The relationship between smoking degree based on the Brinkman index with the neutrophil lymphocyte ratio, lymphocyte platelet ratio and serum MPV/platelet values in healthy adult smokers

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ABSTRACT

Smoking has been shown to increase systemic inflammation. Platelet lymphocyte ratio (PLR) and mean platelet volume (MPV) are indicators of increased platelet activation associated with venous thromboembolic events (VTE). We aim to determine the relationship between smoking degree based on the Brinkman index and the level of inflammation. An observational analytic study using a cross-sectional approach was conducted from October to December 2019 involving all adult smokers in the city of Denpasar, Bali, who meet the inclusion and exclusion criteria. Correlation test was used to determine the relationship between smoking degrees and NLR, PLR, and MPV/platelets in the subject. It was found that the research subjects had an average age of 37.13 years. Brinkman Index (IB) obtained IB 25-1000 (average 275.5506), with the ratio of mild: moderate: severe degrees are 44:45:11 people. The serum NLR value was 0.09 to 4.49 (mean 1.6633), PLR value was 47.97 to 188.81 (mean 104.4154), and MPV/Plt value 0.02 to 0.08 (mean 0.03). The degree of smoking based on the Brinkman Index in 100 samples, consisting of 44% of light smokers, 45% of moderate smokers and 11% of heavy smokers. Heavy smokers have higher NLR and PLR and lower MPV/Plt. There was no significant relationship between smoking degrees and NLR, PLR, and MPV/Plt (P = 0.494: 0.557: 0.776). All smokers classified as normal nutritional status (22%) and overweight (78%). The Spearman test showed that there was a significant relationship between smoking age and smoking degree (P = 0.000), but none for nutritional status (BMI) and smoking degree (P = 0.155). Pearson correlation test showed that the age of the smoker have no relationship with the ratio of NLR, PLR and MPV/Platelet (P = 0.670: 0.878: 0.229). T-Test result showed no relationship between NLR and PLR with nutritional status (P = 0.074: 0.116). The Mann-Whitney test also showed no relationship between MPV/Plt and nutritional status (P = 0.498). In conclusion, the higher the degree of smoking based on the Brinkman index, the higher the NLR and PLR values. There was no significant relationship between smoking degrees based on the Brinkman Index and serum NLR, PLR, and MPV/Platelet in healthy adult smokers.

Keywords: Smoking degrees, Brinkman index, neutrophil lymphocyte ratio, lymphocyte platelet ratio, serum MPV/platelet.

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INTRODUCTION

Cigarettes are the number 3 killer in the world after heart disease and cancer. Based on data from the World Health Organization, smoking causes 6 million deaths worldwide each year. Passive smoking has a higher risk
of suffering from cardiovascular disease, lung cancer and other diseases (WHO, 2018).

Smoking has been shown to increase systemic inflammation. Neutrophil lymphocyte ratio (NLR) is an indicator of subclinical inflammation (Alan et al., 2015; Ljungstrom et al., 2017; Tanacan et al., 2020). Platelet lymphocyte ratio (PLR) and mean platelet volume (MPV) are indicators of increased platelet activation associated with venous thromboembolic events (VTE). NLR, PLR, and MPV examinations to determine the degree of systemic inflammation and as prognosis indicators of critical patients during postoperative and intensive care have also been reported (Djordjevic, 2018; Lee, 2018; Tanacan et al., 2020). One study reported improved blood test results 5 years after quitting smoking. Several previous studies reported reduced leukocyte percentage and platelet activity in smokers (Forget, 2017).

Cigarette smoke can activate the release of inflammatory mediators such as cytokines, TNFα, and attract monocytes and neutrophils to the area of inflammation (Franco et al., 2015; Kastelein et al., 2017). Platelets and leucocytes are reported to play a role in hemostatic processes and immunity. Several studies have found platelets to be a central factor in the inflammatory process, which can influence leukocytes and inflammatory signaling. Platelets interact with granulocytes, blood vessel walls, and pathogens, regulating the inflammatory response through both anti-inflammatory and pro-inflammatory mechanisms (Franco et al., 2015; Salciccioli, 2015; Sonmez and Sonmez, 2017).

Research on the relationship between smoking degree and NLR, PLR, and serum MPV/platelet ratio is still very limited. We aimed to determine the relationship between smoking degrees based on the Brinkman index with lymphocyte neutrophil ratio, lymphocyte platelet ratio, and serum MPV/platelet ratio. This research was conducted in the city of Denpasar, Bali, in October-December 2019. The research sample was all adult smokers in the city of Denpasar, Bali, who met the inclusion criteria and agreed to participate in the study. The sample size was calculated according to the correlation coefficient formula, the minimum sample size was 41 samples. The mean age of the sample was 39.1 years.

**Materials and Methods**

This study was an observational analytic study using a cross-sectional design to determine the relationship between smoking degrees based on the Brinkman index with lymphocyte neutrophil ratio, lymphocyte platelet ratio, and serum MPV/platelet ratio. This research was conducted in the city of Denpasar, Bali, in October-December 2019. The research sample was all adult smokers in the city of Denpasar, Bali, who met the inclusion criteria and agreed to participate in the study. The sample size was calculated according to the correlation coefficient formula, the minimum sample size was 41 samples. The mean age of the sample was 39.1 years.

**Results**

This study involved 100 research subjects who were adult active smokers (≥ 18 years) in the city of Denpasar who were collected from October to December 2019 using consecutive sampling technique. The research subjects consisted of 100 men (100%).

**Samples characteristics**

The research subjects had an average age of 37.13 years, with an age range of 20 to 79 years. Calculation of the Brinkman Index (IB) based on the multiplication of
length of smoking in years with the number of cigarettes per day obtained IB 25-1000 (average 275.5506), with the ratio of mild: moderate: severe degrees is 44:45:11 people. Body Mass Index (BMI) ranged from 17.30 to 41.23 (average 25.3501). As many as 22% of subjects with normal nutritional status, and 78% with excess nutritional status. From the research, it was found that the serum NLR value was 0.09 to 4.49 (mean 1.6633), PLR value was 47.97 to 188.81 (mean 104.4154), and MPV/Plt value 0.02 to 0.08 (mean 0.03) (Table 1).

The relationship between smoking degree and NLR, PLR, and MPV / Plt

The frequency distribution of smoking degrees based on the Brinkman Index for the 100 research samples can be seen in Table 2. Table 3 shows the NLR, PLR, and MPV/Platelet values based on smoking degree (Brinkman Index). Heavy smokers have higher NLR and PLR and lower MPV/Plt than light and moderate smokers. The bivariate correlation test using the Pearson correlation test was used to determine the relationship between smoking degrees and NLR, PLR, and MPV/Plt because the data were normally distributed (Table 4). It was found that there was no significant relationship between smoking degrees and NLR, PLR, and MPV/Plt (P = 0.494: 0.557: 0.776).

Relationship between smoking degree and age and BMI (nutritional status)

The study sample consisted of 22 people (22%) with a BMI of 18.5 to 24.9 (normal nutritional status), and 78 people (78%) with a BMI of 25 to 29.9 (excess nutritional status). Among the 22 people with normal nutritional status, 54.5% were light smokers, 27.3% were moderate smokers, and only 18.2% were heavy smokers. Meanwhile, among 78 people with excess nutritional status, 41% were light smokers, 50% were moderate smokers, and only 9% were heavy smokers. The Spearman correlation test was conducted to assess the relationship between smoking degrees and age of the smoker and nutritional status (BMI) because the data were not normally distributed. The Spearman test results showed that there was a significant relationship between smoking age and smoking degree (P = 0.000), but there was no significant relationship between nutritional status (BMI) and smoking degree (P = 0.155) (Table 5).

Table 1. Characteristic of the research samples (N = 100).

| Characteristic       | Mean ± SD | Minimum | Maximum |
|----------------------|-----------|---------|---------|
| Age (year)           | 37.13 ± 12.778 | 20      | 79      |
| Smoking duration (year) | 16.49 ± 10.546 | 1       | 50      |
| Number of cigarettes/day | 16.17 ± 6.933 | 4       | 30      |
| BW (kg)              | 72.46 ± 11.016 | 50      | 115     |
| BH (cm)              | 169.04 ± 4.544 | 155     | 185     |
| BMI (kg/m²)          | 25.3501 ± 3.69021 | 17.3    | 41.23   |
| RBS (mg/dl)          | 100.97 ± 14.432 | 70      | 128     |
| Neutrophil (10³/µl)  | 4.5205 ± 1.31931 | 0.25    | 9.04    |
| Lymphocyte (10³/µl)  | 2.8971 ± 0.80905 | 1.34    | 4.80    |
| Platelet (10³/µl)    | 281.36 ± 51.880 | 154     | 429     |
| MPV                   | 10.0770 ± 0.74696 | 8.60    | 12.20   |
| Brinkman index       | 275.5506 ± 221.09156 | 25      | 1000    |
| NLR                  | 1.6633 ± 0.63355 | 0.09    | 4.49    |
| PLR                  | 104.4154 ± 33.14981 | 47.97   | 188.81  |
| MPV/Plt              | 0.0373 ± 0.000879 | 0.02 (2%) | 0.08 (8%) |

*BW: body weight, BH: body height, BMI: body mass index, RBS: random blood sugar, MPV: mean platelet volume, NLR: neutrophil lymphocytes ratio, PLR: platelet lymphocyte ratio.

Table 2. Distribution sample data based on Brinkman Index.

| Brinkman index | Frequency (people) | Percentage (%) |
|----------------|--------------------|----------------|
| Light          | 44                 | 44             |
| Moderate       | 45                 | 45             |
| Heavy          | 11                 | 11             |
Relationship between NLR, PLR and MPV/Plt with age of smokers and nutritional status (BMI)

Pearson correlation test was carried out to assess the relationship between NLR, PLR and MPV/Plt with Smoker's Age and Nutritional Status (BMI) because the data were normally distributed, it was found that the age of smokers had no statistically significant relationship with the ratio of NLR, PLR and MPV/Platelet (P = 0.670: 0.878: 0.229). This can be seen in Table 6.

All smokers, whether mild, moderate, or severe, had a classification of normal nutritional status (22%) and excess nutritional status (78%). Table 7 shows the distribution of NLR, PLR, and MPV/Platelet data based on nutritional status. Samples with excess nutritional status had lower mean NLR and PLR and higher MPV/Plt than samples with normal nutritional status. Samples with excess nutritional status had an average NLR of 1.6150, and an average PLR of 101.9070, while the MPV/Platelet values in samples with excess nutrition had an average of 0.0368. T test was carried out on the relationship between NLR and PLR with nutritional status, it was found that there was no significant relationship (P = 0.074: 0.116). The results of the correlation test with the Mann-Whitney test also showed no relationship between MPV/Plt and nutritional status (P = 0.498). This can be seen in Table 8.

DISCUSSION

The research subjects are characterized by an average age of 37.13 years, with an age range of 20 to 79 years. These data are consistent with the study of Jitnarin et al. (2014). In Italy, the mean age of male smokers was 47.1 ± 20.8 years. Lee (2018) study on smokers in North Korea also reported that adult male smokers had a median age of 47 years. Duration of smoking has a range of 1-50 years (mean 16.49). Body Mass Index (BMI) ranged from 17.30 to 41.23 (average 25.3501). This is

Table 3. Distribution of NLR, PLR, and MPV/platelet values based on smoking degree.

| Ratio  | Smoking degree | Mean    | SD     | Confidence interval (95%CI)     | Range   |
|--------|----------------|---------|--------|---------------------------------|---------|
| NLR    | Light          | 1.7715  | 0.62786| 1.5806-1.9624                   | 0.88-3.61|
|        | Moderate       | 1.5163  | 0.50269| 1.3653-1.6674                   | 0.09-2.51|
|        | Heavy          | 1.8314  | 0.99347| 1.1640-2.4988                   | 1.00-4.49|
| PLR    | Light          | 110.5555| 35.51736| 99.7572-121.3537                | 57.25-188.52|
|        | Moderate       | 95.7631 | 27.35666| 87.5442-103.9819                | 47.97-153.97|
|        | Heavy          | 115.2509| 39.11548| 88.9727-141.5290                | 71.39-188.81|
| MPV/Plt| Light          | 0.367   | 0.00982| 0.0338-0.0397                   | 0.02-0.08 |
|        | Moderate       | 0.380   | 0.00817| 0.0355-0.0404                   | 0.03-0.06 |
|        | Heavy          | 0.365   | 0.00879| 0.0317-0.0414                   | 0.03-0.05 |

*MPV: mean platelet volume, NLR: neutrophil lymphocytes ratio, PLR: platelet lymphocyte ratio.

Table 4. The relationship (correlation) between smoking degrees with NLR, PLR, and MPV / Plt based on the Pearson correlation test (significant correlation if P ≤ 0.01).

| Correlation | NLR     | PLR     | MPV/Plt |
|-------------|---------|---------|---------|
| Brinkman Index | Pearson correlation | -0.069 | -0.059  | -0.0390 |
| Sig (2-tailed) | 0.494  | 0.557   | 0.776   |
| N           | 100     | 100     | 100     |

Table 5. The relationship between smoking degree and smoker's age and nutritional status (BMI) based on the Spearman correlation test.

| Correlation | Age | Nutritional status (BMI) |
|-------------|-----|--------------------------|
| Brinkman Index | 0.701 | 0.143                    |
| Sig (2-tailed) | 0.000 | 0.155                    |
| N          | 100  | 100                      |
consistent with the data in the study of Jitnarin et al. (2014), that the BMI of male smokers is 21.6 ± 3.4 kg/m². Data in Lin’s study also reported that the mean BMI of male smokers was 25.47 kg/m² (Lin, 2016). The Spearman test results showed that there was a significant relationship between age and smoking degree (P = 0.000). The higher the age the higher the degree of smoking because the longer smoking time is accumulative and will affect the calculation of smoking degrees. This is in accordance with Lin's study of smokers in the Netherlands, that the accumulation of pack years increases with increasing age of the smoker (Lin, 2016). In this study found that smokers with excess nutritional status dominate in every degree of smokers. This is in accordance with the study by Taylor (2019). That increasing the intensity or severity of smoking is associated with an increase in BMI.

The NLR value is known to be a predictor of mortality in patients with acute coronary syndrome and some cancers. This marker has also been reported as a prognostic predictor of critical illness in patients in the intensive unit. In general, a high NLR is associated with a high mortality rate and a poor prognosis for a condition or disease (Tanacan et al., 2020). Based on Aksoy’s study of exacerbated COPD patients, the cut-of-point mortality for the NLR value was 8.01 10³/µl, with a sensitivity of 78% and a specificity of 60%. The cut-of-point mortality PLR value was 228.87 10³/µl. In this study, the highest average NLR found in heavy smokers (1.8314 10³/µl). This data is consistent with the data in Lee (2018) study that the mean NLR value across all ages was 1.65 10³/µl. The PLR value in this study was higher than the PLR in Lee (2018) study and consistent with Lin (2016) study. The MPV results in this study are in accordance with the MPV in Lee (2018) study, which is an average of 10.02. The highest range of NLR values was found in heavy smokers compared to normal NLR values in healthy people. The highest PLR value range was also found in heavy smokers (71.39 to 188.81 10³/µl), compared to the PLR value in healthy people (46.794 to 218.006 10³/µl), but with a smaller mean. Meanwhile, the MPV value of the dominant study subjects was greater than the range of the normal MPV value in healthy people. The range of MPV/Plt ratios in the study was inversely related to NLR and PLR, with the lowest values being found for heavy smokers (0.03 to 0.05).

In this study, it was reported that in heavy smokers, the average NLR and PLR values were higher and the mean MPV/Plt values were lower than light and moderate smokers. These data are in accordance with the results of research by Tulgar (2016). There is a positive linear correlation between pack year and NLR. The higher the

Table 6. Relationship between NLR, PLR, and MPV / Plt with age of smoker.

| Correlation     | NLR   | PLR   | MPV/Plt |
|-----------------|-------|-------|---------|
| Age of smoker   |       |       |         |
| Pearson correlation | -0.003 | -0.031 | 0.110   |
| Sig (2-tailed)  | 0.670 | 0.878 | 0.229   |
| N               | 100   | 100   | 100     |

Table 7. Distribution of NLR, PLR, and MPV / platelet based on nutritional status (BMI).

| Ratio | Nutritional status | Mean   | SD     | Confidence interval (95%CI) | Range       |
|-------|--------------------|--------|--------|-----------------------------|-------------|
| NLR   | Normal             | 1.8345 | 0.81474| 1.4733-2.1957               | 0.93-4.49   |
|       | Overweight         | 1.6150 | 0.56947| 1.4866-1.7434               | 0.09-3.61   |
| PLR   | Normal             | 113.3087 | 38.84443 | 96.0861-130.5314         | 57.25-188.81|
|       | Overweight         | 101.9070 | 31.18329 | 94.8762-108.9377        | 47.97-183.60|
| MPV/Plt| Normal             | 0.0388 | 0.01078 | 0.0340-0.0435            | 0.02-0.08   |
|       | Overweight         | 0.0368 | 0.00818 | 0.0350-0.0387            | 0.02-0.06   |

Table 8. Relationship between NLR, PLR, and MPV / Plt with nutritional status (BMI).

| Correlation     | NLR   | PLR   | MPV/Plt |
|-----------------|-------|-------|---------|
| Nutritional status |      |       |         |
| Coefisien correlation | 0.043 | 0.016 | 0.121   |
| Sig (2-tailed)  | 0.450 | 0.444 | 0.442   |
| N               | 100   | 100   | 100     |
degree of smoking based on the pack year, the higher the NLR. However, after doing the Pearson correlation test, it was found that there was no significant relationship between smoking degrees and NLR, PLR, and MPV/Platelet in this study (P = 0.494: 0.557: 0.776). This is consistent with the results of the study by Tulgar (2016), that there was no significant relationship between smoking and NLR and PLR, possibly due to the small number of samples in their study. Tulgar (2016) research reference data also states that in studies with large samples, an increase in NLR was reported in smokers.

The results of the analysis test showed that there was no significant relationship between NLR, PLR, and MPV/Platelet, possibly because these inflammatory markers were examined in peripheral blood (systemic). Cigarette exposure will first hit the respiratory tract (local in nature), so the inflammatory reaction will first occur locally in the respiratory tract before going systemic. In Karim's study, on Broncho alveolar lavage (BAL) in adult smokers in Germany, it was found that the lymphocyte and neutrophil cell counts of smokers had a positive correlation with the accumulation of smoking habits. The concentration of all inflammatory cells was reported to be significantly increased in smokers compared to non-smokers or ex-smokers. Experiments on mice exposed to cigarette smoke for a certain time by Qiu (2017). Showed that, there was an increase in the frequency and number of neutrophils, and a decrease in the number of lymphocytes in the airway LAB of mice exposed to cigarette smoke compared to mice not exposed to cigarette smoke. Apart from modulating respiratory immune cells, cigarette smoke is also said to modulate the profiles of airway cytokines, such as IL-1β, IL-6, and TNF-α. This suggests that smoking has an effect on LAB fluid cells, depending on smoking history, which can have clinical implications. In this study, LAB examination was not carried out due to the difficulty of investigating LAB in healthy people in the general population. Complications arising from the long-term effects of cigarette smoke exposure often produce contradictory results, referring to the variety of cigarette smoke chemicals and modes of exposure. The amount of total particulate matter inhalation (TPM) has a major influence on the natural response and the continuation of the body's immune process to cigarette smoke exposure. Therefore, a partial correlation test of the independent and dependent variables was carried out on the confounding variables studied in this study, namely the age of the smoker and nutritional status (BMI).

The relationship between age and NLR, PLR, and MPV/Platelet ratio was tested using the Spearman correlation test which showed that there was no relationship between age and NLR, PLR, or MPV/Platelet ratio. The results of this study differ from those of Lin (2016) who reported that increasing age was associated with increased NLR and PLR. This is because age reflects the underlying condition or disease in the older population, even though in that study the sample is the immunologically healthy population and the sample health data. In this study, there was no possible relationship because the average age of the sample was 37.13 years, or under 40 years, so that the general condition was still good.

The T test for NLR and PLR based on nutritional status, and the Mann-Whitney test for MPV/Platelet ratio on nutritional status found that both NLR, PLR and MPV/Platelet had no significant relationship with nutritional status (P = 0.074: 0.116: 0.498). This is consistent with the results of Lin (2016) study, that in male smokers, NLR and PLR were not affected by BMI, but age and BMI were simultaneously associated with increased NLR and PLR. This is due to a lower BMI at older age.

CONCLUSION

In conclusion, the higher the degree of smoking based on the Brinkman index, the higher the NLR and PLR values. There was no significant relationship between smoking degrees based on the Brinkman Index and serum NLR, PLR, and MPV/Platelet in healthy adult smokers.

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