National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Total and differential leukocyte count and oxygen saturation of hemoglobin changes in healthy smokers and non-smokers

Ashish Goel[1], Priyanka Gupta[1], Desh Deepak[1], Hari Shankar Pandey[2], Arsalan Moinuddin[3]

[1]Department of Physiology, Government Doon Medical College, Dehradun, Uttarakhand, India, [2]Department of Pathology, Government Doon Medical College, Dehradun, Uttarakhand, India, [3]Department of Kinesiology and Sports Management, Texas Tech University, Texas, USA

Correspondence to: Ashish Goel, E-mail: dr.ashishgoel2012@gmail.com

Received: May 04, 2020; **Accepted:** May 23, 2020

ABSTRACT

Background: Inflammatory markers are altered in smokers and smoking has been proved as one of the main causative factors for cardiovascular diseases (CVDs), inflammatory diseases, and oxidative stress stimulation. An alteration in hematological parameters and oxygen saturation of hemoglobin is expected with smoking tobacco. **Aim and Objective:** This study has been planned with the aim to evaluate the changes associated with the extent of adverse effects of tobacco smoking in total and differential leukocyte count (DLC) and oxygen saturation of hemoglobin in healthy smokers and non-smokers. **Materials and Methods**: A total of 150 healthy adults were participated in this cross-sectional study. Anthropometric measurements such as height, weight, and body mass index were taken, and information of smoking habits were obtained by a questionnaire. Blood samples were taken for the estimation of total and DLC using MS-9 automated hematology cell counter. Oxygen saturation of hemoglobin was done using fingertip pulse oximeter Nidek Medical 5300. **Results:** Our results showed a statistically significant rise in the total leukocyte count (TLC) ($P < 0.001$), lymphocyte count ($P < 0.001$), granulocyte count ($P < 0.01$), and monocyte count ($P < 0.02$) and showed a statistically significant decrease in SpO_2 ($P < 0.04$) in smokers as compared to non-smokers. **Conclusion:** The study has shown that altered values of TLC and DLC and oxygen saturation of hemoglobin in smokers should be considered during diagnosis, interpretation of result, and treatment of patients. A high TLC and DLCs exhibited in this research may be responsible for chronic inflammation and subsequent high risk of CVD in smokers. Therefore, quitting smoking should be encouraged for better health.

KEY WORDS: SpO_2; Oxygen Saturation of Hemoglobin; Total and Differential Leukocyte Count; Smokers

INTRODUCTION

Cigarette smoking is one of the leading cause of death globally.[1] Numerous studies indicated that smoking had adverse effects on human health and represented a predisposing factor for the development of various pathological conditions and diseases, such as the chronic obstructive pulmonary disease, cancer, pancreatitis, gastrointestinal disorders, periodontal disease, metabolic syndrome, and some autoimmune diseases. Smokers have elevated risk of all varieties cardiovascular diseases (CVD), peripheral vascular disease, and cerebrovascular diseases, for example, stroke.[2]

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2020.10.05116202023052020	

Hematological parameters varied greatly with smoking as there are as many as 4000 chemicals found in cigarette smoke which can instigate inflammation. Smokers inhale a variety of harmful substances including nicotine, free radicals, carbon monoxide, and other gaseous products. To be more specific on a mechanistic basis, inhaled nicotine mocks nicotinic acetylcholine, which binds to nicotinic

acetylcholine receptors and prevents acetylcholine uptake which subsequently results in ACh accumulation at neuromuscular junctions. During the past 10 years, cigarette smoking is thought to affect the blood parameters that may result in death with smokers exhibiting higher white blood cell (WBC) counts than non-smokers.[3] Cigarette smoking in healthy men was accompanied by significant effects on platelet indices, such as higher mean platelet volume and platelet distribution width in smokers as compared to non-smokers.[4]

Leukocytes have emerged not only as empirical markers of inflammation but also as strong predictors of both cardio and cerebrovascular disease in smokers. The afore-mentioned contents of cigarette smoke increase leukocytes count with nicotine playing a dominant role in this context. Evidence suggests that nicotine stimulates hormonal secretion that boosts leukocyte count. Furthermore, it is worth discussing that smoke irritates respiratory mucosa that leads to inflammation and synthesizes cytokines, which subsequently raise the leukocyte count.[5]

It is evident that respiratory system is primarily meant for oxygenation. Once oxygen reaches the alveoli (mostly transported by hemoglobin), a small fraction of it is remains molten. The amount of dissolved O_2 in the bloodstream 0.003 ml/100 ml, with 1 g of hemoglobin carrying around 1.34 ml of O_2. The amount of oxygen in the bloodstream which is transported by the hemoglobin is known as oxygen saturation (SpO_2). Smoking is a major cause of both morbidity and mortality, with a prevalence rate of 20–40% in females and 30–40% in males in developed countries while 2–10% in females and 40–60% in males in developing countries. Thus, cigarette smoking damages lungs and affects other organ systems in different ways.[6] Our study is thought to give some input to the literature on the effect of smoking on arterial blood and hemoglobin saturation. The present study thus investigates the effect of tobacco smoking on total and differential leukocyte count (DLC) and oxygen saturation of hemoglobin for better diagnosis, interpretation of results, and treatment.

MATERIALS AND METHODS

Study and Study Participants

The present study was carried out in the Department of Physiology at Himalayan Institute of Medical Sciences, Swami Ram Nagar, Dehradun, India. A total of 150 clinically healthy volunteers of Dehradun, in the age group of 21–55 years participated in the present study. Individuals with a history of smoking cigarettes/bidis daily for at least 1 year were considered as smokers. Ex-smokers or past smokers were excluded from the study. Classification criteria as suggested by WHO (1998) were used as below: Smokers are defined as someone who, at the time of the study, smokes any tobacco product either daily or occasionally, while a non-smoker is someone who, at the time of the study, does not smoke at all. Moreover, an ex-smoker is someone who was formerly a daily or occasional smoker but currently does not smoke at all.[7] Unhealthy adults with any history of acute or chronic illness, bleeding and bleeding disorders, drug addiction, and if they had donated blood within the previous 6 months were not included in the study. Pregnant women and those who had delivered within 3 months were also excluded from the study.

Ethical Approval

Approval of the study was taken from the Institutional Ethical Committee. Written informed consent was taken from the subjects.

Data Collection

1. Anthropometric parameters which include height, weight, and body mass index (BMI) were taken. Information of the smoking habits was obtained by a questionnaire
2. Estimation of total, DLC, and oxygen saturation of hemoglobin: After taking antiseptic precautions, blood samples were taken from the antecubital vein and collected into 3 ml ethylenediaminetetraacetic acid (EDTA) vacutainers (Akuret, eastern medkit limited). The EDTA blood samples were processed using MS-9 automated hematology cell counter for total leukocyte count (TLC) (in thousands) and DLC (in percentage). Samples were processed on the same day approximately within 3–5 hours of collection. Oxygen saturation of hemoglobin was done using fingertip pulse oximeter Nidek Medical 5300. Subject was made to sit quietly for 5–15 min. A non-invasive sensor was placed on the index finger (occasionally on another finger if a reading was not obtained promptly or if the index finger was missing), waited for 10–15 s after the first reading on the screen and then noted six consecutive readings at an interval of 10 s. Average of six measurements was taken as final value.

Statistical Analysis

Normality and variance were assessed using Kolmogorov–Smirnov and Shapiro–Wilk tests, "probability" plots and through quantification methods (skew/kurtosis). Keeping these results in perspective, independent "t" was used to analyze the difference between all continuous hematological measurements. Simple correlation analysis was performed to evaluate the association between the above said parameters. Alpha levels were set at $P < 0.05$ and data are expressed as mean ± standard deviation. All

statistical analyses were completed with SPSS version 26 (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 1 shows that in a total of 150 subjects (108 non-smokers and 42 smokers cases), in which baseline demographic parameters (age and BMI) are compared between smokers and non-smokers. No significant difference between the baseline demographic parameters between the smokers and non-smokers ensures optimum comparison avoiding bias.

Table 2 shows the difference between TLC, lymphocyte count, monocyte count, granulocyte count, and oxygen saturation of hemoglobin among smokers and non-smoker subjects. The mean values of TLC ($P < 0.001$), lymphocyte count ($P < 0.001$), monocyte count ($P = 0.02$), and granulocyte count ($P = 0.01$) were significantly higher in smokers as compared to non-smokers, while the mean values of SpO_2 ($P = 0.04$) were significantly lower in smokers as compared to non-smokers.

Figure 1 depicts a significant difference ($P < 0.001$) in TLC between non-smokers and smokers ($10^3/mm^3$). Smoking builds an inflammatory environment in the human body which, in turn, triggers immune response in general, subsequently raising the leukocyte count.

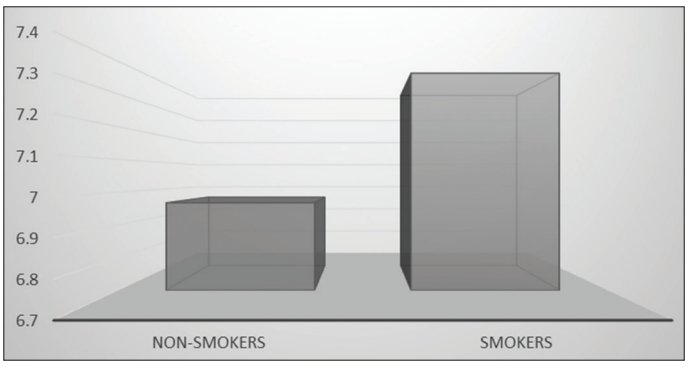

Figure 1: Graphical representation of total leukocyte count ($10^3/mm^3$) in non-smokers and smokers

DISCUSSION

Tobacco smoking has been associated with a variety of major hematological ailments. The results of our study showed a significant increase in the total WBC, lymphocyte count, monocyte count, and the granulocyte count in smokers as compared to non-smokers. We have also exhibited that oxygen saturation of hemoglobin was found to be lower in smokers than in non-smokers.

Pedersen *et al*. in the Copenhagen general population study found that smoking causes increased blood leukocytes, neutrophils, lymphocytes, and monocytes.[8] Asif *et al*. in their study also found that regular smokers exhibited significantly greater WBCs count compared to non-smokers ($P = 0.027$).[9] They also found that the WBC count among male smokers was higher which also suggests that they may have greater risk of developing both atherosclerosis and CVDs than female smokers and non-smokers.[9] Airway epithelium acts as a physical barrier obstructing the entry of inhaled noxious particles into the submucosa. Leukocytosis has emerged as a potential marker of tissue damaged caused by cigarette smoke. Moreover, a rise in its count may account for an increased incidence of CVD through a plethora of postulated pathogenic mechanisms that mediate inflammation, block microvasculature at various junctures, and induce hypercoagulability. Gitte and Taklikar also found in their study a sharp increase in total leukocyte count values of smokers with respect to the non-smokers.[10] Anitha and Manjunath also confirm this empirical positive association between smoking and total leukocyte count.[11] Our study also aimed at DLCs due to a probable association between cigarette smoking with TLC. Evidence suggests a strong possibility of this association, however, its effect on the DLC is still a matter of debate. In our study, it was also demonstrated that there was a statistically significant increase in all leukocyte subtypes. Zei-Shung *et al*. in their study also found significantly higher TLCs along with its subtypes in smokers.[12] One of the possible mechanistic hypotheses of this increased TLC is the extracted glycoprotein from the tobacco leaf, which stimulates lymphocyte proliferation and differentiation by intermingling with a specific membrane

Table 1: Comparison of baseline demographic parameters of smokers and non-smokers subjects

	Descriptive statistics						
Smoking status	***n***	**Range**	**Minimum**	**Maximum**	**Mean**		**Standard deviation**
Non-smoker							
Age	108	35	20	55	31.87	0.903	9.385
BMI	108	20.42	16.02	36.44	24.1931	0.30505	3.17014
Smoker							
Age	42	29	21	50	32.74	1.277	8.276
BMI	42	11.69	18.07	29.76	25.1419	0.45720	2.96299

BMI: Body mass index

Table 2: Comparison of TLC, DLC, and oxygen saturation among smokers and non-smoker subjects

Parameter	Non-smokers (*n*=108)	Smokers (*n*=42)	*P*-value
TLC	6.9674	7.3633	<0.001
Lymphocyte count	0.3717	0.3664	<0.001
Monocyte count	0.053	0.0557	0.02
Granulocyte count	0.5738	0.58	0.01
SpO2	0.9869	0.9829	0.04

TLC: Total leukocyte count, DLC: Differential leukocyte count

component, commonly seen in antigenic response.[13] As for lymphocyte count, Shenwai and Aundhakar reveal that the lymphocyte count increases significantly from 32.4% in non-smokers to 38.3% in smokers, while neutrophil count showed a slight fall in smokers than non-smokers, however, the difference for neutrophil count is statistically non-significant. Furthermore, no significant change was observed in eosinophil, basophil, and monocyte counts.[14] It is quite evident that lymphocytosis is attributed to both chronic tissue damage and inflammation produced by toxic substances found in tobacco smoke. It has also been suggested that smoke causes stimulation of respiratory bronchial tract inflammatory markers, thus inducing their increase in the blood. Moreover, nicotine induces an increase in blood lymphocyte counts too.[9] Cigarette smoking encompasses a myriad of effects on the immune response of lymphocyte cells. Some of the noteworthy examples include immunoglobulin production, T4/T8 lymphocyte ratio change, enhanced NK activity, and low mitogen-induced lymphocyte transformation.[11] In his research, Silverman *et al*. found that that smokers exhibit marked elevation in leukocytes especially "T" lymphocytes.[15]

We are aware that saturation of arterial blood to oxygen is essential for all individuals. Ozdal *et al*. reported that non-smoker individuals had significantly higher oxygen saturation of hemoglobin than smoker individuals ($P < 0.05$) which was similar as found in our study. The two main ingredients of cigarette smoke that potentially reduces oxygen supply to all tissues of the body are nicotine and carbon monoxide by combining themselves to transport proteins such as hemoglobin and myoglobin.[6]

The strength of our study was that the authentic subject selection was done on the basis of inclusion and exclusion criteria. Meticulously statistical analysis was done and p value was obtained to prove statistically significance. Earlier detection of respiratory damage in asymptomatic smokers will prevent future complications. Reduction in smoking may prove useful in subjects undergoing treatment and can surely serve pivotal and an empirical cornerstone in people who are resistant to quitting. Limitations involve the limited sample size; the research should be carried out with larger sample sizes. Future direction in this kind of research is necessary to determine whether smoking cessation is advantageous and if yes to what extent smoking needs to be reduced for health benefits to occur.

CONCLUSION

To sum up, smoking is nonetheless one of the major preventable risk factors for CVD mortality and morbidity. Cigarette smoking enhances inflammatory responses which is exhibited in our study by increasing levels of WBCs count and its subtypes. This study has shown that the total and DLC were altered in smokers and thus should be considered during diagnosis, interpretation of result, and treatment of patients. Tobacco smoking has a negative impact on oxygen saturation of hemoglobin. Reduction in smoking can improve the changes which are sensitive to change in smoking intake. We advise regular monitoring of the above-mentioned hematological parameters in smokers to detect early changes and avoid future catastrophic outcomes.

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How to cite this article: Goel A, Gupta P, Deepak D, Pandey HS, Moinuddin A. Total and differential leukocyte count and oxygen saturation of hemoglobin changes in healthy smokers and non-smokers. Natl J Physiol Pharm Pharmacol 2020;10(09):726-730.

Source of Support: Nil, **Conflicts of Interest:** None declared.