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To assess increases in overall and Differential Leukocyte Count (DLC), as well as oxygen saturation of haemoglobin in stable smokers and non-smokers: An observational study

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Abstract
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 haemoglobin in healthy smokers and non-smokers.
Materials and Methods: This observational study was carried out in the Department of Physiology, Mamata Medical College, Khammam, Telangana from August 2019 to March 2020. A total of 200 clinically healthy volunteers of Khammam, in the age group of 20–60 years participated in the present study. Individuals with a history of smoking cigarettes/bidis daily for at least 12 months were considered as smokers. Another 100 non-smokers of the same age group were included separately in this study as a control group. TLC, DLC and other parameters were analysed using standard methods.
Results: A total of 200 subjects (100 non-smokers and 100 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. The difference between TLC, lymphocyte count, monocyte count, granulocyte count, and oxygen saturation of haemoglobin among smokers and non-smoker subjects.
Conclusion: The study has shown that altered values of TLC and DLC and oxygen saturation of haemoglobin in smokers should be considered during diagnosis, interpretation of result, and treatment of patients.

Keywords: SpO2, Oxygen Saturation of Haemoglobin, Total and Differential Leukocyte Count, Smokers

Introduction
The most serious public health issue is smoking. Many studies have shown that it has negative effects on many organ systems, including the respiratory, reticuloendothelial, and cardiovascular systems [1]. According to figures from the World Health Organization, approximately 2.4 billion individuals have eaten tobacco in any way, such as smoking, chewing, snuffing, or dipping. According to the WHO, tobacco-related deaths will reach 8.3 million in 2030 and one billion in the twenty-first century [2]. Each cigarette smoked is expected to cost an average of 7 minutes of life, about the time it takes to smoke one [3]. A individual who starts smoking at the age of 15 has an average of 8 years less life expectancy, whereas someone who starts after the age of 25 has an average of 4 years less life expectancy. The bulk of excess death associated with tobacco smoking is due to coronary heart disease, cancer, and other respiratory diseases [4]. When compared to non-smokers, smokers have a 16-fold elevated risk of lung cancer, a 12-fold increased risk of COPD, and a two-fold increased risk of developing a myocardial infarction. Several studies have demonstrated a causal link between smoking, haematological parameters, peripheral vascular disease, and stroke since the early 1950s [5]. While the association between smoking and respiratory disorders was discovered in the 1870s, it wasn't until 1964 that the US Surgeon General issued a study warning of a possible link between smoking and emphysema [6]. In 30-40 year olds who are likely to be free of all myocardial risk factors, heavy smoking is the most frequent source of ischemic heart failure and death. The high risk of occlusive vascular disease in chronic smoking could be due to changes in haematological parameters [7].

Chronic smoking seems to trigger an upward change in the haemoglobin dissociation curve, thereby reducing the effectiveness of haemoglobin levels in the diagnosis of anaemia in smokers. This suggests that haemoglobin cut-off values for smokers should be changed to allow for the masking effect of smoking on anaemia detection [8]. It is self-evident that the respiratory system's primary function is to provide oxygen. A small percentage of oxygen that enters the alveoli (mostly through haemoglobin) remains molten. 0.003 ml/100 ml of dissolved oxygen in the bloodstream, with 1 g of haemoglobin carrying about 1.34 ml of oxygen. The amount of oxygen carried by haemoglobin in the bloodstream is referred to as oxygen saturation (SpO2). Smoking is a significant cause of morbidity and mortality in developed countries, with a prevalence rate of 20–40% in females and 30–40% in males, compared to 2–10% in females and 40–60% in males in developing countries. As a result, tobacco smoking harms the lungs and has an effect on other organ systems [9]. The present study thus investigates the effect of tobacco smoking on total and differential leukocyte count (DLC) and oxygen saturation of haemoglobin for better diagnosis, interpretation of results, and treatment.

Materials and Methods

This observational study was carried out in the Department of Physiology, Mamata Medical College, Khammam, Telangana from August 2019 to March 2020, after taking the approval of the protocol review committee and institutional ethics committee. After taking informed consent detailed history was taken from the Participant.

Methodology

A total of 200 clinically healthy volunteers, in the age group of 20–60 years participated in the present study. Individuals with a history of smoking cigarettes/biddies daily for at least 12 months were considered as smokers. Ex-smokers or past smokers were excluded from the study. 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.

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 were also excluded from the study.

Anthropometric parameters which include height, weight, and body mass index (BMI) was taken. Information of the smoking habits was obtained by a questionnaire.

Estimation of total, DLC, and oxygen saturation of haemoglobin: After taking antiseptic precautions, blood samples were taken from the antecubital vein and collected into 3-5 ml Ethylenediaminetetraacetic acid (EDTA) vacationers. The EDTA blood samples were processed using automated haematology cell counter for total leukocyte count (TLC) (in thousands) and DLC (in percentage). Oxygen saturation of haemoglobin was done using fingertip pulse oximeter.

Statistical analysis

The data were analysed using statistical software, SPSS (ver. 20.0) (IBM Inc., Armonk, New York, USA). Descriptive statistics and bivariate and regression analysis were carried out to find association and correlation and considered significant at $P<0.05$. The internal consistency, i.e., Cronbach's alpha value was 0.87 that was suggestive of high reliability.

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

Smoking status	N=200	Range	Minimum	Maximum	Mean	Standard deviation
Non-smoker						
Age	100	33	18	61	32.67	9.457
BMI	100	21.37	17.04	37.08	23.1897	3.16224
Smoker						
Age	100	30	20	57	33.24	7.276
BMI	100	12.78	19.11	30.87	25.1528	2.85245
p-value	>0.05					

Test applied: student t-test, BMI: Body mass index

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

Parameter	Non-smokers (N=100)	Smokers (N=100)	*P*-value
TLC	6.8787	7.3577	<0.001
DLC			
Lymphocyte count	0.3619	0.3723	<0.001
Monocyte count	0.061	0.0537	0.01
Granulocyte count	0.5878	0.50	0.01
SpO2	0.9767	0.9830	0.02

Test applied: student t-test

TLC: Total leukocyte count, DLC: Differential leukocyte count

Discussion

In smokers, there was a substantial increase in overall WBC, lymphocyte count, monocyte count, and granulocyte count as compared to non-smokers, according to our findings. We have discovered that the oxygen saturation of haemoglobin in smoking is lower than in non-smokers. In a Copenhagen general population study, Pedersen *et al.* discovered that smoking induces a rise in blood leukocytes, neutrophils, lymphocytes, and monocytes [10].

In their research, Asif *et al.* discovered that daily smokers had a slightly higher WBC count than non-smokers (P=0.027). 11 They also discovered that male smokers have

a higher WBC count, implying that they are at a higher risk of developing atherosclerosis and CVDs than female smokers and non-smokers [11]. Airway epithelium acts as a physical barrier obstructing the entry of inhaled noxious particles into the sub mucosa. Leucocytosis 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 [12]. Anitha and Manjunath also confirm this empirical positive association between smoking and total leukocyte count [13]. Our study also aimed at DLCs due to a probable association between cigarette smoking's 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 [14]. 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 component, commonly seen in antigenic response [15]. 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 [16]. 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 [10]. 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 mutagen induced lymphocyte transformation [11]. In his research, Silverman *et al*. found that that smokers exhibit marked elevation in leukocytes especially T lymphocytes [17]. 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 haemoglobin 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 haemoglobin and myoglobin [9].

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

This study found that smokers' total and DLC levels were changed, which should be taken into account during patient evaluation, perception, and care. Tobacco use has a detrimental effect on haemoglobin oxygen saturation. Smoking cessation can help to enhance changes that are vulnerable to changes in smoking intake. We recommend that smokers have their haematological parameters monitored on a routine basis to spot early shifts to prevent potential complications.

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