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Correlation of Cigarette Smoking to Differential Leukocyte

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Abstract

The relation between cigarette smoking and differential leukocyte count has not been well described. The present study was carried out to observe the changes in total and differential count of white blood cells of clinically healthy smokers in Ozamiz City, Philippines. Simple random sampling was used in selecting the healthy 30 smokers (15 male, 15 female) and 30 non-smokers (15 male, 15 female) from the target group. Standard blood collection procedure was utilized and blood samples were analyzed using SysmexXS-1000i. Results showed a significant increase in the total leukocyte count of smokers. Granulocytes and monocytes levels were significantly higher in smokers compared to the non-smokers. Among the granulocytes, there was a significant increase in eosinophil count in smokers, whereas the neutrophils were significantly lower. The lymphocyte and basophils were not significantly affected by smoking. The number of cigarettes smoked and number of days of smoking are positively correlated with the total leucocyte count. Cigarette smoking is associated with alterations in inflammatory markers among smokers.

Keywords: eosinophils, granulocytes, monocytes, neutrophils, smokers

Introduction

Despite the significant health risks resulting from cigarette smoking, the number of smokers worldwide remains high (Rom et al., 2013). Globally, cigarette smoking is the second leading cause of death and essential public health challenge. It has both acute and chronic effects on hematological parameters (Vardavas et al., 2010; Overbeek et al., 2011; Shenwai & Aundhakar, 2012; Asif et al., 2013). The main addictive component of cigarette smoke is nicotine, which was first prescribed as a medical drug to treat rodent ulcer and constipation. Smoking is an easy way to administer multiple doses of the psychoactive drug, nicotine. Cigarette smoking can therefore lead to nicotine addiction and is the largest cause of preventable death (WHO, 2010; Vardavas et al., 2010; Mukherjee & Chatterjee, 2013).

Cigarette smoking contributes significantly to cardiovascular morbidity and mortality (Ambrose & Barua, 2004). Widely known, smokers have the higher risk for hypertension, stroke, clotting disorder, inflammation, respiratory disease including chronic obstructive pulmonary disease (COPD) and lung cancer (Andreoli et al., 2015). Cigarette smoking accelerates pathogenesis in lung, pancreas, breast, liver, and kidney cancer. It also enhances pH in the stomach that results in peptic ulcers and gastric diseases (Haider & Rauf, 2010; Shenwai & Aundhakar, 2012; Asif et al., 2013).

Smoking is also associated with immune changes, independent of genetic background and environmental conditions (Andreoli et al., 2015). During the past decade, it had been reported that cigarette smoking affects the blood characteristics that leads to death. White Blood Cell (WBC) count is perhaps the most useful, inexpensive, and simple biomarker for endothelial damage (Shenwai & Aundhakar, 2012; Mukherjee & Chatterjee, 2013). White blood cell plays a significant role in the body's immune system, destroying invading viruses, bacteria, and fungi before they can cause a particular disease (Bishop et al., 2010; Rodak et al., 2013). Hence, people tend to think that elevated levels of WBC are beneficial. This notion is not necessarily the case. A high WBC count is not a specific disease but can indicate the presence of infection, stress, inflammation, trauma, allergy, or certain diseases. Hence, a high WBC count usually requires further investigation.

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A study showed that quitting smoking results to a relative decrease in white blood cell count (Lee et al., 2014).

The WBC count can predict mortality from myocardial infarction, ischemic heart disease, cerebrovascular disease, cancer and an independent risk factor for carotid atherosclerosis (Wu et al., 2013; Swirski & Nahrendorf, 2013; Portegies et al., 2015). These associations may not necessarily indicate causality, but increased WBC reflects cellular injury in both cardiovascular disease (CVD) and cancer (Andreoli et al., 2015). Mortality in cardiovascular diseases and cancer cannot be readily studied in pre-adult populations. However, the relationship between cigarette smoking and WBC can be explored in any age group in which cigarette smoking is prevalent. Nevertheless, such studies have typically been conducted in adult populations.

Several researchers have reported the association of total leukocyte count with cigarette smoking, but conflicting results have been obtained about the effects of smoking on differential leukocyte count (Farsalinos & Romagna, 2013; Praslickova et al., 2015; Andreoli et al., 2015). This study aimed to correlate cigarette smoking with differential leukocytes of smokers. Specifically, this study investigated whether the relationship between cigarette smoking and leukocyte count could also be demonstrated with a population that had been exposed to cigarettes only recently and for a long duration. The paper also correlated the number of cigarettes smoked per day and the duration of smoking with the total leukocyte count of the subjects. Differential leukocyte count was performed among smokers, and the results were compared to non-smokers. Parameters that showed significant difference were also identified.

Materials and Methods

Subject

Simple random sampling was the method used in selecting the subjects for the study. A total of healthy 30 smokers and 30 non-smokers were selected from the target group. The sample size was determined using the Sloven's formula (Olatunde & Joshua, 2012) with 95% level of confidence. The target group includes smokers and non-smokers with no diabetes mellitus, ischemia, peripheral vascular disease, chronic renal disease, and hypertension, no history of drug use, and not currently taking non-steroidal anti-inflammatory drugs.

Informed consent was obtained from all the participants. The study was approved by the ethics committee of the Misamis University before the conduct of research. The subjects were regularly consuming a minimum of three cigarettes per day for at least two years. Face interview was done to obtain the medical history and other relevant information from the subjects. The standard questionnaires on "Tobacco Questions for Surveys" from the World Health Organization (WHO), Center for Disease Control and Prevention and Department of Health and Human Services used in this study were slightly modified. The modifications in the questionnaire include questions on what is the average number of cigarette sticks consumed per day and what time the subjects started smoking to assess the level and duration of smoking.

Laboratory test

Blood samples were collected in the morning between 9:30-11:30 AM to avoid the effect of diurnal variation on blood counts. Two mL of blood was collected into a syringe using a clean venipuncture of the median cubital vein with all aseptic precautions and immediately transferred to K_2 EDTA tubes and mixed gently (Hutchison et al., 2011). The Complete Blood Count (CBC) was measured by registered Medical Technologists within 1-2 hours of blood sampling using Sysmex XS-1000i fully automatic hematological analyzer. The total white blood cell count, neutrophils, eosinophils, basophils, monocyte, and lymphocyte were measured in this study and then compared to the standard data of non-smokers.

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Data analysis

The results were presented as mean ± S.D. All results were analyzed using statistical software MiniTab 17 by applying Z-test to measure any significant difference in the leucocyte count between smokers and non-smokers. The Z and P values were computed. The Z value of > 2 and P value of <0.001 have been taken as statistically significant. Regression analysis was used to determine if the number of cigarettes smoked and number of days of smoking correlate with the total leucocyte count.

Results and Discussion

Table 1 showed a statistically significant increase in the total leukocyte count (TLC) of smokers compared with non-smokers ($P<0.01$ and $Z>2$). A higher count in monocyte, eosinophil and lower count in neutrophil among the differential leukocyte counts were seen in smokers ($P< 0.01$ & $Z >2$).

In this study, a higher total leukocyte count was observed in smokers as compared to non-smokers. Rajan et al. (2014) also showed similar result. Chronic cigarette smoke exposure causes leukocytosis which could be associated with an increase in circulation of polymorphonuclear lecocytes. The result also corroborates with the finding of Tell et al. (1985) who reported high TLC in smokers who have been smoking recently. However, a higher TLC was noted in smokers who have been smoking for a long period (Mukherjee & Chatterjee, 2013). Marked leukocytosis has been reported by several other researchers even in subjects who are smoking less than 10 cigarettes per day (Whitehead et al., 1995). In this study, casual blood samples were examined, and the subjects were not asked to smoke cigarettes or abstain from smoking before the tests.

Table 1. Effect of cigarette smoking on *TLC and **DLC.

Parameters n = 30	Smokers Mean ± SD	Non-Smokers Mean ± SD	Z value	P value
TLC (Cells/cu.mm)	8832.7 ± 1987.7	7442.8 ± 1169.6	3.37	H.S.
Neutrophil (%)	56.63 ± 4.86	61.97 ± 4.32	4.64	H.S.
Lymphocyte (%)	33.08 ± 8.09	32.43 ± 3.95	0.40	N.S.
Monocyte (%)	4.77 ± 1.49	1.48 ± 0.6	11.75	H.S.
Eosinophil (%)	5.31 ± 7.98	1.74 ± 0.87	2.45	H.S.
Basophil (%)	0.23 ±0.27	0.20 ± 0.4	0.38	N.S.

* TLC – Total Leucocyte Count ** DLC – Differential Leucocyte Count
When $P < 0.001$ and Z value is > 2, the result is statistically considered as highly significant (H.S.). N.S. - Not Significant
n = 30 smokers

This study depicts that smoking has an effect on TLC. The increased TLC in smokers may be due to chronic tissue damage (Mehde & Salim, 2015). Smoking can have an irritant effect on the respiratory tree with resultant chronic inflammation (Vestbo et al., 2013). Prolonged smoking impairs ciliary movements, causes hypertrophy and hyperplasia of mucus secreting glands, hyper-responsiveness of the airways and causes bronchiolar inflammation (Nilsson et al., 2014; Indira et al., 2014). Airway epithelium serves as a physical barrier preventing the passage of inhaled noxious particles into the submucosa. The release of inflammatory cytokines from the epithelial cells is increased by exposure to smoke. Leucocytosis in smokers can be the result of the influence of these mechanisms on the growth, differentiation, and activation of leucocytes. Nicotine also increases the release of catecholamines which can subsequently increase the TLC (WHO, 2010). Hemoconcentration attributed to cigarette smoking can also be considered as a possible explanation for the elevation of the total leukocyte count (Indira et al., 2014; Legro et al., 2014).

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Nowadays, the progression of coronary heart disease can be associated not only with the known risk factors such as cigarette smoking, diabetes & hypertension but with inflammation as well. Elevated WBC counts as observed in smokers along with high C-reactive proteins (CRP) are also linked with increased incidence and mortality of coronary heart disease (Gillum et al., 1993; Berton et al., 2003; Danesh et al., 2004; Willems et al., 2010).

In the present study, subjects chosen were all active smokers and casual blood samples were taken. The findings revealed that there was a statistically highly significant increase in the monocyte and eosinophil count in smokers than in non-smokers. However, a highly significant decrease in the neutrophil count was seen in smokers compared to non-smokers. Among other parameters, lymphocyte and basophil count showed an insignificant increase in smokers compared to non-smokers. According to several researchers, effect of smoking on differential count is not uniform and is influenced by the current smoking behavior. Some studies have shown an increased neutrophil count, and a decreased lymphocyte count (Nagai et al., 1992; Schwartz & Weiss, 1994; Ogawa et al., 1998; Praslickova et al., 2015), while few studies have shown that both counts are increased (Andreoli et al., 2015; Farsalinos & Romagna, 2013). Smoking may also mediate association between depressive symptoms and increased neutrophil count (Vulser et al., 2015).

The findings of this study also showed that that the higher the number of cigarettes smoked, the greater the tendency to increase leukocyte count. There is a positive linear relationship between the number of cigarette sticks consumed per day and the total leukocyte count (Figure 1). As shown in Table 2, this relationship is found to be significant.

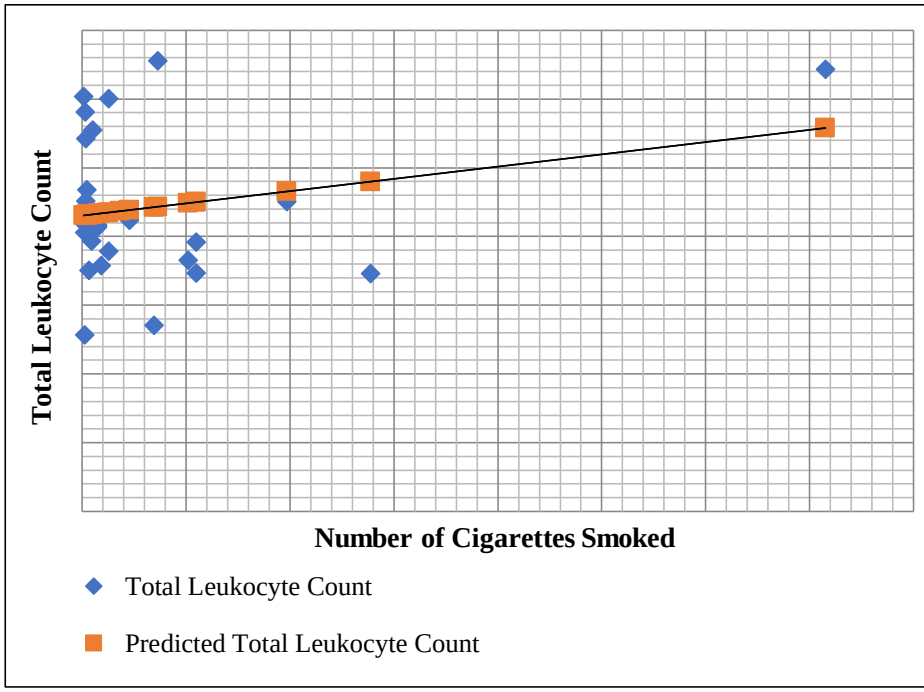

Figure 1. Line fit plot on the number of cigarettes smoked and total leukocyte count.

Table 2. Regression analysis on the number of cigarettes smoked and total leukocyte count.

	df	SS	MS	F	Significance F
Regression	1	7062281.115	7062281.115	1.768885271	0.194253052
Residual	28	111790105.6	3992503.77		
Total	29	118852386.7		t = 21. 3716	

The results of the present study also demonstrate a strongly positive relationship between the total days of smoking and total leukocyte count in healthy current smokers (Figure 2). This relationship is found to be significant (Table 3). The longer the duration of cigarette smoking, the greater the tendencies for the leucocyte count to increase.

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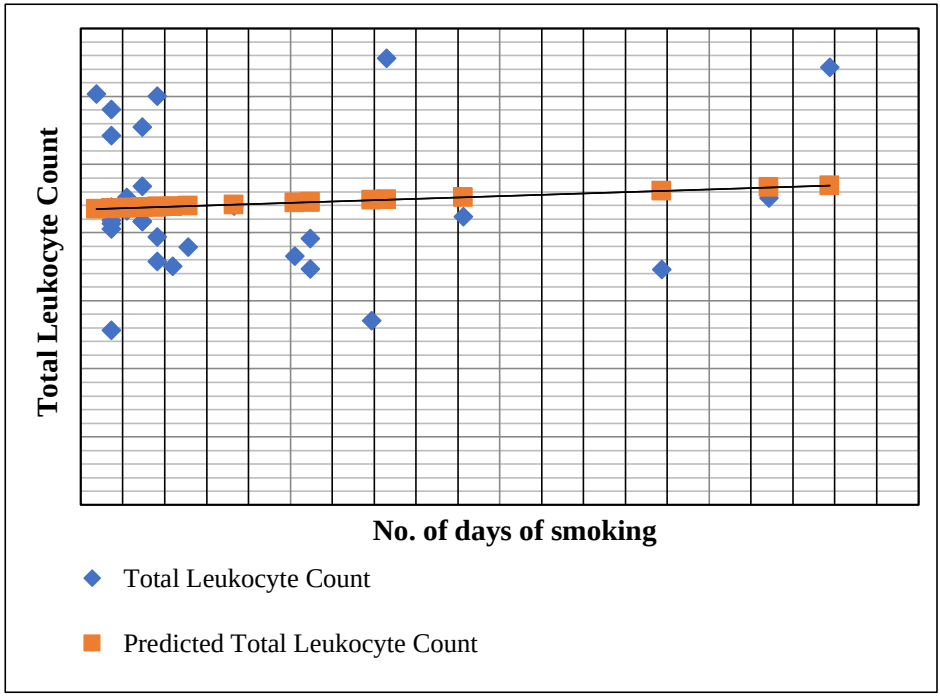

Figure 2. Number of days of smoking and total leukocyte count line fit plot.

Table 3. Regression analysis on the number of days of smoking and total leukocyte count.

	df	SS	MS	F	Significance F
Regression	1	1029546.507	1029546.507	0.244666502	0.624714803
Residual	28	117822840.2	4207958.577		
Total	29	118852386.7		t = 17.88	

Conclusion and Recommendations

There was a significant correlation between cigarette smoking and differential leukocyte. Increased white blood cell count specifically monocytes and eosinophil is shown to be associated with smoking and may increase the mortality rate in patients with ischemic heart disease. Hence, cigarette smoking is associated with alterations in inflammatory markers among smokers.

Additional research is necessary to determine as to what extent smoking needs to be reduced for health benefits and which biomarkers are most sensitive to measuring improved health. Reduction in smoking may be the right approach to engage subjects in treatment and may serve as a good initial step for individuals who resist quitting. Educating patients and families on the harmful effects of smoking by communicating this quantitative information may be a useful tool to assist cessation, promote long-term abstinence in smokers, and thus provide smoke-free environment.

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