Evaluation of Blood Cell Count and Red Cell Indices of Active and Secondhand Smokers in Association with the Degree of Smoking

Claudine F Arro, Maica F.Magpantay, Ann Kathleen A. Rubico, Cristelle M.Sobejana,and Dr. Leah F. Quinto
LPU-St. Cabrini College of Allied Medicine

ABSTRACT

Cigarette smoking is considered as an independent risk factor for coronary heart disease and one of the predisposing factors which contributes to the progression of numerous chronic and age-related disease processes. The mechanism behind the development of this disease incorporates hemostatic instabilities and vascular dysfucnction which is induced by the chemicals contained in the cigarette. The main concern of this study is to know if passive smokers are being affected the same way as active smokers knowing the acute and chronic effect of smoking on hematological parameters. Thus, this study aimed to compare the blood cell count and red cell indices of active smokers, secondhand smokers and non-smokers.

A total of 60 male participants, ages 25 to 40 from Sto. Tomas, Batangas were enrolled in the study with 20 individuals for active, secondhand and non-smokers respectively. The smokers were further classified into four groups (1-5 cigarettes/day, 6-10 cigarettes/day, 11-20 cigarettes/day, and >20 cigarettes/day) which determined the degree of smoking. Five milliliters of blood in EDTA tube was collected and analyzed by Sysmex Hematological Analyzer. The study reveals that passive smokers have higher total white blood cell count (TWBC) compared to active smokers. TWBC particularly neutrophils, lymphocytes, and monocytes are significantly increased in both active and secondhand smokers. Red cell indices show no significance in active, secondhand and non-smokers. Nevertheless, significant difference in mean corpuscular hemoglobin concentration (MCHC) was found in active smokers when compared to non-smokers. It has also been found that the red blood cell count, TWBC particularly neutrophils, monocytes, and basophils have a positive association with the degree of smoking. Other parameters and red cell indices shows no significance with the degree of smoking.

Key words: Smoking, Blood cell count, Red cell indices, Active smokers, Secondhand smokers

INTRODUCTION

Smoking continues to be a major public health concern accounting for roughly six million premature deaths annually (World Health Organization, 2017). It contributes significantly to morbidity and mortality across the world which affects various organ systems primarily the cardiovascular, reticuloendothelial and respiratory system (Inal, Hacıbekiroglu, Cavus, Musaoglu, Demir, & Karadag, 2014). Moreover, pathogenesis of chronic obstructive pulmonary disease (COPD) is attributed to cigarette smoke which causes cellular senescence (Ahmad, Sundar, Lerner, Gerloff, Tormos, Yao, & Rahman, 2015). The occurrence of this disease is due to the chemicals contained in the cigarette which emitted smoke with oncogenic, toxic, inflammatory and mutagenic effects to the body. One of the few highly burden countries is the Philippines. According to Department of Health (DOH) (2016), heart and vascular diseases, COPD, cancer and diabetes mellitus are the four main non-communicable leading cause of death among the Filipinos putting them at high risk of developing such diseases in exposure to tobacco smoke.

Health hazard is not only limited to active smokers but extends in every person who inhaled someone else's smoke. Passive smokers or commonly known as secondhand smokers are believed to be in greater menace compared to main smokers because the smoke directly absorbed by them known as side-stream smoke is actually more harmful than the mainstream smoke, as there is no filter it must pass through (Irish Cancer Society, 2016). Since 1964, around 2,500,000 non-smokers have died due to health problems

acquired from side-stream smoke (U.S. Department of Health and Human Services, 2014). It mostly affects children which is manifested by respiratory infections such as bronchitis and pneumonia. Similarly, adults who never smoked may develop heart disease and lung cancer (Centers for Disease Control and Prevention, 2017).

The mechanism behind the danger of having cardiovascular illness in connection to smoking incorporates hemostatic instabilities, vascular endothelial dysfunction and inflammation. Leukocyte specifically neutrophils participate in the latter process which makes it a useful prognostic indicator of coronary heart disease that is broadly accessible in medical practice (Uysal, Dağlı, Akgüllü, Avcil, Zencir, Ayhan, & Sönmez, 2016). Smoking damages the blood vessels hence, restricting oxygen from reaching the tissues and promotes elevation of cholesterol and other unhealthy fats in the circulation. If the disease progresses, it will lead to chronic inflammatory disease brought by lipids and leukocytes in broken artery (Swirski & Nahrendorf, 2013). This complication is known as atherosclerosis which clinically features inflammation. Many studies established the relationship of white blood cells (WBCs) and smoking and it has been found that smokers tend to have leukocytosis or increase in WBCs compare to non-smokers. It was proven in numerous literatures and studies that neutrophils increase remarkably in conjunction with smoking cigarette. However, little is known on the effect of cigarette smoking to the other subtypes of WBCs and its association with the number of cigarette consumption daily. This is also true on passive smoking, where leukocyte count is poorly explored.

Likewise, cigarette users have an altered red blood cell indices, such as mean corpuscular volume (MCV) as well as hemoglobin concentration (Hb), hematocrit (Hct), , and red cell count (RBC) (Malenica, Prnjavorac, Bego, Dujic, Semiz, Skrbo, & Causevic, 2017). Also, smoking has been identified to have antagonistic effects on cardiovascular risk profile causing higher blood viscosity, tissue plasminogen activator antigen and lower levels of an acute phase reactant, albumin (Sherke, Vadapalli, Bhargava, Sherke, & Gopireddy, 2016). Platelet, in the same manner is related to smoking (Nadi, Shamseldein, & Sara, 2015). Nevertheless, there is inconsistency on the results of platelet count and various RBC indices like mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC).

Considering the diseases and fatalities associated with smoking, a hypothesis arose that the hematological picture of the two different types of smokers mentioned above are relatively different from non-smokers and may further alter blood values and produce complication if exposure to cigarette smoke continues. Due to limited published literatures regarding the said matter, the researchers wanted to conduct a study concerning its effect on various cell counts and RBC indices. This study is significant to increase the awareness of chronic smokers and those who started recently on the prevention of developing smoking-induced diseases and more importantly, on restoration of blood values upon smoking cessation. This will also contribute to the awareness of the public on the deleterious effect of secondhand smoke. Consequently, this study aims to further investigate and assess the effect of mainstream and second-stream smoke on cell counts and various blood parameters in connection with the intensity of smoking. Specifically, the study aims to determine if there is a connection between smoking and other WBC subpopulation aside from neutrophils.

Objectives of the Study

This study evaluates blood cell count and red cell indices of active and secondhand smokers in association with the degree of smoking. It aims to assess the adverse effect of smoking on the hemtological picture of an individual. Specifically, it seeks to attain the following objectives 1) to compare the blood cell counts of active smokers, secondhand smokers and non-smokers in terms of: a. total white blood cell count and differential count, b. red blood cell count, and c. platelet count. Second, the study aims to compare the red cell indices of active smokers, secondhand smokers and non-smokers in terms of: a. mean corpuscular volume, b. mean corpuscular hemoglobin, and c. mean corpuscular hemoglobin concentration. Third, it

aims to determine the significant difference of white blood cell count, red blood cell count, and platelet count with the degree of smoking in terms of : a.1-5 cigarettes/day, b. 6-10 cigarettes/day, c. 11-20 cigarettes/day, and d. more than 20 cigarettes/day. Lastly, to determine the significant difference of red cell indices with the degree of smoking in terms of: a. 1-5 cigarettes/day, b. 6-10 cigarettes/day, c. 11-20 cigarettes/day, and d. more than 20 cigarettes/day.

MATERIALS AND METHODS

In order to satisfy the objectives of this study, an experimental design specifically, a posttest- only non-equivalent control group design was employed. This type of experimental method of research involved a control group and an experimental group which is not created through random assignment. This method was utilized to evaluate the changes on the hematological parameters of smokers and passive smokers through assessing and comparing the blood taken from the control group. The latter groups included persons who are not into smoking while the tested groups are the cigarette users and secondhand smokers. All groups underwent blood test to determine the effect of smoking on the total counts and subtypes of leukocytes, platelet count and selected characteristics of red blood cells and its association with the intensity of smoking.

The experimental type of research is the primary approach used to investigate the cause and effect relationship as well as the connection between variables. In this way, researchers were able to control the factors that might affect the outcome of experiment.

All necessary tests and experiments were conducted in the laboratory of LPU- St. Cabrini School of Health Sciences, Inc.

Blood samples were collected from 60 healthy male participants residing in Sto. Tomas, Batangas. They were selected purposively by asking a pre-qualifier question. They should report their age, comorbidities, concurrent medications, recent smoking status, history of blood transfusion, and the total number of cigarettes smoked per day. The total population is comprised of smokers, secondhand smokers and non-smokers with 20 individuals respectively. The group of smokers were further subdivided according to cigarette consumption and were identified as Group A- Smokers (1-5 cigarettes/day); Group B - Smokers (6-10 cigarettes/day); Group C - Smokers (11-20 cigarettes/day); and Group D - Smokers (more than 20 cigarettes/day) with five participants each.

The inclusions of the study were apparently healthy male ages 25 to 40 years old. Since inflammatory markers could be affected, subjects with any known comorbidities (such as diabetes mellitus, systemic hypertension, and thyroid disease) and current infections were excluded. Individuals with anemia, allergy, bleeding disorders and other blood disorders which may affect blood values were also eliminated. Exclusion criteria also included persons on concurrent medication (Nonsteroidal anti-inflammatory drug) and those who participated in blood transfusion/donation in the last six months.

The participants for passive smokers were the family members, co-workers and peers of smokers who often inhaled smoke but are never cigarette users. Both the classification of smokers and criteria of choosing the participants were based on the study conducted by Vadapalli et. al (2016) who covered a topic similar to this study.

The subjects were chosen accordingly using a predesigned and pretested checklist to determine the biosocial information of participants, such as age, smoking frequency and duration, medications being taken, diseases, blood disorders or infection and blood transfusion activity. Once qualified individuals were identified, an informed consent was asked before collecting blood samples. Afterwards, the researchers as well as the respondents agreed on the day and time of specimen collection and the processes along the experimentation.

Five milliliters of venous blood sample was collected in ethylenediaminetetraacetic acid (EDTA) tube from each participant. The specimens were kept steady at room temperature (20°C- 24°C) during

transport and other succeeding tests was performed upon its arrival on the testing area. In case of delay on processing, the blood is viable within six hours.

In Complete Blood Count, Sysmex automated hematological analyzer is a machine designed to evaluate different blood cells present in the blood with high accuracy and precision. It employed the Coulter principle of sizing and counting particles. It is based on measurable changes in electrical impedance produced by nonconductive particles suspended in an electrolyte.

The protocol of the study is to ensure the confidentiality of results and identity of all the participants. With regards to collecting blood sample, written informed consent was sought before extracting blood which testifies the agreement between the researchers and respondents. The standard operating procedure was ordered in accordance with the normal laboratory practice to prevent any harm and error. Lastly, aseptic technique which is a vital part of blood collection was strictly observed during the specimen collection.

The data were analyzed using SPSS (Statistical Package for Social Sciences) installable software that enabled the assessment of data using several statistical functions. Analysis of variance (ANOVA) and paired t- test were utilized as a treatment to assess the difference in the variables concerning smoking intensity and to compare the hematological picture of non-smokers, secondhand smokers and smokers. A p-value of < 0.05 will be considered significant.

RESULTS AND DISCUSSION

This presents the findings and outcomes of the experimental research. The results obtained are put through statistical analysis and are presented in this chapter. For better understanding, the results were divided and presented under the following tables. Discussion part provides information which supports the findings of this study.

The results show the average blood cell count of the control group (non-smokers) and experimental group (active and secondhand smokers). It reveals that the secondhand smokers have the highest total white blood cell count (WBC) with a mean of 8.18 $x10^3/\mu L$ and a standard deviation of 2.22. Neutrophil and lymphocyte counts are also higher in secondhand smokers with a mean of 4.31 $x10^3/\mu L$ and 2.82 $x10^3/\mu L$ respectively when compared to active smokers who have a mean neutrophil count of 4.05 $x10^3/\mu L$ and mean lymphocyte count of 2.72 $x10^3/\mu L$. On the other hand, red blood cell count of active smokers is higher than secondhand smokers with an average of 5.41 $x10^6/\mu L$ and a standard deviation of 0.90. Also, active smokers have the highest platelet count of 298.25 $x10^3/\mu L$ and a standard deviation of 56.45. It clearly shows that the blood values of active and secondhand smokers are being altered upon exposure to cigarette smoke. Exposure to side-stream smoke brings excessively higher cardiovascular risk than active smoking and is dependent to dose-response relationship (Lu, 2017).

Table 1. Blood Cell Count of Non-smokers, Active Smokers, and Secondhand Smokers

	Non-smokers		Active Smokers		Secondhand Smokers	
BLOOD COUNT	Mean	SD	Mean	SD	Mean	SD
TWBC	6.55	1.33	8.10	1.88	8.18	2.22
Neutrophils	3.27	0.99	4.05	1.47	4.31	1.61
Lymphocyte	2.28	0.53	2.72	0.56	2.82	0.92
Monocytes	0.50	0.13	0.63	0.21	0.63	0.15

Eosinophil	0.45	0.45	0.65	0.63	0.36	0.23
Basophils	0.05	0.02	0.05	0.02	0.05	0.02
RBC	5.25	0.74	5.41	0.90	5.39	0.66
Platelet	274.15	57.29	298.25	56.45	292.70	78.62

Table 2 discusses the comparison of blood cell count among active smokers, secondhand smokers, and non-smokers. There is a statistically significant increase in the total WBC count particularly, lymphocytes and monocytes at 0.39 and 0.19 respectively. The results corroborate with the findings of Shipa et al. (2017) and Aula and Qadir (2013), however, the study showed significant difference in all types of WBC subpopulation. Lymphocytosis is primarily attributed to increase T lymphocytes which explains the high risk of smokers to infections and neoplasia (Shenwai & Aundhakar, 2012). Aside from the inflammatory effect of smoking that causes increase in neutrophil count, another possible mechanism of leukocytosis is that nicotine triggers the release of catecholamine which can lead to elevation of WBCs. It is said to be that secondhand smokers have also higher risk for cardiovascular disease because of the smoke containing chemicals that affects the human body and has no safe level on exposure to smoke (National Toxicology Program, 2016).

Table 2. Comparison of Blood Cell Count among Non-smokers, Active Smokers, and Secondhand Smokers

BLOOD CELL COUNTS	F	Sig.	Interpretation
TWBC	4.969	.010	Significant
Neutrophils	3.051	.055	Not Significant
Lymphocytes	3.431	.039	Significant
Monocytes	4.232	.019	Significant
Eosinophil	2.001	.145	Not Significant
Basophils	.575	.566	Not Significant
RBC	.239	.789	Not Significant
Platelet	.756	.474	Not Significant

The difference between the blood values of active smokers and non-smokers were tabulated below. The statistical analysis discloses a significant difference in the total WBC count with a p-value of .005. Specifically, the marked increase in total WBC count is due to the elevation of lymphocyte and monocyte count which are also statistically significant with a p-value of 0.015 and 0.021 respectively. The result is similar with the study of Sherke et al. (2016) who reported significantly different total leukocyte count including neutrophils, lymphocytes, monocytes and eosinophils, however, red blood cell count was also said to be significant. However, both WBC and RBC count are significantly increased in active smokers (Bashir et al,. 2016; Nadia, Shamseldein, & Sara, 2015).

Table 3. Comparison of Blood Cell Count between Non-smokers and Active Smokers

BLOOD CELL COUNTS	t	p-value	INTERPRETATION
TWBC	-3.013	.005	Significant
Neutrophils	-1.958	.059	Not Significant

Lymphocytes	-2.557	.015	Significant
Monocytes	-2.429	.021	Significant
Eosinophil	-1.144	.260	Not Significant
Basophils	-.983	.333	Not Significant
RBC	-.587	.561	Not Significant
Platelet Count	-1.340	.188	Not Significant

The table reveals the comparison between the hematological parameters of secondhand smokers and control group. It reveals significant difference in the total leukocyte count particularly in neutrophils, lymphocytes, and monocytes at .020, .030, and .005 respectively. According to the study of Flouris et al. (2010), secondhand smoke significantly influences the likelihood of acquiring diseases related with cardiovascular, respiratory and metabolic system. In the experimental research of Dinas et al. (2014), it revealed significantly increased in WBC count after an hour of exposure to secondhand smoke when compared to the initial value. Their findings also noted a positive association between white blood cells and blood cotinine levels. It was believed that side-stream smoke is more harmful than the mainstream smoke thus, putting passive smokers to greater health hazard (Irish Cancer Society, 2016).

Table 4. Comparison of Blood Cell Count between Non-smokers and Secondhand Smokers

BLOOD CELL COUNTS	t	p-value	INTERPRETATION
TWBC	-2.825	.008	Significant
Neutrophils	-2.455	.020	Significant
Lymphocytes	-2.273	.030	Significant
Monocytes	-2.973	.005	Significant
Eosinophil	.795	.434	Not Significant
Basophils	-.482	.632	Not Significant
RBC	-.626	.535	Not Significant
Platelet Count	-.853	.400	Not Significant

The comparison of red cell indices among active smokers, secondhand smokers, and non-smokers were explained in the table. It reveals that the non-smokers have the highest mean corpuscular volume (MCV) with a mean value of 87.24 fL and a standard deviation of 3.44. Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are also higher in non-smokers with a mean of 29.29 pg and 33.35 g/dL as compared to active smokers with an average of 28.51 pg (MCH) and 32.84 g/dL (MCHC) and secondhand smokers being the least with a mean value of 27.93 pg (MCH) and 32.88 g/dL (MCHC). Changes in hematological parameters from the previous studies were also reported to be significantly high particularly in MCV and MCH levels, but smokers MCHC were significantly lower as compared to non-smokers (Bashir et al., 2016). This result supported by the study of Malenica et al. (2017) who stated that MCV and MCH were also significantly elevated.

Table 5. Red Cell Indices of Non-smokers, Active Smokers and Secondhand Smokers

RBC INDICES	Non-smokers		Active Smokers		Secondhand Smokers	
	Mean	SD	Mean	SD	Mean	SD
MCV	87.24	3.44	86.42	9.41	84.61	8.22
MCH	29.29	1.45	28.51	3.77	27.93	3.48
MCHC	33.55	0.86	32.84	1.26	32.88	1.31

Table 6 shows the evaluation of red cell indices among active smokers, secondhand smokers, and non-smokers. It reveals that none of these parameters are statistically significant. The following results are in congruent with the study of Ahmed (2017) who found that red blood cell count, red cell distribution width, hemoglobin, hematocrit, MCV, MCH, MCHC, and glucose levels are all not significant. Based on another study, mean cell volume and mean cell hemoglobin concentration in tobacco users has unaffected

RBC INDICES	F	Sig.	INTERPRETATION
MCV	.739	.482	Not Significant
MCH	.978	.382	Not Significant
MCHC	2.347	.105	Not Significant

results compared to the control group (Sultana et al., 2013). In addition, several factors interfere with the values of RBC indices which include hydration status, stress, and high altitudes. On the other hand, Nadia, Shamseldein, and Sara (2015) showed significantly increased in mean cell hemoglobin concentration (MCHC) and same remarks were noted by Asif et al. (2013) who included mean cell hemoglobin (MCH) in the parameters that is significantly increased.
showed significantly increased in mean cell hemoglobin concentration (MCHC) and same remarks were noted by Asif et al. (2013) who included mean cell hemoglobin (MCH) in the parameters that is significantly increased.

Table 6. Comparison of Red Cell Indices among Non-smokers, Active Smokers, and Secondhand Smokers

The results on Table 7 show the comparison of red cell indices between non-smokers and active smokers. It reveals that both MCV and MCH were not significant. Nevertheless, MCHC is noted to be significant and with accordance to the study of Ahmed (2017), MCHC was said to be significantly high as pack-years of smoking or duration of smoking increases. This is supported by the study of Nadia, Shamseldein, and Sara (2015) who claimed that MCHC of smokers were significantly increased when compared to non-smokers.

Table 7. Comparison of Red Cell Indices between Non-smokers and Active Smokers

RBC INDICES	t	p-value	INTERPRETATION
MCV	1.062	.301	Not Significant
MCH	.864	.396	Not Significant
MCHC	2.080	.045	Significant

The comparison of red cell indices between non-smokers and secondhand smokers which is clearly showed that there is no significant difference in the RBC indices. MCV and MCHC in tobacco users were not significant when compared to the control subjects (Jain et al., 2014; Sultana et al., 2013).

Table 8. Comparison of Red Cell Indices between Non-smokers and Secondhand Smokers

RBC INDICES	t	p-value	INTERPRETATION
MCV	1.324	.197	Not Significant
MCH	1.609	.120	Not Significant
MCHC	1.899	.066	Not Significant

The evaluation of blood cell count of active smokers in connection with the degree of smoking is shown in this Table 9. Total leukocyte count and total erythrocyte count are both significantly related to the degree of smoking with a p-value of 0.006 and 0.027 respectively. There is an association of elevated white blood cell count and red blood cell count with cigarette smoking (Lakshmi et al., 2014; Shipa et al., 2017; Vadapalli et al., 2016). In differential count of leukocyte subpopulations, neutrophils, monocytes, and basophils show significant relationship. The marked increase in neutrophil is due to the inflammatory reaction induced by the chemicals contained in a cigarette which is also a key prognostic marker for coronary heart diseases (Swirski & Nahrendorf, 2013). Weakened host defense observed in smokers may be due to the defective phagocytic activity of monocytes which is the precursor of tissue macrophages. On the other hand, histamine release from basophils may be a mediator of bronchoconstriction upon exposure to smoke irritant. Tulgar et al. (2016) also reported significant difference in monocyte and basophil count. In contrast, no hematological parameters have been found to have a significant correlation with number of cigarettes smoked per day (p value: TWBC 0.999, RBC 0.738, Hb 0.715, PCV 0.876, platelets 0.890, lymphocytes 0.737 and neutrophils 0.290) (Abdalla, 2016).

Table 9. Evaluation of Blood Cell Count in Association with the Degree of Smoking

The results on Table 20 show that the changes in the red cell indices are not significantly related to the degree of smoking. This is supported by the study of Ahmed (2017) explicating that MCV, MCH, and MCHC were reduced insignificantly in cigarette smoking. Similar study done by Pankaj, Reena, Mal, and Ketan (2014) revealed insignificant results except MCHC. The decreased level in MCHC that indicates hypochromic anemia might be due to lack of folic acid, vitamin B12 or thyroid problems. The differences identified between the compositions of the erythrocyte of smokers might be impressions of gaseous and solid phases of tobacco smoke toxic products effects on the bone marrow as well as the adaptive and immunologic reactions of the body to long haul dynamic smoking (Muhammad et al., 2014). Concerning the blood collection for active smokers, random blood specimen was drawn. The participants were not subjected to smoking beforehand which may have affected the results because the half-life of nicotine in blood is only two hours.

Table 10. Evaluation of Red Cell Index in Association with the Degree of Smoking

BLOOD CELL COUNTS	F	Sig.	Interpretation
TWBC	4.036	.006	Significant
Neutrophils	2.966	.027	Significant
Lymphocytes	1.483	.220	Not Significant
Monocytes	4.004	.006	Significant

Eosinophil	.930	.453	Not Significant
Basophils	7.028	.000	Significant
RBC	2.050	.100	Not Significant
Platelet	3.122	.022	Significant

CONCLUSION AND RECOMMENDATION

In the light of the data presented above, the study concludes that passive smokers have significantly higher total white blood cell count (TWBC) compared to active smokers. Total red blood cell count and platelet count are increased in active and secondhand smokers, but their values remain insignificant. When comparing the blood cell counts of active smokers and non-smokers, TWBC particularly lymphocytes and monocytes reveal significant difference. The same findings are noted to be significant in secondhand smokers with the inclusion of neutrophil count. The comparison among the three groups shows significant difference in TWBC, neutrophil, lymphocyte, and monocyte counts. All levels of red cell indices are greater in the control group compared to the experimental group. Significant difference in mean corpuscular hemoglobin concentration (MCHC) is found in active smokers while secondhand smokers found no significance in all of the indices. With regards to the degree of smoking, total red blood cell count and white blood cell count particularly neutrophils, monocytes, and basophils are continuously altered as the number of cigarette consumption increases. On the other hand, red cell indices and platelet count reveal insignificant results in association with the degree of smoking.

RBC INDICES	F	Sig.	INTERPRETATION
MCV	1.074	.378	Not Significant
MCH	2.133	.089	Not Significant
MCHC	1.166	.336	Not Significant

Based on the findings and conclusions, the study suggests that the time of blood collection should be done approximately within two hours right after they smoke. This is for better observation of the actual and initial effect of smoking on blood cell parameters especially in the red blood cell. Appropriate sample size will produce studies capable of detecting clinically relevant differences and will also make the research more reliable. If future scholars only aims to evaluate the effect of smoking and compare it to non-smokers, it is proposed that smokers who consume larger number of cigarettes regularly will be more suitable in order to investigate its adverse effects. For future studies, utilization of a pretest and posttest design will clearly determine the effect of smoking on hematological parameters. Lastly, a more advanced technology such as flow cytometer should be used to make the study more illuminated in the future research.

REFERENCES

Ahmad, T., Sundar, I. K., Lerner, C. A., Gerloff, J., Tormos, A. M., Yao, H., & Rahman, I. (2015). Impaired mitophagy leads to cigarette smoke stress-induced cellular senescence:

implications for chronic obstructive pulmonary disease. The FASEB Journal, 29(7), 2912-2929.

Ahmed, O. A. (2016). Effects of Smoking Cigarette on White Blood Cell and Platelet Parameter on a Sample of Normal Subject in Rania City. *Imperial Journal of Interdisciplinary Research*, *2*(9).

Ahmed, O. A. (2017). Effect of Pack-Year of Cigarette Smoking on Erythrocyte Parameters and Glucose Level Among Healthy Males. GMJ, 28, 196-199.

Ahmed, R.B. and Abdalla, M.H.A. (2017) Effects of Cigarette Smoking on White Blood Cells Count and von Willebrand Factor Levels in Male Smokers in Khartoum State. Open Access Library Journal, 4: e3506. https://doi.org/10.4236/oalib.1103506

American Cancer Society (2015). Health Risks of Secondhand Smoke. Retrieved from https://www.cancer.org/cancer/cancer-causes/tobacco-and-cancer/secondhand-smoke.html#references

Asif M, Karim S, Umar Z, Malik A, Ismail T, Chaudhary A et al. Effect of cigarette smoking based on hematological parameters: comparison between male smokers and nonsmokers. Turkish Journal of Biochemistry.2013; 38(1): 75- 80. 6.

Bashir, B. A., Gibreel, M. O., Abdalatif, H. M., Mohamed, M. A., Ahmed, E. A., Mohamed, M. S., & Hamid, K. A. (2016). Impact of tobacco cigarette smoking on hematologic parameters among male subjects in Port Sudan Ahlia College, Sudan. Sch. J. App. Med. Sci, 4, 1124-8.

CDC. (2017, February 21). Smoking & Tobacco Use. Retrieved May 23, 2018, from https://www.cdc.gov/tobacco/data_statistics/fact_sheets/secondhand_smoke/general_facts/index.htm

Dinas, P. C., Metsios, G. S., Jamurtas, A. Z., Tzatzarakis, M. N., Koutedakis, Y., Tsatsakis, A. M., & Flouris, A. D. (2014). Acute effects of second-hand smoke on complete blood count. International journal of environmental health research, 24(1), 56-62.

DOH. (2016). What are the leading causes of mortality in the Philippines? | Department of Health website. [online] Available at: https://www.doh.gov.ph/node/1058 [Accessed 03 March 2018].

Flouris, A.D., Vardavas, C.I., Metsios, G.S., Tsatsakis, A.M., & Koutedakis, Y. (2010). Biological evidence for the acute health effects of secondhand smoke exposure. Am J Physiol Lung Cell Mol Physiol 298: L3-L12. First published September 18, 2009; doi: 10.1152/ajplung.00215.2009.

Inal, B., Hacıbekiroglu, T., Cavus, B., Musaoglu, Z., Demir, H., & Karadag, B. (2014). Effects of smoking on healthy young men's hematologic parameters. Northern clinics of Istanbul, 1(1), 19.

Irish Cancer Society (2016, May 30). Secondhand smoke. Retrieved from https://www.cancer.ie/reduce-your-risk/smoking/health-risks/second-hand-smoke#sthash.ab699OgX.dpbs

Jain P, Jain R, Mal KL and Mangukiya K. 2014 Effect of cigarette smoking hematological parameters: comparison between male smokers and non-smokers. International Journal of Science and Nature. 2014; 5(4): 740-743.

Lakshmi, A., Anandhi Lakshmanan, G. K. P., & Saravanan, A. (2014). Effect of intensity of cigarette smoking on haematological and lipid parameters. *Journal of clinical and diagnostic research: JCDR*, *8*(7), BC11.

Lu, Liya (2017) Association between active smoking, secondhand smoke and peripheral arterial disease. PhD thesis, University of Glasgow.

Malenica, M., Prnjavorac, B., Bego, T., Dujic, T., Semiz, S., Skrbo, S., ... & Causevic, A. (2017). Effect of Cigarette Smoking on Haematological Parameters in Healthy Population. Medical Archives, 71(2), 132.

Nadia, M. M., Shamseldein, H. A., & Sara, A. S. (2015). Effects of Cigarette and Shisha Smoking on Hematological Parameters: An analytic case-control study. IMJH, 10, 44-51.

National Cancer Institute (2011). Secondhand Smoke and Cancer. Retrieved from https://www.cancer.gov/about-cancer/causes-prevention/risk/tobacco/second- hand-smoke-fact-sheet

National Cancer Institute Dictionary of Cancer Terms. (2009). Retrieved from https://www.cancer.gov/publications/dictionaries/cancer-terms/def/pack-year

National Toxicology Program (2016). Report on Carcinogens, Fourteenth Edition. Research Triangle Park (NC): U.S. Department of Health and Human Services, Public Health Service [accessed 2018 May 23].

Pankaj Jain, Reena jain, Mal, K. L. & Ketan Mangukiya. (2014). Effect of Cigarette Smoking on Hematological Parameters: Comparison between Male Smokers and Nonsmokers

Shenwai M, Aundhakar MRS. effect of cigarettes smoking on various hematological parameters in young male smokers. Indian Journal of Basic & Applied Medical Research 2012; 5: 386-92. I.J.S.N., VOL.5 (4) 2014: 740-743 ISSN 2229 – 6441740

Sherke, B. A., Vadapalli, K., Bhargava, D. V., Sherke, A. R., & Gopireddy, M. M. R. (2016). Effect of number of cigarettes smoked per day on red blood cell, leukocyte and platelet count in adult Indian male smokers–A case control study. International Journal of Medical Research and Health Sciences, 5(2), 13- 17.

Shipa, S. A., Rana, M. M., Miah, M. F., Alam, M. J., & Mahmud, M. G. R. (2017). Effect of Intensity of Cigarette Smoking on Leukocytes among Adult Men and Women Smokers in Bangladesh. *Asia Pacific Journal of Medical Toxicology*, *6*(1), 12-17.

Sultana, M. F., Sarkar, C. R., Zahid, A. R., & Ahmed, N. 2013 Effect of Tobacco Consumption on Red Cell Indices in Tobacco Users.

Swirski, F. K., & Nahrendorf, M. (2013). Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. Science, 339(6116), 161-166.

Tulgar, Y. K., Cakar, S., Tulgar, S., Dalkilic, O., Cakiroglu, B., & Uyanik, B. S. (2016). The effect of smoking on neutrophil/lymphocyte and platelet/lymphocyte ratio and platelet indices: a retrospective study. Eur Rev Med Pharmacol Sci, 20(14), 3112-8.

U.S. Department of Health and Human Services. How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the

U.S. Department of Health and Human Services. The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General.Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2014 [accessed 2018 May 23].

Uysal, H. B., Dağlı, B., Akgüllü, C., Avcil, M., Zencir, C., Ayhan, M., & Sönmez, H. M. (2016, November). Blood count parameters can predict the severity of coronary artery disease. Retrieved April 08, 2018, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5094927/

Vadapalli, K., Devarakonda, B. V., Sherke, B. A., Vijayalakshmi, A., & Malhotra, V. (2016). Evaluation of the sequential effect of quantity of cigarettes smoked per day on differential leukocyte count among North Eastern Indian adult males. *National Journal of Physiology, Pharmacy and Pharmacology*, *6*(6), 572.

World Health Organization. (2017). Meeting on Accelerating the Raising of Tobacco Taxes and the Ratification of the Protocol to Eliminate Illicit Trade in Tobacco Products in the Western Pacific, Manila, Philippines, 27-29 November 2017: meeting report. Manila: WHO Regional Office for the Western Pacific.