# The Effects of Cigarette Smoking on Peripheral Blood Leukocytes and Lymphocyte Subpopulations: An Urban Population-Based Study in Thailand

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**Abstract**

Preliminary studies for peripheral blood leukocytes and lymphocyte subsets were done in smokers and non-smokers. There were 20 smokers (smoked more than 10 cigarettes per day) for more than a year and 20 non-smokers (smoked less than 20 cigarettes/20 years). Ages of smokers and non-smokers were respectively 21-57, and 18-55 years.

Cigarette smoking was associated with a statistically significant increase in the number of neutrophils, activated lymphocytes, CD25 and CD19; but a statistical decrease in the percentage of CD7 and CD3. ($P<0.05$)

**Key word** : Cigarette Smoking, Leukocytes, Lymphocyte Subsets, Flow Cytometry

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A cigarette is one of the narcotics, consisting of over 4,000 chemical agents. The toxic substances in the cigarette that are detrimental to health are nicotine, carbon monoxide, and nitrous oxide to name a few. The amount of these substances received depends on the number of cigarettes smoked per day and the duration of smoking. A previous report has shown, that individuals who

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began smoking at the age of 25 and continued smoking 20 cigarettes a day had a shorter life expectancy of 4.6 years. If the number of cigarettes is doubled, it will be shortened by 8.8 years[1]. In addition, the duration of smoking is strongly associated with squamous cell carcinoma of the lungs[2-4]. The incidence of lung cancer is highest in patients over 60 years old, followed by individuals aged 40-60 years. Smoking does not only cause lung cancer, it is also the etiology of cardiovascular diseases, especially coronary artery disease, and chronic obstructive pulmonary disease[4]. Lung cancer related death was originally reported in male smokers but was later found in nonsmoking women, who were exposed to the carcinogens from cigarette smoke and nitrosamine as well[3]. Changes in the type and number of lymphocytes due to the mobilization of inflammatory cells from the blood to the inflamed respiratory mucosa[7] may affect the immune system of the body. However, there are only a few studies, with conflicting results, regarding the status of the immune system and the type and number of lymphocytes in the blood of smokers.

We, hereby, studied the effects of smoking on type and number of leukocytes and lymphocyte subsets in Thai smokers.

## MATERIAL AND METHOD

Forty subjects were voluntarily enrolled into the study. They were divided into two groups. Group I consisted of twenty smokers, who had smoked more than 10 cigarettes a day for more than a year. Twenty non-smokers (<20 cigarettes/20 years) comprised group II. All subjects were male, 17-59 years of age with no known chronic lung or respiratory diseases, resided in Bangkok; and had no history of autoimmune disorders or diseases that might affect the immune functions. The median ages of the smokers and non-smokers were respectively 39 and 29 years. The number of cigarettes and duration of smoking in smokers are shown in Tables 1 and 2.

**Table 1. The number of cigarettes smoked per day in the smoker group.**

| Number of cigarette | | Total |
|---|---|---|
| 11 - 20 | ≥21 | |
| 17 (85.0%) | 3 (15.0%) | 20 (100%) |

**Table 2. The number of years of cigarette smoking in the smoker group.**

| Number of years of cigarette smoking | | | | Total |
|---|---|---|---|---|
| 1-5 years | 6-10 years | 11-15 years | ≥16 years | |
| 1 (5.0%) | 3 (15.0%) | 7 (35.0%) | 9 (45.0%) | 20 (100%) |

General physical examination, chest X-ray and serology for HIV were the primary screening methods.

Blood collection : Five ml. of clotted blood was used for HIV serology, 2ml EDTA blood for CBC by automated Cell Dyne (Abbott Diagnostics, Mountain view, CA 94043), and 2ml EDTA for lymphocyte subset analysis.

The number of various lymphocyte subsets in this study were determined by FACSCan flow cytometer (Beckton-Dickinson). The following fluorochrome conjugated monoclonal antibodies to human leukocyte antigens were obtained commercially from DAKO : CD3, CD4, CD8, CD11a, CD16, CD25, (CD3+/CD25+), (CD7+/CD4+), (CD7+/CD4-), CD19, and (CD8+/CD11a+).

Assay for lymphocyte subsets was done according to Lul et al[8]. Briefly, one hundred ml of EDTA whole blood was stained by each pair of fluorescent conjugated monoclonal antibodies. After twenty minutes of incubation in the dark at room temperature, red blood cells were lysed with 2 ml of FACS lysing solution (Beckton-Dickinson) for 10 minutes in the dark at room temperature, and washed twice with phosphate buffer saline (pH 7.6). The pellets were resuspended in 0.5 ml of 1 per cent paraformaldehyde at 4°C until analysis on a FACScan flow cytometer (Becton Dickinson). $IgG_1$ FITC/$IgG_{2a}$PE was used as a negative control. The typical forward and side scatter gate together with a CD45/CD14 gate were set to exclude non lymphocytic cells. Fifteen thousand events within this gate were acquired per sample. Two parameter histograms demonstrating surface markers were created using simulset software. Statistical analysis of the number of various lymphocyte subsets between smokers and non-smokers was performed using Kruskal-Wallis test. ($P < 0.05$)

## RESULTS

The percentages of total white blood cells, neutrophils, lymphocytes, monocytes, eosinophils,

**Table 3. Statistical differences in the total white blood cell counts, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and lymphocyte subsets between smoker and non-smoker groups.**

| | | | Smoker | Non-smoker |
|---|---|---|---|---|
| Total white blood cell count | Median | | 6.4 | 5.5 |
| | Percentile | 5-95 | 4.4-8.6 | 3.4-9.4 |
| Neutrophil (%) | Median | | 57.0 [a] | 47.0 |
| | Percentile | 5-95 | 45.5-75.0 | 33.5-66.0 |
| Lymphocyte (%) | Median | | 34.0 | 36.5 |
| | Percentile | 5-95 | 17.5-46.0 | 26.0-52.0 |
| Monocyte (%) | Median | | 4.0 | 5.0 |
| | Percentile | 5-95 | 3.0-7.0 | 3.0-9.5 |
| Eosinophil (%) | Median | | 3.0 | 3.5 |
| | Percentile | 5-95 | 1.0-7.0 | 1.0-9.0 |
| Basophil (%) | Median | | 1.0 | 0 |
| | Percentile | 5-95 | 0-2.0 | 0-1.5 |
| CD4 (%) | Median | | 36.0 | 36.0 |
| | Percentile | 5-95 | 30.5-45.5 | 31.5-44.0 |
| CD7 (%) | Median | | 67.5 [a] | 74.0 |
| | Percentile | 5-95 | 50.0-77.0 | 60.5-82.5 |
| TH1 (CD7+/CD4+) (%) | Median | | 29.5 | 29.0 |
| | Percentile | 5-95 | 22.0-39.0 | 25.0-37.0 |
| TH2 (CD7-/CD4+) (%) | Median | | 7.0 | 7.5 |
| | Percentile | 5-95 | 3.0-18.0 | 4.0-11.0 |
| CD19 (%) | Median | | 14.0 [a] | 11.0 |
| | Percentile | 5-95 | 10.0-23.5 | 8.0-29.0 |
| Activated lymphocyte | Median | | 5.0 [a] | 3.0 |
| (CD3+/CD25+) (%) | Percentile | 5-95 | 2.0-8.5 | 1.0-5.5 |
| CD3 (%) | Median | | 60.0 [a] | 64.5 |
| | Percentile | 5-95 | 42.5-72.0 | 53.5-72.5 |
| CD25 (%) | Median | | 6.0 [a] | 4.0 |
| | Percentile | 5-95 | 2.0-8.5 | 2.0-6.5 |
| CD8 (%) | Median | | 32.5 | 33.5 |
| | Percentile | 5-95 | 16.0-36.5 | 24.0-38.0 |
| CD11a (%) | Median | | 51.5 | 57.0 |
| | Percentile | 5-95 | 29.0-76.5 | 40.5-77.0 |
| Cytotoxic T-cell | Median | | 24.0 | 24.0 |
| (CD8+/CD11a+) (%) | Percentile | 5-95 | 9.0-31.0 | 14.5-33.5 |
| Suppressor T-cell | Median | | 8.0 | 7.0 |
| (CD8+/CD11a-) (%) | Percentile | 5-95 | 4.0-13.0 | 2.0-13.5 |
| NK (CD16+) cell (%) | Median | | 15.5 | 15.0 |
| | Percentile | 5-95 | 8.5-23.0 | 6.0-26.0 |

[a] Statistically significant difference ($P<0.05$)

basophils and lymphocyte subsets were shown in Table 3.

The smoker group had a statistically significant increase in the percentage of neutrophils, activated lymphocytes (CD3+/CD25+), CD25, and CD19; but a statistically decrease in the percentage of CD7 and CD3, when compared to the non-smoker group. (Table 4) No statistical differences in the percentages of total lymphocyes, monocytes, eosinophils, basophils, CD4(+) T cells, T-helper 1 (TH1) cells (CD7+/CD4+), T-helper 2 (TH2) cells (CD7-/CD4+), CD8, CD11a, cytotoxic T-cells, suppressor-T cells and NK-cells. (Table 3)

## DISCUSSION

Our results showed that smokers, defined as smoking more than 10 cigarettes a day for more than 10 years, had a higher number of neutrophils than the non-smokers. Similar results have been reported by Tadashi et al[9]. Other changes noted in our studies included an increase in the number of activated lymphocytes (CD3+/CD25+) and CD19+

B-lymphocytes; and a decrease in CD7+ and CD3+ T-cells. Our studies also indicated no changes in other white blood cell components, e.g. monocytes, eosinophils, basophils, lymphocytes and cytotoxic/suppressor cells and NK cells along with the lymphocyte adhesion molecule (CD11a) in the smoker group. The above findings suggest a predominant and crucial role of neutrophils, not monocytes, eosinophils, basophils, lymphocytes, cytotoxic/suppressor and NK cells in causing tissue damages and chronic lung diseases in heavy smokers.

Corre and Taylor have shown that destruction of lung tissues was proportional to the increase in number of neutrophils[11,12]. The mechanism might be due in part to the chemotactic factor, IL-8, which was secreted from alveolar macrophages after phagocytosis of foreign substances. IL-8 will attract neutrophils from the bloodstream to dispose foreign substances from cigarettes the lungs[9,13,14]. In addition, nicotine caused the neutrophils in the pulmonary microcirculation to be sequestered longer and suppressed apoptosis[15]. This process resulted in the increased number and life span of neutrophils in lung interstitium and bronchoalveolar spaces[16,17], and caused the loss of equilibrium in the release of toxic substances such as $O_2$ radical and hemolytic enzymes and finally the destruction of the lung tissues[18]. A decrease in CD3+/CD7+ T-cells and an increase in CD19+ B-cells in the smoker group was secondary to the inhibitory effect of T-cell mitogenesis by tar, and P-benzoquinone, a thiol-reactive benzene derivatives in cigarette, whose action is to block a thiol-dependent event that controls IL-2 production[19]. There was no difference in the number of various subsets of CD4, CD8, TH1 and TH2 lymphocytes, NK cells, suppressor cells, cytotoxic cells, and the ratio of CD4/CD8. This study showed that smoking had no effect on the T lymphocyte-mediated immune response except for CD3 and CD7. This finding was different from previous reports in Caucasians, which showed an increased CD4[20,21]. The difference could be due to race, or substances in the cigarettes, which might contribute to different immune responses. Further investigation is needed.

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# ผลของการสูบบุหรี่ต่อเม็ดเลือดขาวและเม็ดเลือดขาวชนิดย่อย การศึกษาในชนบทในประเทศไทย

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การศึกษาเบื้องต้นเกี่ยวกับจำนวนเม็ดเลือดขาว และชนิดย่อยของลิมท์โฟซัยท์ในเลือดระหว่างกลุ่มสูบบุหรี่ 20 ราย อายุ 21–57 ปี (สูบบุหรี่มากกว่า 10 มวนต่อวัน) และกลุ่มไม่สูบบุหรี่ 20 ราย อายุ 18–55 ปี (สูบบุหรี่น้อยกว่า 20 มวนภายใน 20 ปี) พบว่ากลุ่มสูบบุหรี่มีการเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติของอัตราร้อยละของนิวโตรฟิล, ลิมท์โฟซัยท์ที่ถูกกระตุ้น, ซีดี 25, และซีดี 19 แต่มีอัตราร้อยละของ ซีดี 7 และซีดี 3 ลดลง เมื่อเทียบกับกลุ่มไม่สูบบุหรี่ ($P < 0.05$)

**คำสำคัญ** : การสูบบุหรี่, เม็ดเลือดขาว, เม็ดเลือดขาวชนิดย่อย, โฟล ซัยโตเมตรี

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