

RESEARCH ARTICLE

Evaluation of the sequential effect of quantity of cigarettes smoked per day on differential leukocyte count among North Eastern Indian adult males

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ABSTRACT


Background: Cigarette smoking is an established risk factor for various cardiovascular disorders such as ischemic heart disease and systemic hypertension. **Aims and Objectives:** A study was conducted among male smokers of Tezpur, Assam, to ascertain the relation between number of cigarettes smoked per day and the sequential changes in various components of differential leukocyte counts. **Materials and Methods:** A total of 125 consenting participants of which 100 smokers and 25 non smokers were studied. Male smokers, based on number of cigarettes smoked per day, were divided into four groups of sequentially increasing cigarettes smoked per day. Blood samples were processed using hematology analyzer (ABX Micros60[®], HORIBA, Kyoto, Japan). **Results:** It was found that smokers had a significantly different percentage of neutrophils, lymphocytes, monocytes, and eosinophils ($P < 0.001$). This effect was significant irrespective of the number of cigarettes compared to the controls. There was no significant change in the percentage of basophils. **Conclusion:** Cigarette smoking has a significant effect on differential cell counts which changed significantly with increasing number of cigarettes smoked per day among North Eastern Indian male smokers.

KEY WORDS: Smoking; Differential Leukocyte Count; Neutrophils; Lymphocytes; Eosinophils; Basophils; Monocytes

INTRODUCTION

Addiction to tobacco occurs in various forms such as smoking, chewing, or by nasal sniffing of dry snuff. The most common form of tobacco consumption is by smoking manufactured cigarettes.^[1,2] Tobacco smoke is linked to oxidative damage to the lungs.^[3] Smokers have been found to have altered hematological parameters.^[4,5] Smoking adversely affects inflammation and hemostasis by higher viscosity, tissue

plasminogen activator antigen, and lower albumin levels.^[6] Recurrent ischemic events are associated with higher leukocyte and granulocyte counts.^[7] Elevated white blood cell counts are strongly associated with the formation of carotid atherosclerotic plaques.^[8] Among patients with chest pain and early coronary artery disease, smokers had increased levels of neutrophils, lymphocytes, and monocytes when compared to non-smokers.^[9] A large epidemiological study conducted among 4264 male smokers demonstrated a significant increase in leukocytes along with an increase in granulocytes, lymphocytes, and monocytes.^[10] It has been found that peripheral differential counts in humans vary with smoking and body mass index.^[11] The effect of an incremental number of cigarettes smoked per day on various cell counts has been documented by a limited number of studies. The present study ascertains the effect of a number of cigarettes smoked on the differential leukocyte count.

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MATERIALS AND METHODS

Study Design

A study comparing male non-smokers and male smokers from Tezpur, Assam, was performed between November 2013 and January 2014.

Study Subjects

A total of 125 healthy male participants consisting of 25 non-smokers and 100 smokers divided into four groups of 25 each with various reported number of cigarettes smoked were planned to be enrolled into the study after written informed consent. All participants included in the study were employed in tea estates in and around Tezpur. All prospective study participants reported their age, comorbidities, concurrent medications, present smoking status, history of blood transfusion, and the total number of cigarettes presently being smoked per day which was recorded on a pre-specified bilingual format.

Inclusion Criteria

The following individuals were included:

- Apparently healthy males
- Age between 25 and 40 years.

Exclusion Criteria

The following individuals were excluded:

- Any comorbidities (such as diabetes mellitus, systemic hypertension, and thyroid disease)
- Any known allergic conditions
- Any concurrent infection
- Individuals on concurrent medication for any comorbidity
- History of recent blood transfusion/donation (in last 6 months).

Based on the reported data, 25 non-smokers and 100 smokers were sequentially included in the study. Hence, five groups of male study participants were identified as follows:

- Group A - Non-smokers
- Group B - Smokers (1-5 cigarettes/day)
- Group C - Smokers (6-10 cigarettes/day)
- Group D - Smokers (11-20 cigarettes/day)
- Group E - Smokers (more than 20 cigarettes/day).

Study Protocol

After obtaining written informed consent, a total of 5 ml of venous blood sample was collected from all study participants in the morning between 8 am and 11 am after a period of fasting of at least 10 h. All blood samples were collected in ethylenediaminetetraacetic acid (EDTA) vacutainers (plastic whole blood tube with spray-coated K2EDTA, BD® Vacutainer, Becton Dickinson and Company, New

Jersey, USA). All samples were processed using hematology analyzer (ABX Micros60®, HORIBA, Kyoto, Japan). The following blood parameters of differential leukocyte count from the hematology analyzer results were tabulated and statistically analyzed to ascertain difference between smokers and non-smokers:

- Neutrophils (%)
- Lymphocytes (%)
- Monocytes (%)
- Eosinophils (%) and
- Basophils (%).

Sample Size Calculation

The sample size was calculated based on data from pilot survey which showed a mean difference of 3% (standard deviation [SD] \pm 3.5) in neutrophil percentage between smokers and non-smokers. Keeping the level of significance at 1% and power of test at 80%, a sample size of 25 for each of 5 groups was obtained from a standard reference table.^[12]

Statistical Analysis

All data were tabulated in Microsoft Excel worksheets. Data were analyzed using WinPepi statistical software.^[13] Continuous data were presented as mean \pm SD. Statistical significance of the difference in the mean values was tested by independent sample *t*-test. A $P < 0.05$ was considered statistically significant.

RESULTS

A total of 125 adult males were included in the study, of which, 25 were non-smokers and 100 were smokers. There was no statistically significant difference in the baseline characteristics between non-smokers and smokers ($P = 0.21$ by ANOVA) (Table 1).

Comparison between Non-smokers and Smokers

Smokers demonstrated statistically significantly different percentage of neutrophils, lymphocytes, eosinophils, and monocytes when compared to non-smokers ($P < 0.001$ by ANOVA) (Table 2). Neutrophils and lymphocytes were detected to be significantly higher while monocytes were found to be decreasing among smokers in (Group C, D, and E)

Table 1: Age characteristics in each group

Groups (<i>n</i> =25 in each group)	Age (mean \pm SD)	<i>P</i> value (of ANOVA)
Group A (non-smokers)	28.8 \pm 4.5	0.21
Group B (1-5 cigarettes/day)	27.6 \pm 5.1	
Group C (6-10 cigarettes/day)	29.2 \pm 3.9	
Group D (11-20 cigarettes/day)	30.2 \pm 6.8	
Group E (>20 cigarettes/day)	31.1 \pm 6.6	

Table 2: Comparison of hematological parameters between non-smokers ($n=25$) and (smokers) $n=100$

Type of WBC (as percentage)	Mean \pm SD ($n=25$)					“P” value ANOVA
	Group A (non-smokers)	Group B (1-5 cigarettes/day)	Group C (6-10 cigarettes/day)	Group D (11-20 cigarettes/day)	Group E (>20 cigarettes/day)	
Neutrophils	64.2 \pm 4.2	65.3 \pm 3.8	67.4 \pm 3.4	68.2 \pm 4	69.6 \pm 3.6	<0.001*
Lymphocytes	34.4 \pm 3.8	35.6 \pm 3.7	37.1 \pm 4.03	38.9 \pm 3.22	39.6 \pm 4.01	<0.001*
Monocytes	5.4 \pm 1.01	4.9 \pm 1.06	4.42 \pm 0.88	4.36 \pm 1.2	3.9 \pm 0.98	<0.001*
Eosinophils	4.6 \pm 1.2	5.25 \pm 0.86	5.6 \pm 1.01	5.8 \pm 0.95	5.93 \pm 0.95	<0.001*
Basophils	0.33 \pm 0.31	0.29 \pm 0.6	0.21 \pm 0.56	0.2 \pm 0.46	0.15 \pm 0.48	0.70

* $P < 0.001$ -Statistically significant. WBC: White blood cell, SD: Standard deviation. Comparison of neutrophils (%), lymphocytes (%), monocytes (%), eosinophils (%) and basophils (%) between non-smokers and four groups of smokers with different numbers of cigarettes smoked per day ($n=25$ in each group)

when compared to non-smokers. With the increase in the number of cigarettes smoked per day (Group B - Group E), neutrophils and lymphocytes increased progressively while monocytes reduced (Table 2). With increase in the number of cigarettes smoked per day (Group B - Group E), eosinophils increased progressively (Table 2). There was no significant change in the percentage of basophils among smokers when compared to non-smokers ($P = 0.70$) (Table 2).

DISCUSSION

Smoking causes alterations in various hematological parameters.^[4,5,14,15] Various studies in the literature have compared hematological parameters of smokers and non-smokers.^[4,5,14,15] Robust scientific evidence exists to support a relationship between smoking and cardiovascular illness, cancer or chronic obstructive pulmonary disease. Endothelial injury,^[16] progression of coronary plaque,^[17] and dyslipidemia^[18] have been described as adverse effects of smoking. However, the literature reporting the effects of different numbers of cigarettes smoked per day on differential leukocyte count is limited.

The present study enrolled adult male participants reporting to be smoking different numbers of cigarettes per day to understand the changes in various hematological parameters with increasing number of cigarettes smoked per day. When compared to assessment by pack-years, the current smoking habit has a stronger effect on white blood cell (WBC) count.^[19] Specifically, in this study, changes in differential leukocyte count were studied among smokers when non-smokers were used as controls for comparison.

Effect on Differential Leukocyte Count

Smokers have increased granulocyte count.^[19] As a marker of inflammation, WBC count was found to be independently associated with incident heart failure in men.^[20] Smokers demonstrated a significant rise in eosinophil count, all types of WBC and neutrophil count.^[21-23] In the present study, neutrophil, lymphocytes, monocytes, and eosinophils show

a statistically significant change among current smokers ($P < 0.001$). Monocytes were found to decrease among smokers when compared to non-smokers. However, there was no significant difference in the percentage of basophils ($P = 0.70$). We have demonstrated similar results in a study conducted among male smokers in Hyderabad, India.^[4]

Limitations

Larger sample size and a long-term follow-up would bring about more clarity in the association. Whether social determinants were also the cause of increased cell counts among smokers as compared to non-smokers is a limitation of this study.

CONCLUSION

Results of our study conclude that there is a significant change in differential leukocyte count among smokers when compared to non-smokers among North Eastern Indian males.

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