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Original Article

Others Section

Inflammatory Markers in Lung Cancer- A Comparative Cross-sectional Study

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ABSTRACT

Introduction: Lung cancer is the most common cause of cancer mortality worldwide, principally because of its late diagnosis. Chronic inflammation plays a significant role in tumour growth and progression via increasing the levels of inflammatory markers in blood. Inflammatory markers are expected to be valuable prognostic biomarkers in cancer. Markers like Absolute Neutrophil Count (ANC), Absolute Lymphocyte Count (ALC), platelet count, Neutrophil Lymphocyte Ratio (NLR) and Platelet Lymphocyte Ratio (PLR) may aid in assessing prognosis of lung cancer.

Aim: To study the inflammatory markers (ANC, ALC, Platelet count, NLR and PLR) in lung cancer and correlate these markers with cancer stage and histopathological type.

Materials and Methods: A hospital-based comparative cross-sectional study was conducted in lung cancer patients at Institute of Respiratory Diseases, SMS Medical College, Jaipur, Rajasthan, India. Sixty patients with lung cancer and sixty controls were included. The clinical characteristics, ANC, ALC, platelet count, NLR and PLR of cases and controls were assessed and compared. Also, the comparison of these inflammatory markers with Tumour, Nodes and Metastases (TNM) staging and histopathological type of lung cancer were documented and results were interpreted. The data analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, (Ver.) 26.

Results: Mean age (years) of the case and control groups was 60.17 and 61.03, respectively. Distribution of gender in cases and controls was comparable. The major histology of lung cancer was Non-Small Cell Lung Cancer (NSCLC, 81.66%) out of which squamous cell carcinoma constituted 55.00% and adenocarcinoma 21.67%, followed by small cell carcinoma at 18.33%. Overall, 46.67% of the patients belonged to TNM staging IVA followed by IIIB (25.00%). The levels of ANC, platelet count, NLR and PLR were significantly elevated and ALC was decreased in cases as compared to control. There was no statistically significant association between the inflammatory markers ANC, ALC, platelet counts, NLR and PLR with the histopathological type of lung cancer. Mean ANC, platelet count, NLR and PLR were found to be significantly elevated in late stages of lung cancer, whereas, ALC had no such association with the stages of lung cancer.

Conclusion: Inflammatory markers (ANC, platelet count, NLR, PLR, ALC) can serve as valuable prognostic biomarkers in lung cancer and can easily be used in resource-limited areas.

Keywords: Absolute neutrophil count, Lymphocyte count, Neutrophil lymphocyte ratio, Platelet lymphocyte ratio, Platelet count

INTRODUCTION

Globally, lung cancer contributed to 2.21 million (11.4%) of all new cases diagnosed in 2020. Lung cancer is the most common cause of cancer mortality worldwide accounting to 1.80 million deaths (18%) in the year 2020 [1]. Smoking is the major risk factor for lung cancer which leads to chronic inflammation. Inflammation itself is recognised both as a condition that leads to cancer development and also as a condition that arises due to oncogenic changes in cancer cells. Inflammation plays a critical role in the progression of many cancers, stimulating cancer cell-proliferation and angiogenesis. The ability of oncogenic cells to establish themselves in a niche and subsequently metastasise depends not only on their own intrinsic cellular signalling pathways but also on complex interaction with immune cells. Predominant cells involved in tumourigenesis are neutrophils, lymphocytes and platelets and these cells serve as inflammatory biomarkers in different cancers [2].

Neutrophils are the dominant leukocytes in the blood, and are the first line of defense against inflammation and infection [3]. The evaluation of tumour associated neutrophils as prognosis index in many tumours has been clearly assessed high neutrophil number and/or elevated NLR do correlate a poor outcome of the patient [4]. Lymphocytes are major cells involved in defense against tumour formation in the body. Decreasing levels of tumour-infiltrating lymphocytes are associated with a poor prognosis in lung cancer [5]. A prognostic significance between platelet count and lung cancer has been identified. Elevated pretreatment platelet counts are related to poor Overall Survival (OS) and Disease-Free Survival (DFS)/Progression-Free Survival (PFS)/Time To Progression (TTP) in lung cancer patients and are an independent prognostic predictor of lung cancer patients [6]. Other simple indices of measuring the levels of tissue inflammation are the NLR and PLR. In NSCLC, an elevated PLR is associated with poor OS and PFS [7]. A meta-analysis demonstrated that high pretreatment NLR was closely related to poorer PFS and OS in patients with lung cancer [8].

Inflammatory markers are expected to be valuable prognostic biomarkers in cancer. A limited number of studies are available, correlating the degree of systemic inflammatory marker levels at the time of diagnosis in patients with lung cancer. Thus, in the present study various blood inflammatory markers (platelet count, ANC, ALC, NLR, PLR levels) have been evaluated in patients with lung malignancy at the time of diagnosis and their relationships are assessed with cancer stage and cell type.

MATERIALS AND METHODS

This was a hospital-based comparative cross-sectional study carried out in the Department of Respiratory Medicine, Institute of Respiratory Diseases, SMS Medical College, Jaipur, Rajasthan, India. Approval was taken from Research Review Board (RRB) and Institutional Ethics

Committee of the institute (Letter no.140/MC/EC/2020). The study was done from January 2020 to December 2021.

Sample size calculation: Sample size was calculated at 80% study power and alpha error of 0.05% assuming severity of NLR being 86% for detection of lung cancer. The required sample size for this study was 45. This was rounded off to 60 patients in both groups, expecting 20% attrition.

Inclusion and Exclusion criteria: Sixty patients of biopsy-proven lung cancer were recruited after excluding those having active infection, haematological disease, autoimmune disorders, history of thromboembolic disease, those who have already received chemotherapy or radiotherapy or having any other type of cancer. Also, sixty age and gender matched healthy controls (attendants of patients) were enrolled.

After recruitment, the patients and controls underwent blood investigations like complete blood count using Automated Elite 580 Haematology Analyser. The results of ANC, ALC, platelet count, NLR and PLR from lung cancer patients and healthy controls were compared. Also, intragroup comparison of the blood values of inflammatory markers of cases was done with staging and histopathological types.

STATISTICAL ANALYSIS

Collected data were entered in Microsoft excel data sheet which was tabulated for data interpretation. This was then subjected to statistical analysis using appropriate statistical tests and results were interpreted. The presentation of the categorical variables was done in the form of number and percentage (%) and the quantitative data were presented as the means±SD. The data normality was checked by using Kolmogorov-Smirnov test. For statistical significance, p-value of less than 0.05 was considered statistically significant. The statistical tests applied for the results were Mann-Whitney Test (for two groups) used for calculation of smoking status, ALC and NLR in cases and controls, Kruskal Wallis test (for more than two groups) for association of histology and staging of lung cancer with ALC and NLR, independent t-test (for two groups) for calculation of age, ANC, platelet count and PLR in cases and controls, ANOVA test (for more than two groups) for association of histology and staging with ANC, platelet count and PLR, Chi-square test for pack years and gender distribution in cases and controls and Fisher's-exact test for distribution of BMI in cases and controls. The data analysis was done with the use of SPSS software, IBM manufacturer, Chicago, USA, (Ver.) 26.

RESULTS

The distribution of age and gender of cases and controls was comparable. Proportion of smokers was significantly higher in cases as compared to controls. BMI in cases was significantly lower as compared to controls [Table/Fig-1].

Demographic characteristic	Cases (n=60)	Controls (n=60)	Total	p-value
Age (years)				
Mean±SD	60.17±9.87	61.03±8.92	60.6±9.38	0.615
Gender				
Female	6 (10%)	7 (11.67%)	13 (10.83%)	0.769
Male	54 (90%)	53 (88.33%)	107 (89.17%)	
Smoking status				
Non smoker	5 (8.33%)	53 (88.33%)	58 (48.33%)	<0.0001
Smoker	55 (91.67%)	7 (11.67%)	62 (51.67%)	
Pack years				
Mean±SD	42.15±22.52	13.14±5.55	38.87±23.18	<0.0001
Body mass index (kg/m²)				
Mean±SD	18.76±2.85	22.13±2.23	20.44±3.06	<0.0001

[Table/Fig-1]: Demographic characteristics.

The major histology of lung cancer was NSCLC (81.66%), followed by small cell carcinoma (18.33%); 46.67% of patients belonged to TNM staging IVA followed by IIIB (25.00%), IIIA (20.00%), IVB (5.00%) and IIIC (3.33%) [Table/Fig-2]. Mean ANC (cells/µL), platelet count (10^5×cells/µL), NLR and PLR in cases was significantly higher as compared to controls, whereas that of ALC (cells/µL) was significantly lower as compared to controls [Table/Fig-3]. There was no statistically significant association found between the levels of inflammatory markers ANC, ALC, platelet counts, NLR and PLR with the histopathological type of lung cancer [Table/Fig-4]. Mean ANC, platelet count, NLR and PLR were found to be elevated in late stages of lung cancer as compared to early stages showing statistically significant association; whereas ALC had no such association with the stages of lung cancer [Table/Fig-5].

Histology	Cases (N)	Percentage
Adenocarcinoma	14	23.33%
Large cell carcinoma	1	1.67%
NSCLC (NOS)	1	1.67%
Squamous cell carcinoma	33	55%
Small cell carcinoma	11	18.33%
TNM staging		
IIIA	12	20%
IIIB	15	25%
IIIC	2	3.33%
IVA	28	46.67%
IVB	3	5%

[Table/Fig-2]: Lung cancer histology and TNM staging.

Markers	Cases (n=60)	Controls (n=60)	Total	p-value
ANC (cells/µL) Mean±SD	6306.32±1280.45	4765.97±1191.53	5536.14±1454.29	<0.0001
ALC (cells/µL) Mean±SD	1668.17±295.13	2765.78±545.98	2216.98±703.35	<0.0001
Platelet count (10^5×cells/µL) Mean±SD	5.09±0.68	2.21±0.5	3.65±1.56	<0.0001
NLR Mean±SD	3.8±0.55	1.73±0.33	2.76±1.13	<0.0001
PLR Mean±SD	313.24±73.89	83.65±26.75	198.44±127.87	<0.0001

[Table/Fig-3]: Comparison of inflammatory markers between cases and controls.

Markers	Adenocarcinoma (n=14)	Large cell carcinoma (n=1)	NSCLC (NOS) (n=1)	Small cell carcinoma (n=11)	Squamous cell carcinoma (n=33)	Total	p-value
ANC (cells/µL) Mean±SD	6214.79±1549.09	7469±0	7565±0	5910.73±1038.05	6403.64±1245.93	6306.32±1280.45	0.554
ALC (cells/µL) Mean±SD	1613.64±242	2134±0	2225±0	1610.45±262.7	1679.55±310.14	1668.17±295.13	0.318
Platelet count (10^5×cells/µL) Mean±SD	5.39±0.61	5.1±0	5±0	5.27±0.74	4.91±0.67	5.09±0.68	0.212

NLR Mean±SD	3.82±0.55	3.5±0	3.4±0	3.68±0.39	3.85±0.61	3.8±0.55	0.733
PLR Mean±SD	342.11±72.62	239±0	224.7±0	334.92±70.36	298.7±72.22	313.24±73.89	0.143

[Table/Fig-4]: Association of inflammatory markers with histology of lung cancer.

Markers	IIIA (n=12)	IIIB (n=15)	IIIC (n=2)	IVA (n=28)	IVB (n=3)	Total	p-value
ANC (cells/μL) Mean±SD	5583.33±1456.57	6083.52±900.14	5470.5±1618.57	6392.36±1517.77	7314.5±945.07	6306.32±1280.45	0.013
ALC (cells/μL) Mean±SD	1810.56±443.55	1701.57±246.56	1512±390.32	1580.14±317.41	1626.08±185.59	1668.17±295.13	0.539
Platelet count (10^5×cells/μL) Mean±SD	4.8±0.79	4.86±0.63	5.2±0.85	5.49±0.54	5.27±0.65	5.09±0.68	0.038
NLR Mean±SD	3.08±0.19	3.58±0.24	3.6±0.14	4.04±0.43	4.5±0.36	3.8±0.55	<0.0001
PLR Mean±SD	281.93±92.67	291.63±59.2	348.25±33.73	358.18±69.85	319.86±74.46	313.24±73.89	0.048

[Table/Fig-5]: Association of inflammatory markers with TNM staging of lung cancer.

DISCUSSION

Inflammation is the hallmark of any neoplastic process going on in the body. The body tries its best to check oncogenesis through its immune system. The immune system of the lung is represented by cells and cytokines, which have different functions under physiological conditions [9]. Smoking is the most important modifiable risk factor of lung cancer. This study also showed a greater proportion of smokers in the lung cancer group as compared to the control group and the number of pack years was also significantly higher in the lung cancer group. This is due to the fact that smoking leads to chronic inflammation, which in turn, triggers the process of oncogenesis and that the risk for lung cancer increases with the duration of smoking and the number of cigarettes smoked per day. An average smoker has an approximately 10-fold risk for lung cancer, whereas heavy smokers had atleast a 20-fold risk [10].

The index study concluded that the levels of ANC were significantly elevated in patients with lung cancer as compared to controls, which is similar to a study done by Şahin F and Aslan AF [11]. Neutrophils are important cells for oncogenesis. They drive angiogenesis in malignancy by providing a significant source of MMP-9 which acts to release VEGF from the Extracellular Matrix (ECM) [12]. In addition to roles in angiogenesis, MMP-9 is also postulated to aid the direct invasion of tumour cells via degradation of ECM/basement membrane, thus helping in metastasis.

The present study also observed that the levels of lymphocytes were significantly lower in the lung cancer patients as compared to the control group, similar to a study by Suzuki R et al., [13]. This indicates that low level of ALC is associated with poor prognosis in lung cancer patients. Pretreatment elevated platelet counts in lung cancer patients have been observed in various studies. The present study also supports this observation where the levels of platelet count in lung cancer patients were significantly higher than control group as reported in a systematic review and meta-analysis [6]. Thrombocytes increase as a reactive response to cancer in the body due to release of factors like Platelet-derived Growth Factor (PDGF), thrombopoietin etc., and they also play a significant role in the growth, progression and tumour spread, as shown by the association of the prevalence of elevated platelet counts with advancing disease stage reflecting the actual tumour load [14]. It was also seen that the NLR and PLR levels were significantly raised in lung cancer group than in the control group which is similar to a study done by Zhu X et al., [15].

In this study, no statistically significant association was found between the inflammatory markers ANC, ALC, platelet counts, NLR and PLR with the histopathological type of lung cancer. This may be due to the fact that all histopathological subtypes of lung cancer exert more or less similar inflammatory response in the body.

In the present study, mean ANC, platelet count, NLR and PLR were found to be elevated in late stages of lung cancer. Whereas, ALC had no such association with the stages of lung cancer. With increasing stages of lung cancer, the degree of systemic inflammation increases which recruit more and more inflammatory cells like neutrophils and platelets with compensatory decrease in lymphocytes. These cells, through various mechanisms are responsible for angiogenesis and metastatic invasion so the levels of these blood inflammatory markers also increase proportionally.

Limitation(s)

This being a single-centred study is an important limitation. Majority of the cases belonged to late stage disease.

CONCLUSION(S)

This study sheds light on the importance of assessing systemic inflammation in lung cancer patients. As inflammation increases with increasing stages of cancer so the inflammatory markers like ANC, ALC, platelet count, NLR and PLR can be used as prognostic indicators of lung cancer. These are simple and affordable tests which can be applied even in remote health facilities. By correlating the clinical history and physical examination with these inflammatory markers, physicians may get a clue to prognosticate lung cancer patients and hence it can aid in treatment planning and outcome.

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