Diagnostic Utility of Neutrophil - Lymphocyte Ratio in Head and Neck Cancers

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ABSTRACT

BACKGROUND

The ratio between the serum neutrophil count and the serum lymphocyte count is termed as Neutrophil to Lymphocyte Ratio (NLR). It is considered to be an individualistic prognostic indicator in hepatocellular carcinoma, colorectal carcinoma and various ovarian tumours. We wanted to assess the Neutrophil-to-Lymphocyte Ratio (NLR) in the head and neck cancer patients and compare it with those of normal subjects.

METHODS

This is a prospective study involving two cohorts i.e. 1) patients of head and neck cancers and 2) healthy normal subjects of the same age group. Total study duration was 3 years and the study sample included 49 patients and 49 healthy subjects.

RESULTS

Results of the present study showed that the Neutrophil-to-Lymphocyte Ratio (NLR) of histopathologically proven cases of Squamous Cell Carcinoma of the oral cavity (OSCC) showed a significantly higher median of 2.9 ($p < 0.001$) compared to the NLR in normal (control) individuals.

CONCLUSIONS

NLR may be helpful in identifying patients with adverse tumour biology as it is a feasible, cost-effective, and potential biomarker.

KEYWORDS

Neutrophil-to-Lymphocyte Ratio, Squamous Cell Carcinoma, Head and Neck, Malignancy

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BACKGROUND

Various kinds of inflammatory, infective and neoplastic lesions can occur inside the oral cavity. Certain diversified inflammatory conditions of the oral cavity such as sub-mucous fibrosis (SMF), oral lichen planus (OLP), and chronic non-healing and idiopathic ulcers are involved in the pathogenesis of Oral Squamous Cell Carcinoma (OSCC). Chronic inflammation leads to oncogenes activation and inactivation of tumour suppressor genes which further facilitates the process of carcinogenesis. Cancers of the head and neck region is a major public health problem universally despite the availability of newer treatment modalities, this is due to the local and regional metastasis of the tumour which further decreases the overall five-year survival rate. Among the head and neck cancers, most commonly encountered histological type of carcinoma is Squamous Cell Carcinoma (SCC) which originates from the epithelial cells of the oral cavity.[1] A few haematological and biochemical markers of inflammation such as leukocyte or total white blood cell (WBC) count, neutrophils to lymphocyte ratio (NLR) & C-reactive protein (CRP) levels were estimated to be coupled with the presence or prognosis of various carcinomas. Among the above mentioned haematological and biochemical markers, NLR is one of the extensively studied and confirmed prognostic marker. Few authors reported that, elevated NLR was shown to be correlated with poor survival in oesophageal, lung, breast, gastric, colorectal, and genitourinary system cancers.[2-9] There is also growing evidence about potential diagnostic and prognostic role of NLR in head and neck malignancies. Recently, elevated peripheral blood NLR before starting the treatment has been introduced as an independent prognostic factor linked with relatively poor survival in different types of malignancies. It has been also attributed that neutrophils and lymphocytes play these vital roles in the tumour inflammation and immunology. There is some evidence that NLR can also be used as a predictive biomarker of outcome in patients treated with systemic therapy for metastatic renal cell carcinoma, pancreatic cancer, ovarian cancer, and gastric cancer. In terms of response to treatment, NLR was also shown to be predictive so that high NLR before neoadjuvant chemoradiation for rectal cancer or systemic chemotherapy for lung cancer and oesophageal cancer was a reliable predictor of poor clinical or pathologic response to treatment.

METHODS

This study was a prospective study involving two cohorts i.e., 1) patients of head and neck cancers and 2) healthy normal subjects of the same age group. This study was approved by the Ethics Committee of the institute after the fulfilment of the ethics committee protocol. Total duration of this study was 3 years (between January 2016 and December 2018), number of cases included were 49 (excluded all other lesions and considered only malignancies) and the total healthy subjects included were also 49. The inclusion criteria employed for this study was patients in any age group who presented with chronic non-healing ulcer or lesion or nodule or swelling in the oral cavity or in the head and neck region (only freshly presented cases were included in this study). Exclusion criteria employed in this study was patients who had a report of carcinoma, patients with infections or inflammatory conditions such as oral lichen planus, pemphigus and bullous pemphigoid, patients with haematological disorders, history of corticosteroid therapy in the last 2 months, renal insufficiency and patients with recurrence of the lesion were excluded from this study. Healthy subjects included in this study were 49 and they were chosen with certain close characteristics to that of the patients, like smoking habits (cigarette, beedi), tobacco and gutkha chewing, no systemic diseases or oral lesion.

After complete general physical examination of the patient, necessary imaging studies to define TNM staging of all the included patients were done, two peripheral blood smear (PBS) slides were obtained (by patient's fingertip blood-without ethylene diamine tetra-acetate - EDTA) and let them for drying, these peripheral blood smear slides (from the patient suspicious of cancer and normal subject of same age group) were sent to the hospital's laboratory for staining and report by the pathologist. Patients and study subjects were instructed not to smoke or use tobacco at least 24 hours before sample collection. All samples were collected in the morning by finger prick method. Two pathologists separately viewed the stained slides under a light microscope using oil immersion magnification and the mean percentage of neutrophils and lymphocytes and NLR for each patient were calculated. With a previously prepared fact sheet, the following data for each patient were collected: patient's age, sex, weight and height, date of diagnosis, primary tumour site, histological grade of primary tumour, TNM stage, and location of metastasis if present. All patients were given quality cancer treatment protocols and regimens based on primary tumour site, size, and stage of the disease and patient's general physical health status. After undergoing initial primary treatment, patients were followed up regularly in the surgical oncology clinic for every three months.

SPSS version V26 was used for statistical analysis. Continuous variables were tested for normality using Kolmogorov-Smirnov or Shapiro-Wilks test depending on number of subjects. χ^2 test used for comparison of gender distributions. In all the tests $p < 0.05$ was considered to be significant statistically.

RESULTS

A total of 49 patients (test group) and 49 normal healthy subjects (control group) was included in this study. The minimum age of the carcinoma patient was 28 years and the maximum age was 62 years. The mean age was 46.22 years age groups and the number of cases is tabulated in Table / Figure 1. Male patients were 29 and Females 20. Total numbers of malignant lesions are tabulated in Table / Figure

2. The most common malignant lesion was squamous cell carcinoma, followed by mucoepidermoid carcinoma and the least common in this study was adenoid cystic carcinoma.

Age Group	Number	Percentage
20 - 29 Years	06	12.25
30 - 39 Years	10	20.40
40 - 49 Years	14	28.57
50 - 59 Years	07	14.28
60 - 69 Years	12	24.50
Total	**49**	**100**

Table 1. Age Wise Distribution of Cases

Type of Lesion	Number	Percentage
Squamous Cell Carcinoma (SCC)	34	69.38
Mucoepidermoid Carcinoma (MEC)	09	18.37
Adenoid Cystic Carcinoma (ACC)	06	12.25
Total	**49**	**100**

Table 2. Histological Variants of Malignancies in the Head and Neck Region

The results of the present study showed that the NLR of histopathologically proven cases of OSCC showed a significantly higher median of 2.9 ($p < 0.001$) compared to the NLR in normal (control) individuals (range of 1.4 - 2.1 with a median of 2.0) using one sample Wilcoxon-signed rank test. In this present study, NLR was found to be high or increased in poorly differentiated carcinomas (05 cases out of 34) when compared to moderately differentiated carcinoma (08 cases out of 34 cases) and well-differentiated carcinomas (21 cases out of 34). Other parameters of the complete blood picture are tabulated in Table / Figure 3.

	Study Group	Control Group	P Value
Number of Cases / Subjects	49	49	
Gender (Male / Female)	29 / 20	29 / 20	
Age	46.22 ± 11.68	46.22 ± 11.68	< 0.001
Hemoglobina	13.12 ± 1.14	14.61 ± 1.35	< 0.005
Mean (RBC) Corpuscular Volume (MCV)	84.14 ± 5.02	88.65 ± 7.57	< 0.005
Mean (RBC) Corpuscular Haemoglobin (MCH)	28.16 ± 1.77	31.14 ± 2.80	< 0.005
Mean (RBC) Corpuscular haemoglobin Concentration (MCHC)	30.66 ± 1.02	32.01 ± 1.26	< 0.003
Total WBC / Leukocyte Count (TLC)	9.24 ± 1.66	7.63 ± 2.10	< 0.003
Neutrophil	7.59 ± 1.98	4.65 ± 1.39	< 0.005
Lymphocyte	2.91 ± 1.01	3.77 ± 1.21	< 0.003
Neutrophil to Lymphocyte Ratio (NLR)	6.33 ± 1.43	4.77 ± 1.24	< 0.003
Platelet Count	1.88 ± 29.31	2.14 ± 45.50	< 0.005
Mean Platelet Volume (MPV)	7.98 ± 1.10	8.28 ± 3.61	< 0.005

Table 3. Comparison of Demographic Data and Haemogram Parameters between Study and Control Groups

In this study, from the above table, it is evident that patients with malignancies had higher NLR, higher neutrophils, white blood cell (WBC) count and low haemoglobin, MCV, MCH, MCHC and platelet counts were noted.

DISCUSSION

Inflammatory cells such as neutrophils and lymphocytes play a very vital role in inflammation which is an integral part of carcinogenesis and tumour molecular environment. Neutrophils, also known as neutrocytes or heterophils are the most abundant type of granulocytic cells present in the blood constituting about 45 - 70 %. Neutrophils are a type of phagocytes which help in fighting with the bacteria. Neutrophils are one of the first responders of inflammatory cells which migrate towards the site of inflammation. Neutrophils are the hallmark of inflammation.[10] Inflammation plays a very noteworthy role in tumour prognosis. Other causes of inflammation are diseases of immune system, malignant and benign lesions or tumours and various pathological entities which recruit cells responsible for inflammation at specific sites into the body. Inflammation adversely helps the development and progression of different kinds of malignancies. Inflammation anywhere in the body can be identified by a simple and useful test (CBP / CBC - Complete Blood Picture or Count) by using peripheral blood of the patient.

According to the various recent studies on various cancers of the body, it was documented that there is a relationship between the inflammatory surroundings of a lesion and systemic feedback influenced by the tumour. The uptrend of neutrophils and / or downtrend of lymphocytes may suppress lymphokine-activated Natural Killer Cells (NK-Cells).[11] In malignancies, decreased lymphocyte count may indicate a generalized state of immune suppression.[12]

Several animal experimental studies have shown that deficiency or lack of development of function of CD4 + Th1 helper cells, CD8 + cytotoxic T cells or natural killer (NK) cells makes the living being more susceptible to carcinogenesis.[13] NLR may show the two opposing inflammatory and immune pathways existing together in cancer patients.[14] Tumour-associated inflammatory response has been long recognized as a vital constituent of the spectrum of factors that curtail neoplastic cell progression and directly influence the grade and prognosis of the disease. The cells commonly observed to be a part of the histology of various malignant tumours include macrophages, neutrophils, mast cells, natural killer (NK) cells, and lymphocytes. Neutrophils, which represent 50 % - 70 % of the total circulating leukocytes, have been found to comprise a significant portion of the leukocytic infiltrate in a wide variety of human cancers.

NLR is increased in initial phase of tumour formation or carcinogenesis as a response given by the immune system of the host to the recognition of the "foreign nature" of tumour in the connective tissue zone. In advanced stages of tumour progression, there is moderate to severe lymphopenia further leading to relative increase in NLR. This is well supported in our study as a progressive increase in the NLR is evident in cases showing poorer differentiation. Poorly-differentiated tumours have a greater genomic instability which could be attributed to the increase in the infiltration of neutrophils. As the tumour grows, it integrates the function of neutrophils with it to further increase the neutrophils and NLR. Haqqani et al. have proved that the reactive nitrogen oxygen species that are produced by the activated neutrophils in the tumour milieu are to an extent responsible for the accumulation of mutations that are responsible for tumour progression.[15]

Various studies on NLR and its prognostic significance and implication as a systemic inflammatory marker had been documented and postulated in various solid organ malignancies. In a few recent studies with over 40,000 patients has demonstrated a negative correlation between high NLR.[16] Some more studies by authors highlighted the prognostic significance of NLR in the nasopharyngeal cancers from the China, Singapore and Bangladesh.[17,18] However; the role of NLR in oral squamous cell carcinoma has largely remained unexplored and lot of research is going on this topic in various countries across the globe. In a study, out of total 46 sample size, the average age of 54.2 years (27 – 82 years). The mean NLR of the sample population of patients was 2.8. The average NLR was dramatically low in the group with higher response when comparison was made with the no-response group (2.5 and 5.8, respectively; $p = 0.027$). The differences were also significant for percentage of neutrophils and lymphocyte between these study groups. A NLR $\geqq 3$ was significantly associated with advanced clinical American Joint Committee on Cancer (AJCC) 7th stage, higher clinical T classification, oral cavity primary tumour site, and alcohol history. In the study by Lai HL et al, the overall response rates of induction chemotherapy were 70 % and 50 % ($p = 0.022$) in patients with a NLR < 3 and NLR $\geqq 3$ and 78 % and 52 % ($p = 0.008$) in patients with a PLR < 120 and PLR $\geqq 120$, respectively. In the same study, univariate analysis exhibited 5 year cancer progression free survival (PFS) rates of 58 % and 32 % ($p < 0.001$) in the patients with a NLR < 3 and NLR $\geqq 3$ and 59 % and 38 % ($p = 0.022$) in those with a PLR < 120 and PLR $\geqq 120$, respectively. In multivariate analysis, NLR $\geqq 3$ was significantly independently associated with worse PFS ($p = 0.018$, odds ratio [OR]: 2.11) and OS ($p = 0.026$, OR: 1.87).[19]

In this study, it is evident that patients with head and neck malignancies had higher NLR, higher neutrophils, white blood cell (WBC) count and low haemoglobin, MCV, MCH, MCHC and platelet counts were noted. This study is in close relation with the studies done by various authors Caro JJ et al, Fang et al, Mahalakshmi R et al, Phulari G et al and Duzlu M et al all over the world, where they also have found that NLR, Neutrophils and total leukocyte count was raised in the patients of head and neck cancers and can be used as a early marker to suspect cancers mainly in the head and neck regions.[20,21,22]

CONCLUSIONS

NLR was significantly elevated in oral cancers when compared with benign oral cavity pathologies and healthy control group in this study. Higher NLR was also found in cases that showed the involvement of surgical margins and lymph nodes. NLR may be helpful to identify patients with adverse tumour biology as it is a feasible, cost-effective, and potential biomarker.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.

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