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The effect of smoking on neutrophil/lymphocyte and platelet/lymphocyte ratio and platelet indices: a retrospective study

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Abstract. – OBJECTIVE: Smoking commonly leads to death. Although the neutrophil/lymphocyte Ratio, platelet/lymphocyte ratio and platelet indices have been shown to be important for the diagnosis, prognosis and severity of some diseases, the smoking status of patients in these studies has not been well defined. In this study, we compared ratios derived from complete blood count and platelet indices to smoking status and length in smokers and non-smokers.

PATIENTS AND METHODS: The data of healthy males and females aged between 18-60 years who presented to our institute for a routine check-up were collected, and subjects were divided in two groups – smokers and non-smokers. The presence of medical history or laboratory results which could affect inflammatory response, formed our exclusion criteria. All complete blood count results were noted and persons' smoking habits were calculated as pack/years.

RESULTS: White blood cell, neutrophil, basophil and eosinophil counts; mean corpuscular volume, red cell distribution width and neutrophil/lymphocyte ratio were significantly higher in smokers when compared to non-smokers ($p<0.05$). When smokers were grouped according to smoking habits; positive linear correlations were detected between pack/year and Neutrophil/lymphocyte ratio and also pack/year and plateletcrit in smokers ($p<0.05$).

CONCLUSIONS: Neutrophil/lymphocyte ratio increases in correlation with pack/year while platelet/lymphocyte ratio is not affected and platelet distribution width is increased in smokers. If smokers are not excluded from studies evaluating neutrophil/lymphocyte ratio and platelet distribution width, the relationship between smoking status as well as pack/year must be determined and reported.

Key Words:
Smoking, Neutrophil, Lymphocyte, Neutrophil/lymphocyte ratio, Platelet, Platelet/lymphocyte ratio.

Introduction

According to the World Health Organization, smoking leads to approximately 6 million preventable deaths worldwide, each year. As of 2030, it is estimated that this number will be more than 8 million[1]. Apart from causing cancer, smoking can lead to cardiovascular, neurological and respiratory diseases and may affect every system. Although the detrimental effects of smoking are associated with the number of years and amount of use, the effect of smoking may be reversible and mortality decreases, even if smoking is ceased at a later age[2,3].

Complete blood count (CBC) is a cheap and easily available laboratory test. One of the measures included in a CBC is mean platelet volume (MPV) and measurements obtained from a CBC can be used to calculate the neutrophil-lymphocyte ratio (NLR) and the platelet-lymphocyte ratio (PLR). NLR is accepted as a systemic inflammatory marker and apart from being used for the diagnosis and determination of severity for many disease processes (cardiovascular disease, pulmonary, infections, endocrinological disorders and some cancers), its correlation with prognosis, morbidity and mortality has also been reported[4-13]. PLR and NLR have been the subject of many studies, and it has been suggested that they are relevant

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and may be prognostic markers for some cancer types such as lung and gastrointestinal[8,11,14,15]. It has been demonstrated that these two indexes may increase in psychiatric diseases such as bipolar disorder[16]. Similarly, platelet indexes such as platelet distribution width (PDW), mean platelet volume (MPV) and plateletcrit (PCT) have been shown to be other biomarkers that may change in many conditions, such as infections and respiratory and cardiovascular pathologies[17–20].

While there are many studies of CBC parameters in many areas, we are unaware of a study evaluating the effect of smoking on NLR, PLR and platelet indices. Many of studies including NLR, PLR and platelet indices have either not evaluated smoking as a confounder, or have not collected this data.

In this study, we compared CBC parameters and ratios derived from these parameters such as NLR and PLR to smoking status and length in smokers and non-smokers. Our aim was to define possible effects of the length and amount of smoking on inflammatory markers that could be affected by chronic hypoxia.

Patients and Methods

This retrospective study, performed between January 2014 and July 2015, included healthy males and females aged between 18-60 years who presented to our institute for a routine check-up. Subjects who smoked one or more cigarettes per day were accepted as smokers. As inflammatory markers could be affected, subjects with the following were excluded from this study: presence of chronic diseases such as diabetes mellitus, hypertension, hypo/hyperthyroidism and metabolic syndrome; chronic medication use; use of NSAID in the previous week; steroid use in previous 6 months (including steroid creams); upper respiratory tract infection within the last 3 weeks, pregnant women; subjects whose check-up evaluation form did not have the required medical history information; subjects who during evaluation were found to have hyperlipidemia, anemia, vitamin B12 or vitamin D deficiency, leukocytosis, leukopenia or any other hematological, biochemical or serological abnormalities; subjects with routine alcohol intake and ex-smokers were excluded. The following data was collected; age, gender, height, weight, smoking habits (including pack/year if smoker), CBC parameters including WBC, RDW, neutrophil count, lymphocyte count and platelet indexes. CBC measurements were performed using Sysmex XT 1800i (Sysmex, Munich, Germany) according to quality standards. Pack-years were calculated as number of cigarettes smoked per day x number of years smoked/20.

Statistical Analysis

SPSS 16.0 Statistical package program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Parameters were compared using independent samples test and intergroup comparison was performed using ANOVA test. Relations in between data were analyzed with Pearson correlation analysis. Summaries for groups of cases and correlations of data were shown as graphs using, respectively, define simple boxplot and overlay scatterplot. The p value of <0.05 was regarded as statistically significant.

Results

Data of 243 patients were retrospectively reviewed and after exclusions, 134 patients (43 females, 91 males) were included in this study. Patients were excluded due to alcohol use (n: 23), abnormality in laboratory parameters (n: 22), presence of chronic disease (n: 10), medication use (n: 15), pregnancy (n: 1), obesity or low body weight (n: 26) and insufficient medical history data (n: 12). There were 58 smokers (males: 41, females: 17) and 76 non-smokers. Age and CBC parameters according to smoking status are shown in Table I. Average age of patients was similar between smokers and non-smokers ($p>0.05$). WBC, neutrophil count and MCV were found to be significantly higher in smokers when compared to non-smokers (7.30±1.38 *vs.* 6.68±1.52, p: 0.015; 4.21±1.10 *vs.* 3.59±0.97, p: 0.001 and 86.7±4.7 *vs.* 84.3±5.1, p: 0.006 respectively). When platelet indexes were compared, platelet counts, MPV and PCT were similar between groups. However, smokers had significantly higher PDW measurements when compared to non-smokers (13.46±3.05 *vs.* 12.11±2.82, $p<0.05$). There were no significant differences in other CBC parameters (Table I).

NLR, calculated from parameters obtained from CBC, was found to be significantly higher in smokers (2.14±0.91 *vs.* 1.66±0.60, p: 0.001). PLR was found to be similar between groups.

When smokers were grouped according to pack/year (group 1: <10 pack/year, group 2: 11-20 pack/year, group 3: 21-30 pack/year, group 4:

Table I. The comparison of differences in data values of groups.

	Smokers (N=58) Mean±SD	Non-smokers (N=76) Mean±SD	*p*
Age (years)	35.0±9.2	37.8±10.1	0.098
RBC (10^6/mm^3)	5.005±0.541	5.005±0.483	0.995
Hb (g/dL)	14.44±1.39	14.72±1.59	0.283
Hct (%)	42.0±3.6	43.4±4.1	0.059
MCV (fL)	84.3±5.0	86.7±4.7	0.006**
RDW (%)	13.2±0.9	13.3±1.0	0.663
WBC (10^3/mm^3)	6.69±1.52	7.31±1.39	**0.015***
NEUT (10^3/mm^3)	3.60±0.98	4.21±1.10	**0.001****
LYMPH (10^3/mm^3)	2.29±0.62	2.12±0.61	0.113
MONO (10^3/mm^3)	0.58±0.20	0.64±0.19	0.119
EOS (103/mm^3)	0.19±0.14	0.25±0.13	**0.022***
BASO (10^3/mm^3)	0.026±0.022	0.040±0.042	**0.017***
PLT (10^3/mm^3)	232.7±50.8	229.6±47.35	0.714
PDW (fL)	12.11±2.82	13.46±3.05	**0.010***
MPV (fL)	9.11±0.86	9.03±0.92	0.637
PCT (%)	0.21±0.04	0.20±0.05	0.166
NLR (%)	1.66±0.60	2.14±0.91	**0.001****
PLR (%)	107.7±31.8	114.9±32.6	0.199

p values; Independent Samples Test, Statistical significant (*$p<0.05$, **$p<0.01$)
RBC, Red Blood Cells; Hb, Hemoglobin; Hct, Hematocrit; MCV, Mean Corpuscular Volume; RDW, Red cell Distribution Width; WBC, White Blood Cells; NEUT, Neutrophils; LYMPH, Lymphocytes; MONO, Monocytes; EOS, Eosinophils; BASO, Basophils; PLT, Platelets; PDW, Platelet Distribution Width; MPV, Mean Platelet Volüme; PCT, Plateletcrit

31-40 pack/year, group 5: 40+pack/year), NLR was found to increase as pack/year increased (*p*: 0.014). Although PCT was significantly different between smokers and non-smokers (*p*: 0.007), there was no correlation between PCT and pack/years (Table II). No changes were found between other parameters either (Table II). NLR distribution between smokers and non-smokers are shown in Figure 1. A positive linear correlation was detected between pack/year and NLR in smokers (*p*: 0.005) (Figure 2). A similar positive correlation was detected for pack/years *vs.* PDW (*p*: 0.034) (Figure 3).

Discussion

This study evaluated the effect that smoking has on CBC parameters as well as NLR and PLR that are obtained from CBC measurements. Study data has demonstrated that WBC, neutrophil, basophil, eosinophil counts and MCV, PDW and NLR are significantly higher in smokers when compared to non-smokers. NLR ratio and PDW levels were found to correlate with the number of cigarettes smoked daily and the time of smoking.

Although we are unaware of any study evaluating the effect of smoking status on NLR, PLR or platelet indexes, there are studies of the effect of smoking on hematologic parameters. Khand et al[21] compared CBC, C-reactive protein and magnesium (Mg) levels of 48 smokers and non-smokers and found that neutrophil counts were lower and lymphocyte counts were higher in smokers. Additionally, they reported higher CRP and Mg levels in smokers. Ata et al[22] compared CRP levels in 96 smokers *vs.* non-smokers and found CRP to be higher in smokers. In contrast to Khand et al[21], although we found an increase in neutrophil count in smokers, we did not detect a difference in lymphocyte counts. Both studies found smokers to have an increase in CRP – a marker of immune response[22]. Our study found another marker of immune response – NLR, also elevated in smokers.

Lakshmi et al[23] compared hematological and lipid parameters of 40 smokers and non-smokers each and found no statistical difference in neutrophil, lymphocyte or platelet counts. They also grouped smokers as mild, moderate and heavy

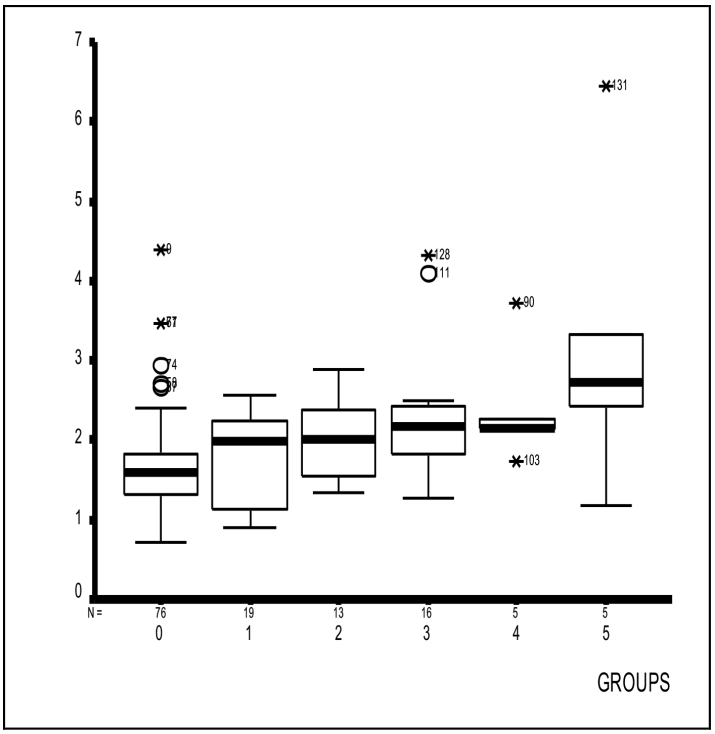

Figure 1. Neutrophil/lymphocyte ratio distribution according to groups in smokers.

smokers and compared hematological parameters between these groups. While there was no statistical difference between lymphocyte counts, a statistically significant increase in WBC and especially neutrophil count was found as pack/year increased. However, this study did not evaluate NLR, PLR or platelet indices. The study reports an increase in neutrophil count in patients with high pack/year yet no change for lymphocytes. Although this data may lead to the conclusion that NLR also increased, the study did not report these findings. Our study differed from Lakshmi et al[23], reporting as we found an increase in neutrophil counts in smokers. However, when pack/year of smokers were evaluated, there was no correlation with neutrophil counts.

There are two reports that indirectly report the link between smoking and MPV[24,25]. Cho et al[24] evaluated the MPV levels of 398 patients without diabetes or hypertension, presenting for checkup and found that those who had stopped smoking had comparatively lower MPV levels. However, they report that lower MPV levels were only statistically significant in women. Another

Table II. The comparison of differences in data values of groups.

	Smokers					
	<10 years N=19	10-19 years N=13	20-29 years N=16	30-39 years N= 5	up 40 years N=5	p
WBC (103/mm3)	7.10±1.42	7.22±1.16	7.52±1.69	7.58±0.62	7.21±1.56	0.907
NEUT (103/mm3)	3.81±1.02	4.13±0.82	4.53±1.11	4.79±0.86	3.52±1.87	0.139
LYMPH (103/mm3)	2.29±0.58	2.13±0.52	2.11±0.70	2.05±0.30	1.46±0.59	0.110
NLR (%)	1.75±0.57	2.00±0.50	2.30±0.83	2.40±0.76	3.21±1.96	0.014*
PLT (103/mm3)	223.1±49.5	231.5±44.6	250.8±39.9	216.0±39.9	195.2±62.7	0.153
PLR (%)	102.3±32.9	114.7±36.5	124.4±27.2	105.3±16.4	123.8±34.6	0.277
PCT (%)	0.20±0.03	0.22±0.04	0.22±0.02	0.19±0.03	0.17±0.05	0.007**
PDW (fL)	12.41±3.00	13.01±2.79	14.95±2.53	12.28±3.15	14.68±3.83	0.085
MPV (fL)	9.11±0.84	9.42±0.77	8.75±0.79	8.80±1.26	8.86±1.45	0.350

p values; One-Way-ANOVA, Statistical significant (*$p<0.05$, **$p<0.01$)

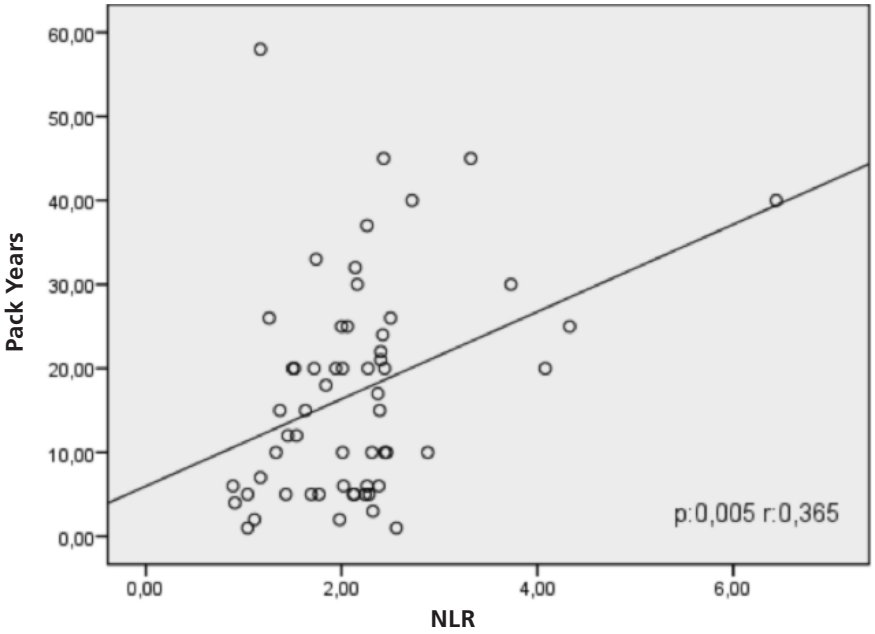

Figure 2. Correlation between smoking density (pack/years) and neutrophil/lymphocyte ratio (NLR).

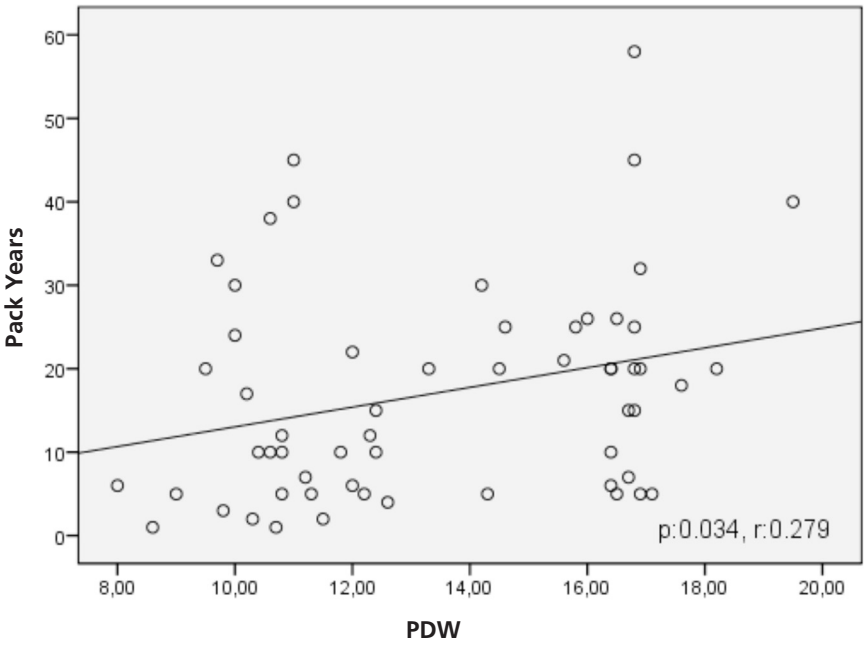

Figure 3. Correlation between smoking density (pack/year) and platelet distribution widht (PDW).

similar study[25] compared MPV levels of 116 smokers (average age 46.3) with 90 healthy volunteers (average age 47.4) and found that when compared to basal levels, MPV was higher in smokers *vs.* Non-smokers (8.8±0.9 *vs.* 8.0±0.8 fL, $p<0.001$). During the study period, 101 smokers quit smoking and their MPV levels were found to be significantly lower compared to their basal levels, 3 m after smoking cessation (8.9±1.0 *vs.* 7.9±0.7 fL, respectively; $p<0.001$). Our study did not find a correlation between smoking and MPV. Ghahremanfard et al[26] evaluated platelet indexes in 542 healthy males according to their smoking status. The study found the average platelet count to be higher and PCT level to be lower in smokers *vs.* non-smokers. Our study has demonstrated that amongst platelet indexes, only PDW was higher in smokers and that PDW increased in correlation with pack/years.

Our study has some limitations. Although inclusion and exclusion criteria were carefully chosen, the retrospective design of this study means

that some of these criteria may not have been noted in patient files. Similarly, the difference between gender distribution between smokers and non-smokers is a result of our retrospective study design.

Although NLR, PLR and platelet indices have been shown to be important for the diagnosis, prognosis and severity of some diseases, the smoking status of patients in these studies has not been well defined.

Conclusions

Our study has shown that NLR increases in correlation with pack/year while PLR is not affected and PDW is increased in smokers. If smokers are not excluded from studies evaluating NLR and PDW, the relationship between smoking status as well as pack/year must be determined and reported.

Conflicts of interest

The authors declare no conflicts of interest.

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