973) for predicting malignancy [Tables 1-4 and Figure 1].

DISCUSSION

NLR revealed significantly higher values in oral cavity SCC compared with premalignant oral cavity lesions and healthy individuals. It is used as a marker of subclinical inflammation and its higher values are an independent predictor of poor prognosis of various cancers.[12] In head-and-neck malignancies, NLR was first studied in nasopharyngeal carcinoma. Elevated NLR was shown be associated with poor survival in nasopharyngeal carcinoma.[13] Later on, other reports were published regarding the association of elevated NLR for laryngeal SCC diagnosis and survival.[14,15] In the study of Perisanidis *et al.*,[16] elevated NLR was shown to be associated with poor survival in patients with oral cavity cancer who were given pre-operative chemoradiotherapy. The age group of patients included in our study in malignant group varied between 21 years and 79 years with mean age 48.19 years. In premalignant group, it was 20–78 years with mean being

44.99 years whereas the overall mean age of all the cases in the study group was 42.89 years. This was found similar to the study conducted by Perisanidis *et al.* with mean age 48.19 ± 10.21.[16]

Our study showed that majority of cases was male in both malignant and premalignant groups. This was similar to the study conducted by Duzlu *et al.*[15] and Perisanidis *et al.*,[16] this is because of a larger population of males consuming tobacco in a chewable form in Indian subcontinent and hence greater incidence among men.

Majority of patients were tobacco chewers (73%) and 27% were non-tobacco chewers. Our study was similar to a study done by Perisanidis *et al.*,[16] Farrag *et al.*,[17] and Tu *et al.*[18] There were significant associations between oral cancer and tobacco chewing. From the study, we concluded that the mean NLR in malignant group was 3.56 ± 1.05 and in premalignant group was 2.21 ± 0.40, also the cutoff value of NLR in control group was 1.98 ± 0.38. This was similar to Duzlu *et al.*,[15] mean NLR for malignant group was 3.07 ± 1.30, and it was 3.46 ± 1.51 from study of Kum *et al.*[14]

In this study, mean NLR level increases as grading of malignancy goes from well differentiated (3.14) to moderately differentiated (3.69) to poorly differentiated subtype (5.37). Furthermore, the majority of the cases fell in well-differentiated category (48% of all cases) followed by MDSCC (42% of all cases) and PDSCC (10% of all cases). The studies by Farrag *et al.*[17] and Perisanidis *et al.*[16] also demonstrated higher NLR in MDSCC and PDSCC.

In this study showed that with increasing tumor size (staging), the value of NLR increases progressively. Majority of our cases fall into T2 stage (70% of total cases) with mean NLR 3.69 ± 1.11 followed by T1 stage

Table 1: Demographic and clinical characteristics of the study population

Age (in years)						
Group	*n*	Mean	SD	Median	Minimum	Maximum	*P*-value
Control	270	35.50	10.56	34.00	17	64	<0.001
Malignant	270	48.19	12.35	45.50	21	79	
Pre malignant	270	44.99	12.31	44.00	20	78	
Total	810	42.89	12.93	42.00	17	79	

Sex	Group					
	Control		Malignant		Premalignant	
	n	%	*n*	%	*n*	%
F	140	51.9	64	23.7	100	37.0
M	130	48.1	206	76.3	170	63.0
Total	270	100.0	270	100.0	270	100.0

Table 2: Comparison of NLR between control, premalignant, and malignant cases of oral cavity

Group	n	Mean	SD	Median	Minimum	Maximum	P-value
				N: L			
Control	270	1.98	0.38	1.90	0.90	3.10	<0.001
Malignant	270	3.56	1.05	3.30	2.10	8.20	
Premalignant	270	2.21	0.40	2.10	1.20	3.00	
Total	810	2.58	0.98	2.30	0.90	8.20	

NLR: Neutrophil-lymphocyte ratio

Table 3: Correlation of NLR with different histological subtypes of oral cavity cancers

HPR	N	Mean	SD	Median	Minimum	Maximum	P-value
				N: L			
MDSCC	115	3.69	0.98	3.60	2.10	8.20	<0.001
PDSCC	23	5.37	1.44	5.00	3.40	8.20	
WDSCC	132	3.14	0.56	3.00	2.10	7.50	
Total	270	3.56	1.05	3.30	2.10	8.20	

NLR: Neutrophil-lymphocyte ratio, WDSCC: Well-differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Poorly differentiated squamous cell carcinoma

Table 4: Comparison of NLR between different TNM staging

TNM staging	N: L
T1	
n	71
Mean	3.11
SD	0.49
T2	
n	188
Mean	3.69
SD	1.11
T3	
n	10
Mean	4.51
SD	1.6
T4	
n	1
Mean	2.7
SD	
P-value	<0.001

NLR: Neutrophil-lymphocyte ratio

(26% of total cases) with mean NLR 3.11 ± 0.49 and T3 (3.8% of all cases) with mean NLR 4.51 ± 1.6. These increased progressively from T1 to T3. While in T4 stage, only 1 case (0.3% of total cases) was seen. Mean NLR was 2.7 in T4 stage which was less than T3 stage which might be by chance as there was only one case of T4 lesion in our random study. This was similar to the study conducted by Perisanidis *et al.*[16] –and Fang *et al.*[5] A coexistence of higher NLR had significant association with pathologic tumor status, Farrag *et al.*[17] and Liu *et al.*[19]

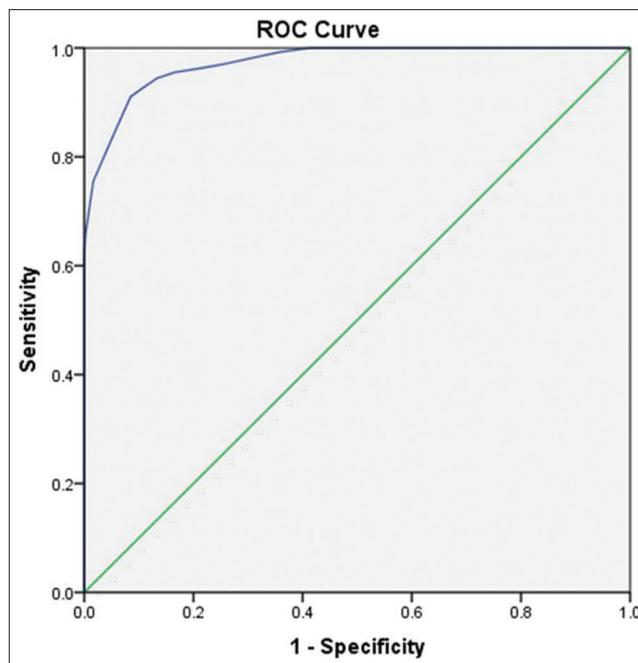Overall, an elevated NLR may be either the result of an excessive but ineffective immune response to the tumor load,

Figure 1: Receiver operating characteristic curve, neutrophil-lymphocyte ratio to predicting malignancy

or it may be a marker of imbalanced inflammatory state which facilitates tumor growth. The ROCs curve analysis suggested cutoff value of 2.75 for NLR in predicting malignancy. In the study by Duzlu *et al.*,[15] the ROC curve analysis suggested cutoff value of 2.88 for NLR in predicting malignancy.

There are several explanations for the relation between elevated NLR and poor prognosis in cancer. The first is that neutrophilia promotes tumor growth. Circulating neutrophils contain and secrete various cytokines, including circulating vascular endothelial growth factor,[20] platelet-derived growth factor, fibroblast growth factor, CXCL8,[21] matrix metalloproteinases,[22] and elastases.[23]

SUMMARY AND CONCLUSION

Inflammation has been shown to play a major role in the development of premalignant lesions and their progression to malignancy. We recommend that hematological investigations like NLR should be routinely evaluated and should be part of standard investigations of oral cavity malignancy, which may help in screening and early diagnosis of oral cavity cancers and their managements.

COMPLIANCE WITH ETHICAL STANDARDS

Consent

written and informed consent taken from all participants.

Ethical approval

Taken from institute ethics committee. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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