



ISSN No: 2319-5886

International Journal of Medical Research &  
Health Sciences, 2016, 5, 2:13-17

## Effect of number of cigarettes smoked per day on red blood cell, leucocyte and platelet count in adult Indian male smokers – A case control study

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### ABSTRACT

The effects of cigarette smoking are fatal. Present study was done to compare cell counts of blood in males smoking different number of cigarettes per day and non smokers of Hyderabad city. 150 consenting subjects of which 30 controls (non-smokers) and 120 cases (smokers) were studied. Smokers were divided into four groups based on number of cigarettes smoked per day. Blood samples processed using Hematology analyser (ABX Micros60®, HORIBA, Kyoto, Japan). The smokers had significantly different red blood cell counts ( $p < 0.0001$ ), white blood cells counts ( $p < 0.0001$ ) including neutrophils, lymphocytes, monocytes and eosinophils. This effect was significant irrespective of the number of cigarettes. There was no significant change in the percentage of basophils and platelet counts. Conclusion: Our findings showed that cigarette smoking has a significant effect on hematological cell counts and these counts changed significantly with increasing number of cigarettes smoked per day.

**Keywords:** Smoking, Total Leucocyte count, Differential Leucocyte count, Red Blood cell count, Platelet count.

### INTRODUCTION

Addiction to tobacco kills prematurely. The most common form of tobacco consumption today is by smoking manufactured cigarettes [1, 2]. Tobacco smoke produced by cigarette contains numerous harmful carcinogenic substances [3] and is also linked to oxidative damage to the lungs [4]. Smokers have been found to have altered White blood cells counts, Red blood cell counts and Platelet counts in addition to changes in Hematocrit, Mean Corpuscular Volume and Mean corpuscular Hemoglobin concentration [5]. Smoking has been demonstrated to have adverse effects on cardiovascular risk profile of an individual by increasing the activation of inflammation and haemostasis in the form of higher blood viscosity, tissue plasminogen activator antigen and lower levels of albumin [6]. The number of cigarettes smoked by individuals studied in various studies published in literature are not uniform. Very few studies documented effect of incremental number of cigarettes smoked per day on various cell counts. The present study aims to understand the effect of number of cigarettes smoked on the total leukocyte count, total red blood cell count, platelet count and the differential leukocyte count.

### MATERIALS AND METHODS

Study design: A case control study comparing male non-smokers and male smokers from Hyderabad, India was performed between April and September 2012.

Study subjects: 150 healthy male subjects consisting of 30 non-smokers and 120 smokers divided into four groups of 30 each with various reported number of cigarettes smoked) were planned to be enrolled into the study after written

informed consent. Male subjects consenting to participate in study were sequentially assessed for suitability to be included in the study. All prospective study subjects reported their age, comorbidities (including thyroid disease), concurrent medications, present smoking status, recent blood donation or transfusion (< 6 months) and the total number of cigarettes presently being smoked per day on a pre-specified bilingual format. Based on the reported data, 30 non-smokers and 120 smokers (were sequentially included in the study. All subjects who reported any comorbidity including diabetes, primary hypertension or concurrent medication for any comorbidity or recent history of blood transfusion/donation (< 6months) were excluded from the study. Hence five groups of male study subjects were identified as follows:

- Group A – Non smokers (Controls)
- Group B – Smokers (1 to 5 cigarettes per day)
- Group C - Smokers (6 to 10 cigarettes per day)
- Group D - Smokers (11 – 20 cigarettes per day)
- Group E - Smokers (more than 20 cigarettes per day)

Study procedure: After obtaining written informed consent, a total of 5 ml of venous blood sample was collected from all study subjects in the morning between 8am and 11am after a period of fasting of atleast 10 hours. All blood samples were collected in 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 analyser (ABX Micros60®, HORIBA, Kyoto, Japan). The following blood parameters from the Haematology analyser results were tabulated and statistically analysed.

- (a) Total Leukocyte count (WBC) (per cu.mm)
- (b) Differential Leukocyte count (Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils) (%)
- (c) Total Red blood cell count (RBC) ( $\times 10^5$  per cu.mm)
- (d) Platelet count ( $\times 10^5$  per cu.mm)

Statistical analysis: All data was tabulated in Microsoft Excel worksheets. Data was analysed using WinPepi statistical software (7). Continuous data were presented as Mean  $\pm$  SD. Statistical significance of difference in the mean values was tested by independent sample t-test. A p value of less than 0.05 was considered statistically significant.

## RESULTS

A total of 150 adult males were included in the study, of which 30 were non-smokers and 120 were smokers. There was no statistically significant difference in the baseline characteristics between non-smokers and smokers (Age (in years):  $27.5 \pm 5.76$  vs  $28.1 \pm 3.37$  p = 0.458).

Comparison between Non Smokers and Smokers: Smokers demonstrated statistically significantly different haematological cell counts (except Basophils and Platelet counts) when compared to Non-smokers (Table 1).

**Table 1. Comparison of Haematological parameters between Controls (Non-smokers) (n = 30) and Cases (Smokers) (n=120).**

Blood cell count	Non smokers (Controls) Mean $\pm$ SD (n = 30)	Smokers (Cases) Mean $\pm$ SD (n = 120)	p value
RBC ( $\times 10^5$ per cu.mm)	5.5 $\pm$ 0.29	6.18 $\pm$ 0.54	<0.0001
WBC count (per cu.mm)	6668.75 $\pm$ 1130.62	9103.75 $\pm$ 1551.8	<0.0001
Neutrophils(%)	59.48 $\pm$ 2.84	62.06 $\pm$ 4.2	0.002
Lymphocytes(%)	31.21 $\pm$ 2.52	28.35 $\pm$ 3.94	<0.0001
Monocytes(%)	4.89 $\pm$ 0.91	3.91 $\pm$ 1.22	<0.0001
Eosinophils (%)	4.2 $\pm$ 1.19	5.54 $\pm$ 1.05	<0.0001
Basophils(%)	0.23 $\pm$ 0.42	0.13 $\pm$ 0.33	0.163
Platelet count	266870 $\pm$ 42090.05	274562.5 $\pm$ 49696.18	0.390

Comparison between Controls (Non-smokers) and Cases (Smokers): When compared to Non-smokers, WBC count and RBC count were significantly higher among all sub-groups of smokers with different number of cigarettes smoked per day. However, the Platelet count showed no difference between smokers and non-smokers, except in subjects who smoked more than 20 cigarettes per day (Table 2).

**Table 2 Comparison of RBC count, WBC count and Platelet count between Controls (Non-smokers) and four groups of Cases (Smokers) with different number of cigarettes smoked per day. (n = 30 in each group)**

Study Parameters	RBC Count (x 10 <sup>5</sup> per cu.mm)		WBC count (per cu.mm)		Platelet count (per cu.mm)	
	Mean±SD	p value	Mean±SD	p value	Mean±SD	p value
<b>Group A (Non-Smokers) (Controls)</b>	5.5±0.29		6668.75±1130.62		266870±42090.05	
<b>Group B (Cases) (1- 5 cigarettes per day)</b>	5.93±0.29	<0.0001	7429.0±1155.63	0.013	254150±50862.06	0.296
<b>Group C (Cases) (6- 10 cigarettes per day)</b>	6.04±0.49	<0.0001	9452.0±1043.2	<0.0001	282450±52724.18	0.211
<b>Group D (Cases) (11-20 cigarettes per day)</b>	6.26±0.47	<0.0001	9505.5±774.6	<0.0001	283700±52631.88	0.177
<b>Group E (Cases) (&gt; 20 cigarettes per day)</b>	6.51±0.68	<0.0001	10028.5±1684.83	<0.0001	287950±39087.58	0.049

Neutrophils, Lymphocytes, and Eosinophils were detected to be significantly higher in blood counts among Smokers when compared to non-smokers. With the increase in the number of cigarettes smoked per day (Group B to Group E), Neutrophils increased progressively while lymphocytes reduced (Table 3).

**Table 3 Comparison of Neutrophils (%) and Lymphocytes (%) between Controls (Non-smokers) and four groups of Cases (Smokers) with different number of cigarettes smoked per day. (n = 30 in each group)**

Subject groups	Neutrophils (as percentage)		Lymphocytes (as percentage)	
	Mean±SD	p value	Mean±SD	p value
<b>Group A (Non-Smokers) (Controls)</b>	59.48±2.84		31.21±2.52	
<b>Group B (Cases) (1- 5 cigarettes per day)</b>	60.9±3.68	0.100	29.4±3.39	0.22
<b>Group C (Cases) (6- 10 cigarettes per day)</b>	61.65±4.97	0.042	27.40±4.92	<0.0001
<b>Group D (Cases) (11-20 cigarettes per day)</b>	62.6±3.7	0.001	28.5±3.98	0.003
<b>Group E (Cases) (&gt; 20 cigarettes per day)</b>	63.10±4.3	<0.0001	28.1±3.32	<0.0001

Monocytes were reduced significantly among smokers when compared to non-smokers. With increasing in the number of cigarettes smoked per day (Group B to Group E), Monocytes decreased while Eosinophils increased progressively (Table 4). There was no significant change in percentage of Basophils among Smokers when compared to non-smokers (Table 4).

**Table 4 Comparison of Monocytes (%), Eosinophil (%) and Basophils (%) between Controls (Non-smokers) and four groups of Cases (Smokers) with different number of cigarettes smoked per day. (n = 30 in each group)**

Subject groups	Monocytes (as percentage)		Eosinophil (as percentage)		Basophils (as percentage)	
	Mean±SD	p value	Mean±SD	p value	Mean±SD	p value
<b>Group A (Non-Smokers) (Controls)</b>	4.89±0.91		4.2±1.19		0.23±0.42	
<b>Group B (Cases) (1- 5 cigarettes per day)</b>	4.2±1.36	0.024	5.35±1.13	<0.0001	0.15±0.36	0.432
<b>Group C (Cases) (6- 10 cigarettes per day)</b>	4.00±0.91	<0.0001	5.50±1.27	<0.0001	0.20±0.41	0.781
<b>Group D (Cases) (11-20 cigarettes per day)</b>	3.95±1.5	0.005	5.60±0.82	<0.0001	0.05±0.22	0.042
<b>Group E (Cases) (&gt; 20 cigarettes per day)</b>	3.5±1.0	0.017	5.7±0.97	<0.0001	0.10±0.30	0.173

## DISCUSSION

Smoking causes alterations in various haematological parameters (4, 5, 8, 9). Various studies in literature have compared haematological parameters of smokers and non-smokers (4, 5, 8, 9). Robust scientific evidence exists to support relationship between smoking and cardiovascular illness, cancer or chronic obstructive pulmonary disease. Endothelial injury (10), progression of coronary plaque (11) and dyslipidemia (12) have been described as adverse effects of smoking. Changes in RBC counts (13), Platelet count, platelet parameters (14), Mean corpuscular

Volume, Mean Corpuscular haemoglobin, Red cell distribution width (5) have been reported in literature. However, the literature reporting the effects of different number of cigarettes smoked per day on differential leucocyte count is limited.

The present study enrolled adult male subjects reporting to be smoking different number of cigarettes per day to understand the changes in various haematological parameters with increasing number of cigarettes smoked per day. When compared to assessment by pack-years, current smoking habit has a stronger effect on WBC count (18). Specifically, in this study, changes in differential leucocyte count were studied among smokers when non-smokers were used as controls for comparison.

**Effect on RBC count:** RBC count has been found to be increased even with minimal amounts of smoking among adult males compared to non-smokers ( $p < 0.0001$ ). This is consistent with published reports (13). Increased RBC count along with WBC count leads to poor blood flow, increased viscosity and enhanced coagulability (16).

**Effect on WBC count:** WBC count is a commonly evaluated haematological parameter. It is a simple marker for endothelial injury (5). We found that WBC count was higher among smokers, irrespective of the number of cigarettes smoked per day, when compared to non-smoker controls ( $p < 0.0001$ ). This is in agreement with published literature (5, 9). In addition, WBC count increased progressively with the increase in number of cigarettes smoked per day. The mechanism for the increase in WBC count is attributed to the inflammatory effects of cigarette smoke on bronchial mucosa. Increase in WBC count has been described as a risk factor for vascular wall thickening and subclinical atherosclerosis (15). Hence, significantly increased WBC counts with any amount of smoking (even less than five cigarette per day) can pose a higher risk of atherosclerosis and cardiovascular risk.

**Effect on Platelet count:** Our study did not find any difference between smokers and non-smokers in terms of Platelet count except for a statistically significant increase only among Smokers consuming more than 20 cigarettes per day ( $p = 0.049$ ). Previous studies have not reported any significant difference in Platelet activation, mean platelet volume or platelet distribution width (5, 14, 17).

**Effect on Differential Leucocyte count:** The EPIC-Norfolk study (19) concluded WBC count, as a marker of inflammation, to be independently associated with incident heart failure in men but not women. Granulocyte count has been reported to increase among smokers (18). Significant rise in eosinophil count, all types of WBC and Neutrophil count has also been demonstrated among smokers (20, 21, 22). In our study, Neutrophil, Lymphocytes, Monocytes and Eosinophils show a statistically significant change among current smokers ( $p < 0.0001$ ) irrespective of the number of cigarettes smoked per day. Monocytes were found to decrease among smokers when compared to non-smokers. However, there was no significant difference in percentage of Basophils.

**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 amongst smokers as compared to non-smokers is a limitation of this study.

## CONCLUSION

Results of our study conclude that there is a significant increase in RBC and WBC count amongst smokers. Increase in cell count would increase the viscosity of blood, reduce the velocity of blood flow thus predisposing to deep vein thrombosis, stroke and embolism. Significant increase in WBC count amongst smokers supports the available evidence in favour of inflammatory changes due to smoking, progression of atherosclerosis and progression of coronary plaque. The implications of this relationship can also help to guide the medical work-up in a smoker with leukocytosis or erythrocytosis.

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