ASSESSMENT OF RED BLOOD CELLS MORPHOLOGY AND THE ASSOCIATED FACTORS AMONG YOUNG CHINESE STUDENTS PURSUING TERTIARY EDUCATION

By

KHOO SIN YE

A project report submitted to the

Department of Allied Health Sciences

Faculty of Science

Universiti Tunku Abdul Rahman

in partial fulfilment of the requirement for the

degree of

Bachelor of Science (Honours) Biomedical Science

April 2023

ABSTRACT

ASSESSMENT OF RED BLOOD CELLS MORPHOLOGY AND THE ASSOCIATED FACTORS AMONG YOUNG CHINESE STUDENTS PURSUING TERTIARY EDUCATION

KHOO SIN YE

Smoking cigarette, vaping, secondary smoker and exercise are several factors that can alter the morphology of the RBC and other blood parameters. Therefore, the main objective of this study was to determine the prevalence of abnormal red blood cells among the young adults and to analyse the associated factors of abnormal red blood cells among the young adults. This study conducted using a cross-sectional survey, using the Google Form questionnaire that was developed. The sampling was started from November 2022 to February 2023, and successful recruited 321 participants in this study. In the clinical assessment, anthropometry measurement, hemoglobin level, blood smearing and blood staining were performed. The microscopic view was conducted to identify the blood morphology abnormalities. Chi-square test was performed to analyse the association between the blood morphology abnormalities with the sociodemographic characteristics, exercise, smoking status, anthropometry measurement and hemoglobin level. The result showed the majority of the

students possessed RBC with smaller and paler in colour than normal. The prevalence of anisocytosis, hypochromic and poikilocytosis are 50.8%, 53.3% and 47.0% respectively among the young adults. Significant associations were discovered between the gender, secondary smokers, height and weight. The variation in RBC colour was found to be significantly affected by gender (χ^2=6.576, p=0.010), height (χ^2=21.040, p=0.000) and weight (χ^2=13.310, p=0.038). In addition, only secondary smokers (χ^2= 7.182, p=0.028) was found to have a significant influence on variation in RBC size. In conclusion, even the participant recruited was healthy, but there were several types of blood abnormalities can be observed from the blood due to some unhealthy lifestyle. Therefore, proper education should be tailored to the young adults in order to raise the awareness of seriousness of smoking that affect the red blood cells morphology as an early underlying symptom for latter diagnosis of chronic disorders.

ACKNOWLEDGEMENT

First and foremost, I would like to express my gratitude and appreciation to my supervisor, Dr Annaletchumy Loganathan, for giving me the chance to conduct this research and provide the assistance and unwavering support at every stage of this research project. I am extremely thankful for her enormous knowledge and experience, which give me a greater help and guidance that allow me to complete this project successfully.

Besides that, I would like to extend my grateful to all the respondents who were willing to participated in this research, otherwise this research would be difficult to carry out smoothly. Furthermore, I forward my appreciation to all my group members in this research, Liow Zhe Ying, Ng Zhen Yong, Wendy Khor and Yeong Shue Rou for their guidance and teamwork.

Last but not least, I would like to thank my dearest family members, especially my parents who always give the support and encouragement throughout my college life. Because of them that I have the opportunity to complete my study.

DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

Khoo Sin Ye

APPROVAL SHEET

This project report entitled **"ASSESSMENT OF RED BLOOD CELLS MORPHOLOGY AND THE ASSOCIATED FACTORS AMONG YOUNG CHINESE STUDENTS PURSUING TERTIARY EDUCATION"** was prepared by KHOO SIN YE and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Biomedical Science at Universiti Tunku Abdul Rahman.

Approved by:

(DR ANNALETCHUMY A/P LOGANATHAN) Date: 7 June 2023

Supervisor

Department of Allied Health Sciences

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: 7 June 2023

PERMISSION SHEET

It is hereby certified that **KHOO SIN YE** (ID No: **19ADB04056**) has completed this final year project entitled **"ASSESSMENT OF RED BLOOD CELLS MORPHOLOGY AND THE ASSOCIATED FACTORS AMONG YOUNG CHINESE STUDENTS PURSUING TERTIARY EDUCATION"** supervised by Dr. Annaletchumy Loganathan from the Department of Allied Health Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

(KHOO SIN YE)

TABLE OF CONTENTS

	PAGE
ABSTRACT	ii
ACKNOWLEDGEMENT	iv
DECLARATION	v
APPROVAL SHEET	vi
PERMISSION SHEET	vii
TABLE OF CONTENTS	viii
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF ABBREVIATIONS	xiii

CHAPTER

1 INTRODUCTION	1
1.1 Research Question	5
1.2 Problem Statement	5
1.3 Hypothesis	5
1.3.1 Null Hypothesis	5
1.3.2 Alternative Hypothesis	5
1.4 Objective	5
2 LITERATURE REVIEW	7
2.1 Effect of Cigarette Smoking on Human Health	7
2.2 Effect of Cigarette Smoking on Hematological Parameters	8
2.3 Effect of High Altitude on Hematological Parameter and Red Blood Cell Morphology	10
2.4 Effect of Exercise on Hematological Parameter	12
2.5 Effect of Alcoholism on Hematological Complications	14
3 METHODOLOGY	17
3.1 Study Area	17
3.2 Study Population	18

3.3 Study Design 19
3.4 Sample Size 19
3.5 Ethical Approval 20
3.6 Data Collection Tool: Cross-Sectional Survey 20
3.7 Data collection Tool: Clinical Assessment 22
3.7.1 Anthropometry Measurement 22
3.7.2 Finger Pricking Technique 24
3.7.3 Hemoglobin Concentration 26
3.7.4 Blood Smearing 28
3.7.5 Blood Staining 29
3.7.6 Microscopic Examination 29
3.7.7 Blood Screening Review 30
3.8 Data Collection Method 30
3.9 Data Analysis 31

4 RESULTS 32
4.1 Socio-demographic Characteristics 32
4.2 Individual's Health Status 35
4.3 Lifestyle Factors 36
4.4 Smoking Status 37
4.5 Anthropometry Measurement 37
4.6 Hemoglobin Measurement 39
4.7 RBC Morphology Characterization 40
4.8 Association of Blood Morphology Abnormalities with Sociodemographic Characteristics 45
4.9 Association of Blood Morphology Abnormalities with Lifestyle Factor of The Participants 49
4.10 Association of Blood Morphology Abnormalities with Smoking Status 52
4.11 Association of Blood Morphology Abnormalities with Anthropometry Measurement 54
4.12 Association of Blood Morphology Abnormalities with Hemoglobin Level 59

5 DISCUSSION 63
5.1 Sociodemographic Characteristics 63

5.2 Smoking Status 63
5.3 Anthropometry Measurement 64
5.4 Hemoglobin Level 65
5.5 Blood Morphology Abnormalities 66
5.6 Association Factor That Cause Blood Morphology Abnormalities 69
5.6.1 Sociodemographic Characteristics 69
5.6.2 Lifestyle Factor 69
5.6.3 Smoking Status 70
5.6.4 Anthropometry Measurement 71
5.6.5 Hemoglobin Level 72
5.7 Limitation and Future Recommendation 72

6 CONCLUSION 74
6.1 Conclusion 74
6.2 Implications 75

REFERENCES 76

APPENDICES 86

LIST OF TABLES

Table		Page
3.1	Inclusion and Exclusion Criteria	18
4.1	Sociodemographic Details of Participants	33
4.2	Medical History of The Participants	35
4.3	Lifestyle Factor of The Participants	36
4.4	Smoking Status of The Participants	37
4.5	Anthropometry Measurement of Participants	38
4.6	The Frequency and Prevalence of Hemoglobin Level among Male and Female	39
4.7	Blood Morphology Abnormalities	41
4.8	Association of RBC Size with Sociodemographic Characteristics	45
4.9	Association of RBC Colour with Sociodemographic Characteristics	47
4.10	Association of Poikilocytosis with Sociodemographic Characteristics	48
4.11	Association of RBC Size with Lifestyle Factor	50
4.12	Association of RBC Colour with Lifestyle Factor	50
4.13	Association of Poikilocytosis with Lifestyle Factor	51
4.14	Association of RBC Size with Smoking Status	52
4.15	Association of RBC Colour with Smoking Status	53
4.16	Association of Poikilocytosis with Smoking Status	54
4.17	Association of RBC Size with Anthropometry Measurement	55
4.18	Association of RBC Colour with Anthropometry Measurement	56
4.19	Association of Poikilocytosis with Anthropometry Measurement	58
4.20	Association of RBC Size with Hemoglobin Level	59
4.21	Association of RBC Colour with Hemoglobin Level	60
4.22	Association of Poikilocytosis with Hemoglobin Level	61

LIST OF FIGURES

Figure		Page
3.1	Map of the UTAR Location in Perak	18
3.2	Correct of posture standing when using Seca scale	23
3.3	Details of the measurement of body composition monitor	24
3.4	Procedures and precaution steps of finger pricking	26
3.5	Steps of blood collection and filling the microcuvette	27
3.6	Steps of blood smear preparation	28
3.7	Morphology of stained peripheral blood smear	29
4.1	Frequency of different types of poikilocytosis	42
4.2	Images of anisocytosis and poikilocytosis of the RBC	42
4.3	Images of normocytic and normochromic	43
4.4	Images of microcytic, hypochromic and macrocytic, hypochromic of the RBC	43
4.5	Images of ovalocytes, target cells, echinocytes and schistocytes	43
4.6	Images of elliptocytes, tear drop cells, and bite cells	44
4.7	Images of spherocytes and heinz bodies	44
4.8	Images of polychromasia and stomatocytes	44

LIST OF ABBREVIATIONS

BMI	Body Mass Index
CO	Carbon Monoxide
EPO	Erythropoietin
EDTA	Ethylenediaminetetraacetic Acid
Hb	Hemoglobin
HbCO	Carboxyhemoglobin
Hct	Hematocrit
K^+	Potassium ion
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Cell Volume
n	Number of Respondent
p	p value
PV	Plasma Volume
RBC	Red Blood Cell
RDW	Red Cell Distribution Width
SA	Sideroblastic Anemia
UTAR	Universiti Tunku Abdul Rahman
WBC	White Blood Cell
χ^2	Chi-square
%	Percentage

CHAPTER 1

INTRODUCTION

Cigarette smoking is one of the health issues affecting the public health around the world (Aldosari, et al., 2020). According to the World Health Organisation (2022), there are 22.3% of the global population was cigarette smokers. 36.7% of the smokers are men and 7.8% of the smokers are women. Smoking prevalence is highest among adults, which is 23% of those 16-24 age group and 24% among those in 25-34 age group. The lowest smoking prevalence is the elderly people aged 60 and above is 8.9%. Based on the statistics, more than 8 million of people die each year due to smoking related disease. More than 7 million of the death cases are due to active smoker while around 1.2 million are caused by second hand smoker. Active smoker is an individual who currently smoked at least one cigarette a day. Secondary smoke is the cigarette smoke released from the cigarette's burning end or the smoke exhaled from the smoker. Quitting smoker is the individuals want to quit for smoke since they become aware from the harmful of the cigarette. However, nicotine contained in cigarette smoke will trigger the highly addictive reaction. Only 4% of the smokers who effort to quit cigarette smoke will succeed (World Health Organization, 2022).

Smoking has brought the detrimental effects with both severe and long-lasting consequence on haematological parameters. Smoking cigarettes is associated with an elevated risk of cardiovascular disease, including myocardial infraction,

coronary artery disease, peripheral vascular disease, ischemic heart disease and atherosclerosis (AlQahtany, et al., 2020; Gallucci, et al., 2020).

Cigarette smoke released the variety of dangerous chemicals which is toxic and carcinogens into human body. Cigarette contains more than 7000 chemical compounds and about 70 of them can cause cancer (Centers for Disease Control and Prevention, 2021). The harmful chemicals enter our lungs through breathing and diffuse into bloodstream and finally the entire organs and body. These chemical compounds will damage DNA and the parts that protect human against cancer. The chemicals causing the cells harder to repair the damaging DNA. The build-up of DNA damage over the time will leads to the cancer in cells (Cancer Research, 2021).

Chemical compounds of the cigarette smoke are considered harmful to the human health, including free radicals, nicotine, tars and carbon monoxide (CO). These chemical compounds are caused the adverse effect to the pharmacological activity (Herath, et al., 2021). CO able to diffuse rapidly to bind firmly with the hemoglobin (Hb) to form carboxyhemoglobin (HbCO) in the alveolar capillaries. The binding ability of CO to the Hb is 200 to 250 times greater than oxygen. The formation of HbCO is leading to tissue hypoxia and increased the levels of Hb and red blood cells (RBCs) (Aldosari, et al., 2020).

In the previous study, Aldosari, et al. (2020) have reported that cigarette smokers may elevate the levels of hematological parameters such as RBC, Hb and hematocrit (Hct). It has been found that the increased number of cigarettes

smoked per day will cause the elevated of RBC count. Morphological changes in complete blood counts show the changes in mean cell volume (MCV) due to effect of CO in RBCs. This situation occurs due to the hypoxia of tissue and increase the oxygen demand of the RBCs. Furthermore, cigarette smoking has been shown to have an adverse effect on white blood cell (WBC) in both male and female smokers. Smokers have significantly higher of WBC count compare to non-smokers. The elevated WBC levels also include neutrophils, lymphocytes, monocytes, eosinophils and basophils. Exposure of the tissues or cells to toxic substances found in the cigarette smoke can leads to the impairment of hematopoietic mechanisms and causing the adverse effect on haematological parameters (Herath, 2021).

In addition, Shamsuddin and Haris, (2000) have reported that factors associated with parental smoking, especially father's smoking behaviour was caused their children to start smoking. According to this study, 41.4% among the male students had tried smoking from the age 16 to 17 years old. This condition can be explained by the role-modelling effect. Children is the one who always imitate the behaviour of the parents (Kandel, 2015). Therefore, the young children who grows up in a home where they frequently interact with a smoking parent has a higher prevalence of beginning to smoke compare with non-smoking parents (Hospice, 2020).

Besides that, peer pressure can also be a factor that cause the young adult to smoke. Peer group can be categories in a variety of ways, including close friendships, romantic relationships or colleague at work (Wood, 2020). Prior to

the work of Robalino and Macy (2018), the results showed a positive association between peer pressure and smoking. This study indicated that 20% of smokers initiate smoking because of peer pressure. The teenagers always encouraged to try smoking by their friend who smoked. As the result, they try smoking but they are unaware that it can lead to addiction (American Lung Association, 2022). Thus, young adult's current smoking habits were significantly associated with the peers who smoked and poor understanding on the negative effect of smoking.

The media is an effective instrument that greatly influences how people perceive smokers. There are several movie characters are smokers, which may lead young people to assume that smoking is attractive and fashionable (Hospice, 2020). The recent study (Shadel, Tharp and Fryer 2008) has shown the young adults smoking is strongly associated to increased exposure to cigarette advertisements. In the past, many cigarette advertisements aimed towards young adults and used imagery of appealing, hip and rebellious people smoking cigarette to associate smoking with good qualities. This commercial always ignored the health consequences of smoking (American Lung Association, 2022).

1.1 Research Question

There have three research question include in this study. Firstly, (1) What is the prevalence of abnormal red blood cells morphology among younger adults? Secondly, (2) What is the associated factors of abnormal red blood cells morphology among young adults? Thirdly, (3) What are the changes in red blood cells morphology due to smoking among young smokers?

1.2 Problem Statement

There is scarcity of the previous studies that focused on the young adults of below 30 years old. Secondly, there is a plausible of the research to studies on the red blood morphology and the associated factors among the young adults.

1.3 Hypothesis

1.3.1 Null Hypothesis

There are no association between the blood morphology abnormalities with the socio-demographic factors among the young adults.

1.3.2 Alternative Hypothesis

There are association between the blood morphology abnormalities with the socio-demographic factors among the young adults.

1.4 Objective

This present study was aim (1) To determine the prevalence of abnormal red blood cells among the young adults. The second objective was (2) To analyse the associated factors of abnormal red blood cells among the young adults. The

third objective was (3) To determine the changes in red blood cells morphology due to smoking cigarette, vape, secondary smokers and exercise among young adults.

CHAPTER 2

LITERATURE REVIEW

According to the statistics reported by Centers for Disease Control and Prevention (2022a), almost 90% of daily cigarette smokers in adulthood start smoking by the age of 18 and 99% start smoking by the age of 26. Smoking can lead to nicotine addiction in young people, early heart damage and reduce lung growth and function. Smoking can cause the impairment of the capacity of smokers to participate in spots and other physical activities even in young smokers. Smoking increases the risk of developing cancer, asthma, bronchitis, certain tooth and gum disease and other immune system problems (Frederick Health, 2021). The development of adolescent brain can be harmed by nicotine. The brain continues to grow until age 25. The regions of brain that regulate attention, learning, emotion and impulse control can damaged in adolescents who use nicotine. Stronger connections or synapses are formed between brain cells when a new memory and new skill is gained. The brain of the younger people developed synapses more quickly than adult brain. Nicotine alters the way these synapses created (Centers for Disease Control and Prevention, 2022b).

2.1 Effect of Cigarette Smoking on Human Health

Smoking increases the build-up of fat, cholesterol and other substances in the blood and leading to the formation of plaque in the blood vessels. This occurrent causing the narrowing and less flexibility of the arteries. The blood flow become interrupt and no longer flow properly to various part of the cells and tissues will

eventually cause the atherosclerosis. Besides that, smoking also increase the risk of stroke. Since the blood flow to the brain is interrupted, lack of the oxygen supply can cause the permanent brain damage and death (Centers for Disease Control and Prevention, 2014). In addition, the short-term effects of smoking may cause some adverse effect such as bad breath, fatigue, dizziness and coughing. If the duration of smoking increase, it may reduce the life expectancy and also cause the asthma, tuberculosis, stroke and heart disease (Davos, 2023).

In addition, cigarette smoking contributes to a major risk of getting cancer. For instances, smoking will increase the chance of cancer in lung, oral cavity, larynx and pharynx. Cigarette smoking not only trigger the morbidity and also the mortality. According to the American Cancer Society, there are 30% of cancer death is due to the cigarette smoking and 80% of deaths is caused by lung cancer (Aldosari, et al., 2020).

2.2 Effect of Cigarette Smoking on Hematological Parameters

The major focus of the hematological parameters is on the red blood cell (RBC) morphology because RBC are oxygen-carrying cells. The abnormality in the RBC count can causing the decrease oxygen carrying capacity ultimately facing the difficulties in the daily life activities. Based on the research of Aldosari, et al. (2020) the study population with 7.54 years of average time to smoking have 40% of the smokers suffered from shortness of breath. 60% of the smokers have shown the RBCs with macrocytic compared with 4% of non-smokers. Besides that, the levels of Hb, Hct, MCV and mean corpuscular hemoglobin (MCH) are significantly elevated in the smokers compare with non-smokers. However, the

differences in red cell distribution width (RDW) and mean corpuscular hemoglobin concentration (MCHC) value is not significant among the smokers and non-smokers. The WBC count, neutrophils and lymphocytes are statistically higher in smokers compare with non-smokers (Çiftçiler, et al., 2019).

RBC count, Hb and Hct levels are statistically increased with the intensity of cigarette smoking among the smokers. The elevation of Hb concentration is due to mediated by exposure to CO and also compensatory mechanism (Çiftçiler, et al., 2019). Increased of RBC count and Hct value in the smokers is caused by tissue hypoxia. Since the formation of carboxyhemoglobin increased, the secretion of erythropoietin also elevated and thus leading to higher erythropoiesis. CO from the cigarette smoke will increase the permeability of the capillaries which reduces the plasma volume and leading to polycythemia. The increasing of the RBCs in the blood volume which is contributed to higher level of hematocrit (Malenica, et al., 2017).

In addition, MCV is a blood test used to measure the average size of RBCs (National Library of Medicine, 2022). The increased levels of MCV and MCH in the smokers is considered that smokers might probably suffer from megaloblastic, hemolytic, pernicious or macrocytic anemia that commonly due to deficiency of vitamin B12 and folic acid (Çiftçiler, et al., 2019). Next, the macrocytic of RBCs in the smoker is one of the RBC morphological changes. The direct toxic effect of cigarette smoke caused the RBC decreased the

oxygen-carrying capacity and become macrocytic. Macrocytosis also caused by the insufficiency of the vitamin B12 and folate (Reilly, et al., 2013).

Furthermore, the WBC count which include neutrophils and lymphocytes are significantly higher in the smoker group. Smoking-induced leucocytosis is cause by several factors. Malenica, et al. (2017) assumed that increase in the level of leukocytes might be due to nicotine induced secretion of catecholamine and steroid hormone from the adrenal glands. Since the secretion of certain endogenic hormone increased, such as epinephrine and cortisol, the number of leukocytes will also be elevated. Moreover, lymphocyte count was elevated with the intensity of cigarette smoking. For instances, atherogenic effect of cigarette smoking may increase the level of leukocytes. Leukocytes count also be a useful basic markers of endothelium injury. The continuously elevated levels of leukocyte considered the chronically endothelium injury (Çiftçiler, et al., 2019).

2.3 Effect of High Altitude on Hematological Parameter and Red Blood Cell Morphology

Blood cell morphology and phenotypic are affected by high altitude. After an individual ascends to high altitude, hypoxia in high altitude areas cause RBC, Hb and Hct levels to significantly increase. Based on the previous study conducted by Zhong, et al. (2015), the Hb level in highland is augmented compared with the lowland. From this study, the average Hb concentration in highland was ranged from 185 to 218 g/L, which was higher compared with the low land Hb concentration, which ranging from 164 to 183 g/L. This is because

at the high altitudes, the oxygen pressure is lower, the human body required more Hb to maintain the oxygen requirement. Thus, the erythropoietic factor will stimulate the bone marrow to increase the RBC production to compensate the insufficient amount of oxygen (Windsor and Rodway, 2007). In addition, the people who living at high altitude in long term period will decreased the affinity of Hb for oxygen and reduced the formation of oxyhemoglobin in body tissues. Deoxyhemoglobin contain the higher level of 2,3-DPG and P50. This combination decreased the affinity of Hb to oxygen when the people stay in highland for a long period. Therefore, the blood from the highland individual will have the weaker oxygen-delivering capacity compared with the lowland (Zhong, et al., 2015).

Furthermore, based on the study from Zhong, et al. (2015), MCV was higher in highland compared with lowland. This considered that RBCs in high altitude became swollen and spherical. The abnormally shaped of the RBCs in highland was gradual increased than that of lowland of RBCs. Based on the finding from scanning electron microscope, the number of reversible shapes which is echinocyte and stomatocyte was found lower in highland RBCs compared with lowland RBCs, whereas the number of irreversible shapes which is spheroechinocyte and spherostomatocyte shapes was found increasingly in highland RBCs than that of lowland RBCs. The alteration of the RBC shape can be caused by osmotic fragility, hemolysis, electrolytes reduction and lower pH. According to osmotic fragility analysis, lowland RBCs have higher osmotic resilience than highland RBCs. The increasingly osmotic fragility of the high-altitude RBCs enhanced cell sphericity and cell volume. The higher osmotic

fragility of highland RBCs was leading to the higher rate of hemolyis and causing the deformability of the RBC. Besides that, RBCs in high altitude had increased level of extracellular K^+ compared with lowland RBCs. Higher extracellular K^+ considered that the RBCs in high altitude lost the intracellular K^+ faster than lowland RBCs. RBCs loss K^+ due to dehydration, causing in shape changes (Zhong, et al., 2015). Dehydration commonly caused by sweating or diarrheal. The ion movements across the RBC membrane will cause the reversible shape change of RBC. Thus, echinocyte formation can be observed when electrolytes reduction (Bogdanova, et al., 2020). Lastly, the pH of high-altitude RBC was gradually lower than those in lowland RBC (Zhong, et al., 2015). The changes in the cell pH cause the alteration of the RBC shape with its membrane potential and cell water cells. The recent study that reported by Gedde, Davis and Huestis (1997) has shown that the human RBC change from discocytes to stomatocytes in low pH condition or echinocytes in high pH environment when the external pH is altered. Therefore, an altered pH environment will lead to the changes RBC's shape.

2.4 Effect of Exercise on Hematological Parameter

The amount of RBC and Hct level in the blood increases with regular exercise. During exercise, Hct level rises due to decrease in plasma volume (PV) when fluid replacement is insufficient. Several factors that can cause the fluid loss, which including sweating during exercise, osmotically active metabolite build up that cause plasma water diffuse to the extracellular space and the filtration process leads to increase in capillary hydrostatic pressure. In addition, the increased amount of RBC in the blood of athletes can be due to the

erythropoiesis. Increased of Hb level and RBC volume in athletes is considered as a significant sign of erythropoiesis that stimulate by exercise. The elevation of pre-mature forms of reticulocytes counts are a sign of activated bone marrow. Androgens increased in response to exercise. Androgen is a factor that stimulate the erythropoiesis by triggering the erythropoietin (EPO) release and increasing the bone marrow activity (Mairbäurl, 2013).

Besides that, exercise-induced intravascular hemolysis can reduce the RBC mass and thus cause the formation of senescent RBCs. The mechanical rupture of the RBC when passing through the blood capillaries and the compression of RBC will lead to the formation of the senescent RBC. For example, foot soles during running or hand palm of the weightlifters will lead to the increased senescent RBC. These changes also caused a decrease of the average age of the circulating RBC especially in the athletes (Mairbäurl, 2013).

Exercise like jogging can raise the heart rate. The blood flow throughout the body increases as the heart rate rises, which then increased the oxygen supply to the body tissues and cells. Jogging is an aerobic activity to generate more effective mitochondria, which increases the amount of energy and oxygen supply for physical activity. A significant increase in metabolic activity causing the decreasing of pH. As a result, more oxygen is released by Hb and increasing the amount of oxygen delivered to the muscles. Hb levels in the blood might rise due to the physical activity or exercise. Thus, this situation is able to explain how exercise can raise the Hb level and RBC mass, which improves oxygen-carrying capacity. The body requires more oxygen during exercise or physical

activity compared with the resting state. The bloodstream in the muscles normally provides the oxygen that required by the cells. Thus, it showed that the increased demand for oxygen during activity is fulfilled by increasing muscle blood flow. The amount of blood and Hb that circulates and is bound by blood was increased during the physical exercise. Based on this fact, the regular exercise can elevate the level of Hb (Sepriadi, Jannah and Eldawaty, 2020).

2.5 Effect of Alcoholism on Hematological Complications

Based on the findings from Latvala (2004), the people with severe alcoholism typically markedly impaired hematopoiesis. Excessive alcohol consumption decreases the amount of blood cell precursors in the bone marrow and results in structural defects that are characteristic of these cells, producing lower amount of non-functional mature blood cells than normal. The RBC precursor cells are affected by the abnormalities of bone marrow due to the severe alcoholics. The formation of numerous big vacuoles in early RBC precursor cells is the most dominant sign of alcohol's adverse effect on bone marrow cells. The vacuoles also produce in the granulocyte precursors that cause the structural changes and interfere with WBC production.

Besides that, a blood disorder, acquired forms of sideroblastic anemia (SA) can be caused by alcohol consumption. SA causes anemia and iron accumulation (Mohan, 2021). The iron is converted into ferritin and accumulate in RBC precursor instead of bound with Hb molecules. These ferritin-containing cells are known as ringed sideroblasts, caused the precursor RBC unable develop into mature function RBCs. Therefore, the RBC count in the blood declines and

develop into anemia. In addition, alcoholics commonly causing megaloblasts produced in the bone marrow which leading to megaloblastic anemia. Megaloblasts is an immature RBC with unusually large of size and structural abnormal that accumulate in the bone marrow. Megaloblastic anemia commonly caused by vitamin B12 and folic acid deficiency (Biggers, 2021). Vitamin B12 and folic acid are important substances in RBC production. However, alcoholics typically also have lower level of folic acid in the RBC. Alcohol consumption cause the deficiency of folic acid by altering the absorption of folic acid from the diet. The deficiency of the folic acid caused the precursor cells unable to divide effectively and large amount of immature and nonfunctional cell build up in the bone marrow instead in the bloodstream. The defective hematopoiesis not only cause the low number of RBC, but also WBC and platelets. The decreased RBC number in the blood leading to anemia (Cylwik, and Chrostek, 2011; Miwa, et al., 2020).

People with heavy drinking can develop macrocytosis in RBC even there are absent with other factors associated with RBC enlargement. In the study by Ballard, (1997), more than 80% of men and 46% of women of alcoholism have been found to be macrocytosis in their RBC. The majority of the macrocytosis are uniformly round, compared to the more oval shape in normal RBC. In the patient with macrocytosis, the MCV also higher than normal. For instances, cells with elevated MCV can found in patient with megaloblastic anemia with folic acid and vitamin B12 deficiency (Miwa, et al., 2020).

Moreover, chronic heavy alcohol consumption can cause the several types of hemolytic anemia, which include stomatocyte hemolysis and spur-cell hemolysis. Stomatocytes and spur cells can be observed in the blood in these two types of disorder. When examined under a microscope, stomatocytes are appeared with mouth-, or stoma- like shape due to the defect of RBC's membrane. Stomatocytes have a shorter lifespan because they are eventually destroying after trapped in the small capillaries of the spleen. Stomatocytes account for less than 5% of RBCs in healthy person whereas, more than 25% of alcoholics has a higher number of stomatocytes. Next, spur cell hemolysis is referred to the RBC that are distorted and have spikelike protrusions on the surface of their membrane that vary in width, length and distribution. The formation of spurs cells is caused by the excessive incorporation of cholesterol into the cell membrane, which leads to an increase in the cell surface area without increase in cell volume. Flattened RBC shape formed when modestly elevated membrane cholesterol levels while the spikey membrane formed in RBC with higher cholesterol levels. Around 3% of alcoholics with advanced liver illness suffer from spur-cell hemolysis, which results in anemia that worsens gradually and eventually fatal (Miwa, et al., 2020).

CHAPTER 3

METHODOLOGY

A cross-sectional study was conducted at Universiti Tunku Abdul Rahman, Kampar campus from November 2022 to February 2023. This study was a type of observational study that allow the researchers to analyse the data at a single point of time across a sample population (Thomas, 2020). The targets population in this study was involved Chinese students pursuing bachelor degree and foundation studies. The Google form questionnaire was filled in by all the participants during the period of data collection. In the clinical assessment, anthropometry measurement was performed. Finger pricking method was used to collect blood sample. Hb level was measured by using hemocue. Blood smearing, blood staining and microscopic examination was performed to analyse the blood morphology characteristics.

3.1 Study Area

The study area for this study was targeted on Universiti Tunku Abdul Rahman (UTAR) Kampar students. UTAR Kampar campus is located in the developing township of Bandar Barat Kampar in Perak. UTAR Kampar campus provide a comfortable and peaceful study atmosphere. The students can access the modern educational facilities and latest learning technologies from this campus to the student (Universiti Tunku Abdul Rahman, 2023). Therefore, block D, block H and block N of UTAR were chosen as the location where we collected the samples. Location of the UTAR Kampar as pursuit in the map below.

Figure 3.1: Map of the UTAR location in Perak
(Adapted from Universiti Tunku Abdul Rahman, 2020)

3.2 Study Population

Chinese students were recruited to take part in this study based on the inclusion and exclusion criteria. Chinese students were chosen as the sampling population because the number of Chinese students in UTAR Kampar is high. This will easily fulfil our target sample size. The inclusion and exclusion criteria for thus study were shown in Table 3.1:

Table 3.1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
UTAR Kampar Chinese students	Physically impaired student
Age (>18 years old)	Mentally impaired student
Gender (both male and female)	Blood related disease student

3.3 Study Design

A cross-sectional study was conducted in this study. This is a type of observation study to analysed the data collected at a single point of time across a sample population. Cross-sectional study allows the researchers to collect the data from a large population and this study design is less-time consuming compared with others types of study design (Thomas, 2020). Descriptive comparative design was employed in this study in order to identify the relationship between the changes in red blood cells morphology with the associated factors, which including smoking cigarette, vaping, secondary smokers, exercise among young cigarette users.

3.4 Sample Size

The Cochran formula was used to calculate the sample size. By using Cochran formula, the desire sample size can be determined based on the desired level of precision, desired level of confidence, and the expected percentage of the characteristic present in the population (Glen**,** 2013). It was calculated based on previous study conducted by the Lim, et al. (2018) in Malaysia whereby the smoking prevalence of those aged 15 and above is 22.8%. A sample size of 298 was obtained after considering a margin of error of 5% and a 95% confidence level. In this study, 10% of the drop-out rate was added in order to compensate for participants that are unable to contact (Duntoye, 2015).

Formula:

$$\text{Sample size: } \frac{Z^2 p(1-p)}{e^2}$$

where,

- Z^2 is 1.96 for 95% confidence interval
- p is the proportion of the population
- e is the margin of error, 5%

(Glen**,** 2013).

Calculation:

$$\text{Sample size} = \frac{(1.96)^2(0.228)(1-0.228)}{0.05^2}$$

$$= 271$$

Estimate a 10% drop out rate:

$$\text{Sample size} = 271 \times 110\%$$

$$\approx 298$$

3.5 Ethical Approval

Ethical approval (U/SERC/242/2022) from Universiti Tunku Abdul Rahman Scientific and Ethical Review Committee was obtained before the data collection. The ethical approval sheet was attached in Appendix A.

3.6 Data Collection Tool: Cross-Sectional Survey

A Google Form questionnaire was the desired data collection tool used in this study. Google form is well-known and wisely used for the majority of the students because it is convenience and easier to understood. This questionnaire

was developed in English language and consisted of four sections; Section A – Sociodemographic Information, Section B – Individuals Health Status, Section C – Smokers, Secondary Smokers and Non-smokers Screening and Section D - Smokers' and Non-smokers' Knowledge, Attitude and Practice on Smoke. The questionnaire was attached in Appendix B.

The purpose of Section A is to collect the sociodemographic information of the respondents. In this section, 10 questions are listed, including name, gender, date of birth, marital status, contact number, faculty, course, year of study, state of the respondents come from and monthly income.

Section B was designed in order to collect the information of the respondents' health status. There are 4 questions composed in this section, with 2 questions for screening the blood related diseases and other diagnosed diseases, and 2 questions for ensure whether the respondents having the exercise for past 1 hour and frequency of the exercise in a week. The respondent must identify the statement either yes or not.

Section C was established to screen the smokers, secondary smokers and non-smokers. There are 2 questions for secondary smokers, which include the duration and environment that expose to smoke. Besides that, the information about the smoking history of smokers was collected based on cigarette and vape smokers. There are 5 questions provided to the cigarette smokers and 6 questions for the vape smokers.

Section D is to assess the understanding of respondents towards the knowledge, perceptions and factors on smoking. This section should be filled in by smoker and non-smoker. Smoker section was categories with the health knowledge, smoking initiation, smoking perceptions. There are 18 questions composed in this section, with 14 questions for smokers and 4 questions for non-smokers.

3.7 Data collection Tool: Clinical Assessment

3.7.1 Anthropometry Measurement

Seca scale was used to measure the height of the participants. The participants should stand barefoot on the platform and make sure the heels of both feet are touching the wall. The body of the participants should be upright and in the middle of the ruler. Before measuring, we should ensure the position of the eyes and the top of the ears are level. The headpiece against the scalp was placed and locked it in order to obtained the height accuracy. The height of the participants directly read out by the users (Seca, 2015). The picture below showed the correct posture of Seca scale measurement.

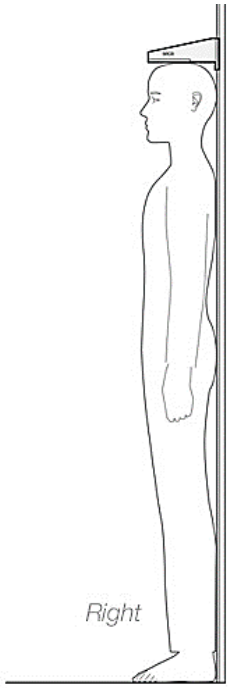


Figure 3.2: Correct of posture standing when using Seca scale
(Adapted from Seca, 2015)

The bioelectrical impedance analysis (BIA) was used to measure the weight and body mass index (BMI) of the participants. Firstly, we should lift up the monitor handle and screen, turn on the power button located on the back of the scale. Next, we enter the age and choose the male or female symbol. The height of the participants was entered into the system. All the participants must stand on scale barefoot, look straight ahead and hold arms out straight at 90° angle. After that, the results include weight, BMI, body fat, muscle mass, body age and visceral fat was displayed on the screen (OMRON Healthcare, 2008). The picture below showed the details of how the body composition monitor work.

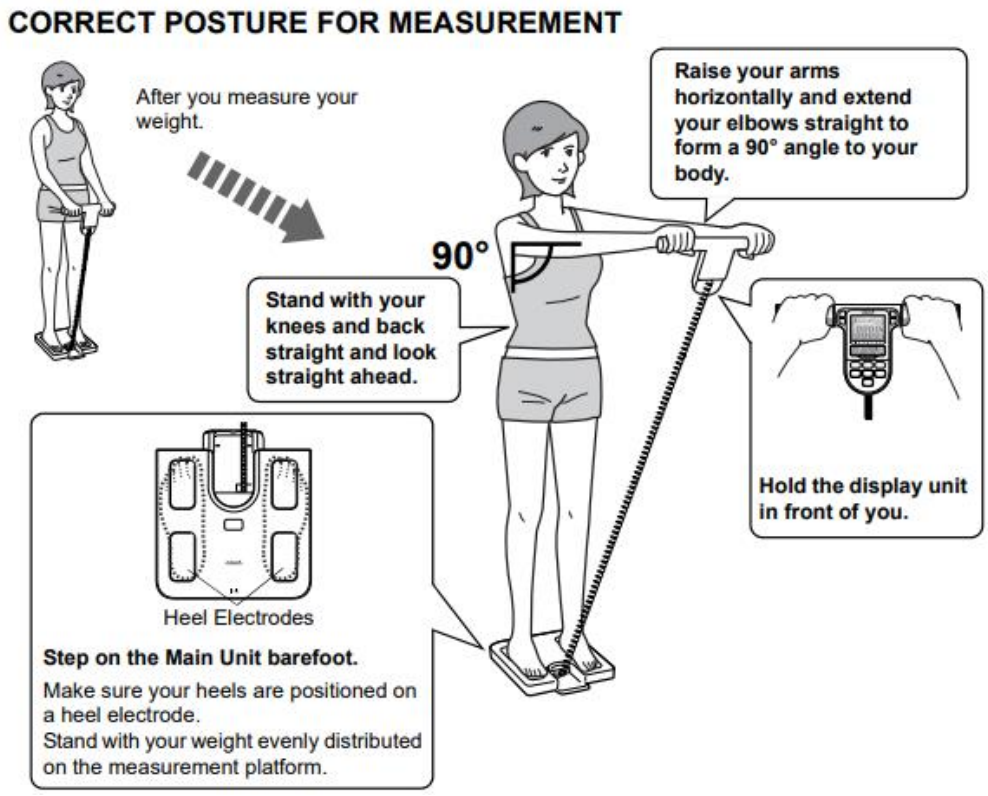


Figure 3.3: Details of the measurement of bioelectrical impedance analysis (Adapted from OMRON Healthcare, 2008)

3.7.2 Finger Pricking Technique

The blood of the participants was collected by finger pricking method. Firstly, the working table was sterile with 70% alcohol and medical examination gloves were worn to prevent the contamination. Next, the participants' preferred finger either the ring finger or the middle finger was chosen for finger prick purpose. With participants' permission the fingers and palm were massage prior pricking their finger. This action was important to warm up their hand in order to increase the blood flow. An alcohol wipe was used to clean the finger of the participants. This is because the participants may have sweats or microbes on their hand, thus it is necessarily to wipe their finger by using the alcohol wipe to prevent contamination of the blood sample. The pressure was applied to the participant's

finger until the fingertip appear with dull red tint. A disposable type of lancet was opened and placed at the side of the finger. The lancet was pressed down until the needle penetrated the skin of the participant's finger. The first drop of the blood was wipe away with a cotton. This is because the first drop of the blood contains the tissue and debris from the prick and it will interfere the purity of the blood sample. The finger was usually pushed down and the blood was collected into the ethylenediaminetetraacetic acid (EDTA) tube. The EDTA tube was labelled according to the number of participants. If there are too less of the blood can be collected from the participant, a permission to perform the second time of finger prick was asked from the particular participant. After the blood have been collected, the participant's finger was cleaned by using a new cotton and a band-aid was placed on their finger to stop the bleeding. Lastly, the lancet, alcohol wipe and cotton which come into contact with blood and body fluids were disposed into a biohazard bag and the lancet was disposed into the sharp bin. The hands and working table were cleaned by 70% alcohol before the blood for the next participant was collected. (World Health Organization, 2016). The figure 3.4 shows the procedures and safety precautions of finger pricking.

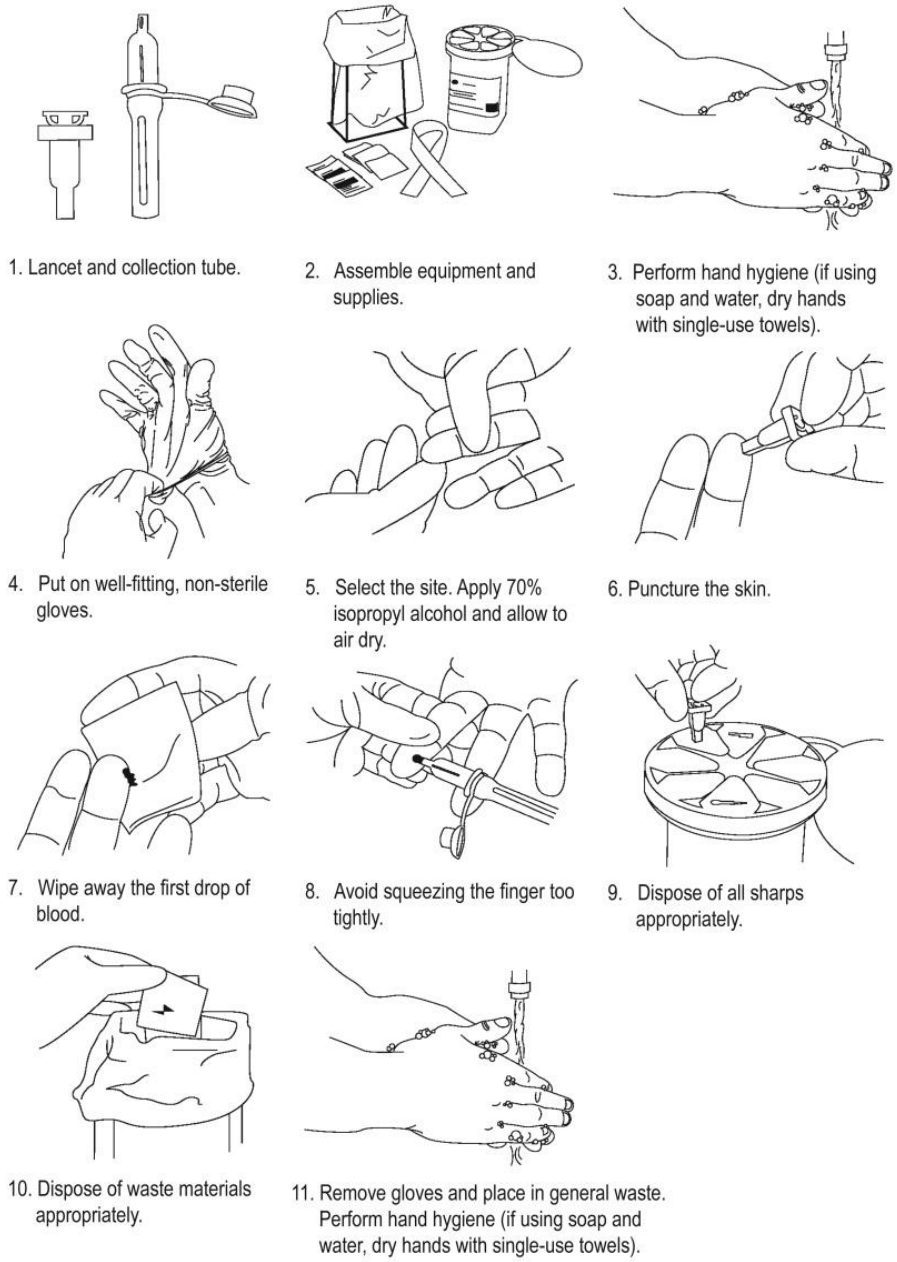


Figure 3.4: Procedures and precaution steps of finger pricking (Adapted from World Health Organization, 2010)

3.7.3 Hemoglobin Concentration

Hemocue was used to measure the Hb concentration. First, the power button of the instrument was turned on. Three flashing dashes which ready for measuring of the Hb level was displayed on the screen. The finger of the participant was cleaned with alcohol wipe and the blood was collected by using the lancet. The

thumb was used to apply some pressure and the fingertip was pressed towards in order to stimulate the blood flow. The first drop of the blood was wipe away by using cotton. Then, the microcuvette was filled with another drop of blood. The tip of the microcuvette was filled with blood and the formation of bubbles were avoided. Before inserting the microcuvette, the excess blood around the microcuvette tip was wipe off by using the clean cotton. Figure 3.5 below shows the details of blood collection and filling the microcuvette. After that, the microcuvette that filled with blood was placed in the cuvette holder and the cuvette holder was pushed to its measuring position. During the measurement, the hourglass symbol was appeared on the screen. The Hb level was displayed on the screen after 15 to 60 seconds. After the test completed, the microcuvette was discarded into the biohazard bag and the cuvette holder was cleaned by alcohol wipe before the next blood sample to be measured (Appn, 2021).

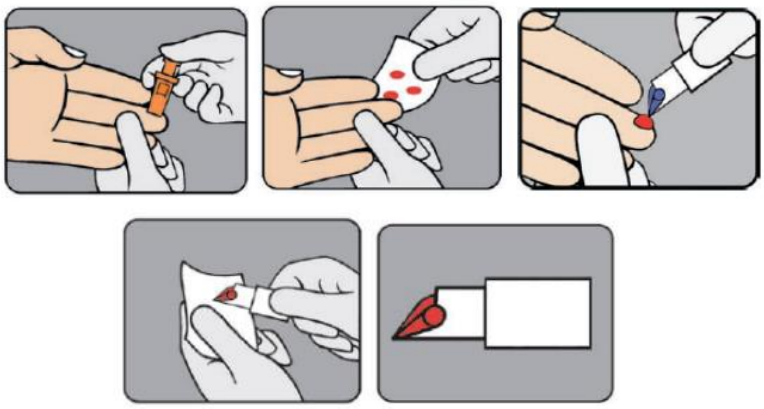


Figure 3.5: Steps of blood collection and filling the microcuvette (Adapted from Appn, 2021)

3.7.4 Blood Smearing

Blood smear was performed by wedge method. The blood from the EDTA tube was pipetted out by using pasteur pipettes. A small drop of blood was dropped on the midline at the end of a labelled glass slide. A spreader was placed in front of the blood drop with 30° to 45° angle. After the blood drop was spread along the edge of the spreader, the spreader was move forward quickly and the blood spread along the glass. A proper pressure was applied when the blood was smeared on the glass slide. Otherwise, the excessive pressure will push too much of blood forward the glass slide without forming a good quality of feathered edge. Thus, the good quality of blood smears was obtained, which include head, body and tail. The angle of the spreader was increased if there was a small drop of blood. Then, the blood smears were allowed to dry in the air. After that, the blood smears were dipped into the methanol for 30 seconds (Centers for Disease Control and Prevention, 2020a). At least two blood smears per participants was prepared. The figure 3.6 performed the steps of blood smearing and showed a good characteristics of blood smear.

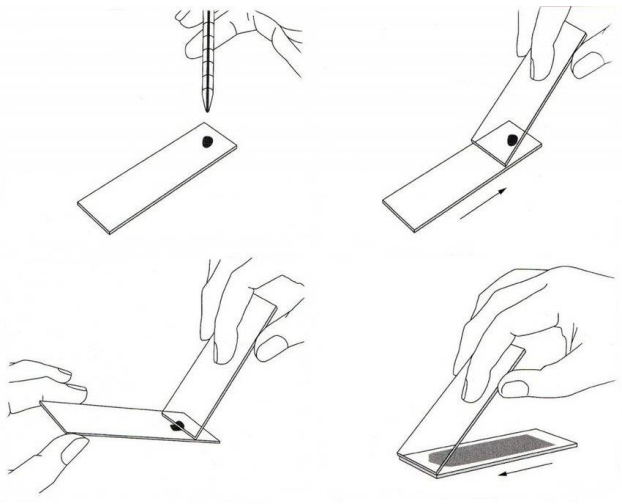


Figure 3.6: Steps of blood smear preparation
(Adapted from Susan, 2016)

3.7.5 Blood Staining

Leishman stain method was used to stain the blood smear. A drop of 0.5 mL of Leishman stain was added to the blood smear and stands for 1 minute. After that, 1 mL of phosphate buffer was added to the slide and stands for 10 minutes. Then, the slide was washed by the distilled water and allowed it to dry (Ruhi, 2018). The image below showed the morphology of stained blood smear after the staining process. After the slide was dry, it's allowed to view under the microscope. Thus, the morphology of the RBCs can be observed and analysed under the microscope. The image of the RBCs was then captured under the microscope.

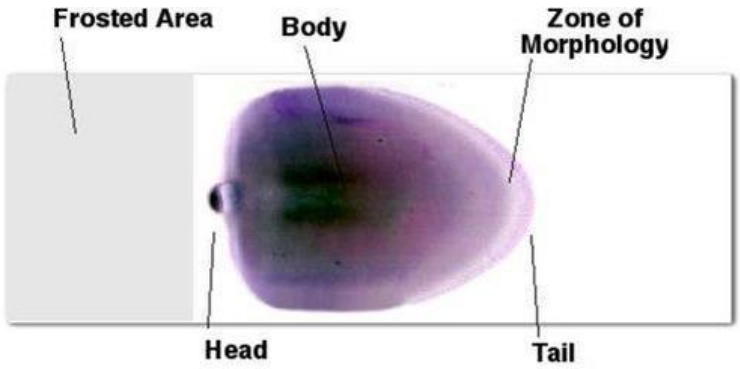


Figure 3.7: Morphology of stained peripheral blood smear
(Adapted from Natalie, 2018)

3.7.6 Microscopic Examination

The microscopic examination was performed to observe and analyse the blood characteristics for each of the participants. Light microscope with 400× and 1000× magnifications were used to observe the morphology of the RBC. The oil immersion method was performed when the RBC morphology was observed with 1000× magnification. A small drop of oil was dropped onto the microscope slide. Oil immersion method was used because this technique was able to

increase the refraction and allowed more light to pass through the microscope slide. Therefore, a higher resolution and clearer image of RBC morphology was obtained. After the observation completed, the image of RBC morphology was captured and the oil on the microscope lenses was wide off with lens paper.

3.7.7 Blood Screening Review

Blood screening were reviewed and discussed with seven times among the researcher and project team mates to reach a mutual agreement and ensure the blood morphology were analysed appropriately. This blood screening review was performed through the physical meeting and online meeting by using the Microsoft Team. The blood morphology of each of the participants have been analysed, which include the size, color of RBC, size variation and the abnormalities of the RBC. All the characteristic and abnormalities of the blood morphology was recorded during the meeting.

3.8 Data Collection Method

In the cross-sectional study, the data was collected through an online platform. Google form was selected to perform the online survey questionnaire. Prior to filling in the questionnaire, a written statement of consent was obtained from the respondents to ensure the confidentiality, protection, security and accuracy of the respondents' information. For the clinical assessment, the anthropometric measurement (height and weight), finger pricking and Hb level were performed and measured for each of the participants.

3.9 Data Analysis

The version 29 of SPSS was used to analyse the data obtained from the questionnaire. All the collected data was key in into SPSS version 29. In this study, descriptive analysis and Chi-square test were used to conduct the statistical analyses. For descriptive analysis, the categorical data of the sociodemographic information was presented by frequency, percentage, mean and standard deviation. Chi-square test was performed to determine the association between sociodemographic characteristics, health status, smoking status and blood morphology of the participants. SPSS version 29 was used because it provides the efficient and reliable data analysis and also simple and easy to use by the student.

CHAPTER 4

RESULTS

4.1 Socio-demographic Characteristics

A total of 342 participants from UTAR, Kampar campus were enrolled in the study, however only 321 participants were successfully completed the study assessments. The response rate of this study was accounted was 93.9%.

The sociodemographic details of participants were summarized in Table 4.1. According to Table 4.1, the highest frequency of the age group was aged 21, which is 24.9%, and the lowest age frequency (2.2%) was aged 23 and above. Besides that, over half of all responses were females (59.5%) and followed by 40.5% of males. According to the course taken, the majority of the students were belonging to Faculty of Science (34.3%), and the least was 1.9% in Faculty of Engineering and Green Technology. In addition, year 3 students account for the majority of the respondents (33.6%). Based on the responses from the questionnaire, a large proportion of students come from Perak (27.7%), while least (0.6%) of the students come from Terengganu. Lastly, 77.9% of the respondents had RM0 to RM700 of monthly income, 17.8% had RM701 to RM1400, followed by 3.7% of RM1401 to RM2100, and the least (0.9%) had RM2100 and above of monthly income.

Table 4.1: Sociodemographic Details of Participants (n=321)

Variables	n	%
Age		
18	53	16.5
19	71	22.1
20	68	21.2
21	80	24.9
22	26	8.1
23	16	5.0
>23	7	2.2
Gender		
Female	191	59.5
Male	130	40.5
Education Background		
Faculty		
Centre for Foundation Studies (CFS)	80	24.9
Faculty of Arts and Social Science (FAS)	24	7.5
Faculty of Business and Finance (FBF)	41	12.8
Faculty of Engineering and Green Technology (FEGT)	6	1.9
Faculty of Information and Communication Technology (FICT)	44	13.7
Faculty of Science (FSc)	110	34.3
Institute of Chinese Studies (ICS)	16	5.0
Year of Study		
Foundation	80	24.9
Year 1	76	23.7
Year 2	51	15.9
Year 3	108	33.6

Table 4.1 continued: Sociodemographic Details of Participants (n=321)

Year 4	6	1.9
State of Origin		
Kedah	22	6.9
Kelantan	3	0.9
Kuala Lumpur	18	5.6
Melaka	9	2.8
Negeri Sembilan	8	2.5
Pahang	6	1.9
Penang	58	18.1
Perak	89	27.7
Perlis	4	1.2
Sabah	7	2.2
Sarawak	15	4.7
Selangor	36	11.2
Terengganu	2	0.6
Financial Status		
Monthly Income (RM)		
0-700	249	77.9
701-1400	57	17.8
1401-2100	12	3.7
>2100	3	0.9

4.2 Individual's Health Status

Table 4.2 described several blood-related disease and other diagnosed disease of the responses. The frequency and duration of exercise among the responses were categorized as Table 4.3 below.

According to the Table 4.2, most of the responses were not diagnosed with any blood-related disease. However, there was 1 student (0.3%) diagnosed with anemia and there were 4 students (1.2%) diagnosed with thalassemia. Besides from blood-related disease, there were 0.3% of students diagnosed with cardiovascular disease, diabetes mellitus and dyslipidemia, followed by 0.6% of students diagnosed by other diseases, which including asthma and milk allergic. These participants that had diagnosed with blood-related disease have been excluded from this study.

Table 4.2: Medical History of The Participants (n=321)

No.	Questions	n	%
a. Blood-related disease			
1	Anemia	1	0.3
2	Hemophilia	0	0
3	Venous Thromboembolism	0	0
4	Thalassemia	4	1.2
5	Von Willebrand Disease	0	0
6	Sickle Cell Disease	0	0
7	Others	0	0
b. Other disease			
1	Cardiovascular Disease	1	0.3
2	Diabetes Mellitus	1	0.3

Table 4.2 continued: Medical History of The Participants (n=321)

3 - Dyslipidemia	1	0.3
4 - Others	2	0.6

4.3 Exercise Status

The frequency and duration of exercise among the responses were categorized in Table 4.3 below. In addition, there were 6.5% of respondents have exercise in past 1 hour compared with 93.5% of responses does not exercise in past 1 hour. There were accounted 45.2% of responses have exercise more than 30 minutes in a week, followed by 29.6% of responses have exercise less than 30 minutes in a week, and lastly there were 25.2% of responses does not exercise in a week.

Table 4.3: Exercise Status of The Participants (n=321)

No.	Questions	n	%
a. Exercise in past 1 hour			
1 - Yes		21	6.5
2 - No		300	93.5
b. Frequency of exercise in a week			
1 - <30 minutes		95	29.6
2 - >30minutes		145	45.2
3 - None		81	25.2

4.4 Smoking Status

Table 4.4 demonstrated that 20 smokers (6.2%) were recruited from UTAR and 94 students (29.3%) were belonging to secondary smokers. In the total of 20 smokers, 5 smokers (1.6%) were tobacco user while 15 smokers (4.7%) were vape users.

Table 4.4: Smoking Status of the Participants (n=321)

No.	Questions	n	%
a. Smoker			
1 - Yes		20	6.2
2 - No		301	93.8
b. Secondary smoker			
1 - Yes		94	29.3
2 - No		227	70.7
c. Tobacco or vape users			
1 - Tobacco smoking		5	1.6
2 - Vape		15	4.7
3 - Non-smokers		301	93.8

4.5 Anthropometry Measurement

Table 4.5 demonstrated the anthropometry measurement of participants. The majority (37.1%) of the students have a height of between 1.6 m to 1.69 m, while the minority (3.1%) of the students have 1.8 m to 1.89 m of height. Besides that, there are 34.0% of the students have 50.0 kg to 59.9 kg in the weight category and there are four students (1.2%) have less than 40 kg in their weight. In addition, BMI of all the participants was measured and grouped into

four groups. which including underweight (<18.5 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (>30 kg/m^2) (Centers for Disease Control and Prevention, 2022d). Among all the responses, there were 203 of students (63.2%) had a normal body mass index, 22.7% of students who were underweight, followed by 10.9% had overweight, and lastly 3.1% of students who were obese.

Table 4.5: Anthropometry Measurement of Participants (n=321)

Variables	n	%
Height (m)		
Mean±SD	1.639±0.086	
1.40-1.49	11	3.4
1.50-1.59	101	31.5
1.60-1.69	119	37.1
1.70-1.79	80	24.9
1.80-1.89	10	3.1
Weight (kg)		
Mean±SD	57.454±12.607	
<40.0	4	1.2
40.0-49.9	95	29.6
50.0-59.9	109	34.0
60.0-69.9	64	19.9
70.0-79.9	27	8.4
80.0-89.9	14	4.4
>90.0	8	2.5
Body Mass Index		
Mean±SD	21.268±3.713	
Underweight (<18.5 kg/m^2)	73	22.7

Table 4.5 continued: Anthropometry Measurement of Participants (n=321)

Normal (18.5-24.9 kg/m^2)	203	63.2
Overweight (25-29.9 kg/m^2)	35	10.9
Obese (>30 kg/m^2)	10	3.1

4.6 Hemoglobin Measurement

The clinical screening of Hb level and the abnormalities of red blood cells morphology are summarized in Table 4.6 and Table 4.7 below as frequencies and percentages.

According to Table 4.6, the majority of the females (78.0%) in the category of normal range of Hb level, followed by lower Hb level (17.3%) and 4.7% of females were categories as higher Hb level. Besides that, most of the males (80.8%) are enrolled in normal range of Hb level. 10.8% of them have lower Hb level while there have 8.4% of males were measured with higher Hb level.

Table 4.6: The Frequency and Prevalence of Hemoglobin Level among Male (n=130) and Female (n=191)

Variables	n	%
Hemoglobin		
Female		
Mean±SD	12.867±1.474	
Low (<11.6 g/dL)	39	20.4
Normal (11.6 – 15 g/dL)	142	74.3

Table 4.6 continued: The Frequency and Prevalence of Hemoglobin Level among Male (n=130) and Female (n=191)

High (>15 g/dL)	10	5.2
Male		
Mean±SD	14.861±1.449	
Low (<13.2 g/dL)	17	13.1
Normal (13.2-16.6 g/dL)	102	78.5
High (>16.6 g/dL)	11	8.4

4.7 RBC Morphology Characterization

According to the Table 4.7, the blood morphology abnormalities were analysed based on the size, colour and shape of the RBC. Anisocytosis defined as the RBC variation in size. Figure 4.1 showed the frequency of different type of poikilocytosis in the pie chart. There was a relatively large proportion of the participants (50.8%) have anisocytosis with their blood morphology. Among all the participants, 45.5% had normocytic, 49.8% had microcytic and 4.7% of participants with macrocytic. In addition, in the abnormalities in colour, the results reveal that 46.7% of participants had normochromic and 53.3% of them had hypochromic. Next, almost half of the participants (47.0%) have defined as poikilocytosis with their blood morphology. The most common abnormalities that can be found in the blood of the participants are elliptocytes (48.9%), target cell (43.0%), tear drop cell (42.1%), ovalocytes (35.2%) and echinocytes (26.8%).

Table 4.7: RBC Morphology Abnormalities

Variables	n	%
a. Size		
Anisocytosis	163	50.8
0 - Normocytic	146	45.5
1 - Microcytic	160	49.8
2 - Macrocytic	15	4.7
b. Colour		
0 - Normochromic	150	46.7
1- Hypochromic	171	53.3
c. Shape		
Poikilocytosis	151	47.0
1 - Elliptocytes	157	
2 - Target cell	138	
3 - Tear drop cell	135	
4 - Ovalocytes	113	
5 - Echinocytes	86	
6 - Schistocyte	48	
7 - Spherocytes	42	
8 - Heinz bodies	39	
9 - Polychromasia	36	
10 - Bite cell	30	
11 - Stomatocytes	18	
12 - Crenated cells	15	
13 - Pencil cell	12	
14 - Acanthocytes	5	
15 - Nucleated RBC	2	

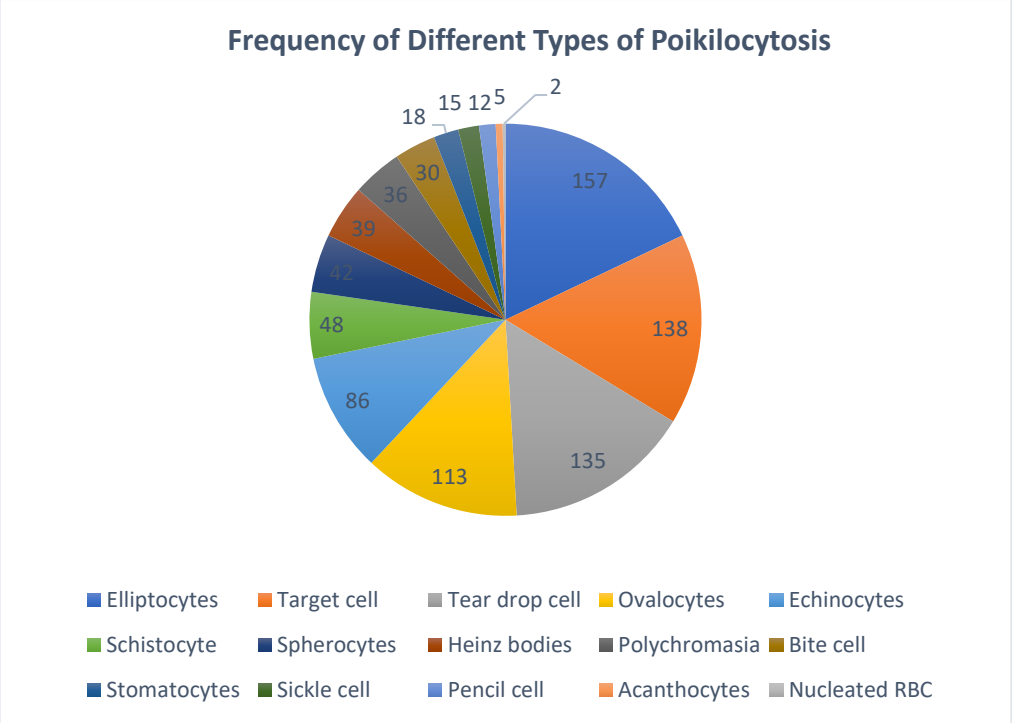


Figure 4.1: Frequency of different types of poikilocytosis

The blood morphology of the participants was analysed under the light microscope with the magnification 400× and 1000×. Oil immersion was used to observed the RBC morphology with 1000× magnification. The results were showed from Figure 4.2 to Figure 4.8 as below.

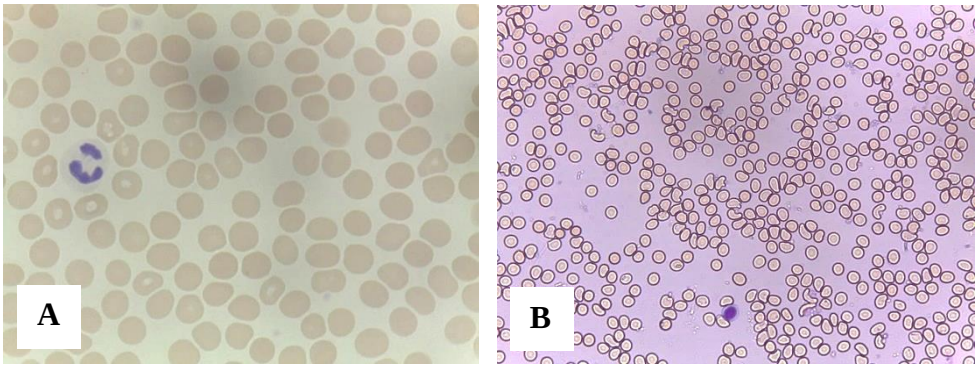


Figure 4.2: Light microscope images of anisocytosis (A) with 1000× magnification and poikilocytosis (B) of the RBC with 400× magnification

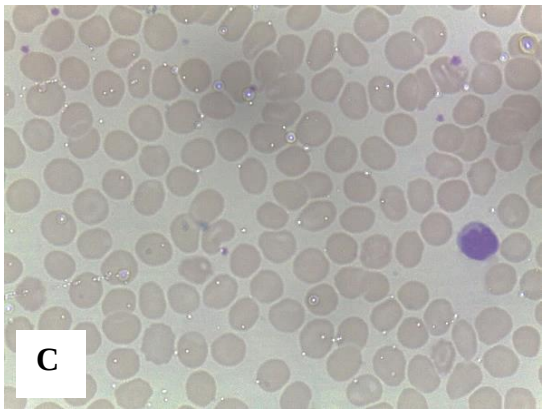


Figure 4.3: Light microscope image of normocytic and normochromic (C) of the RBC with 1000× magnification

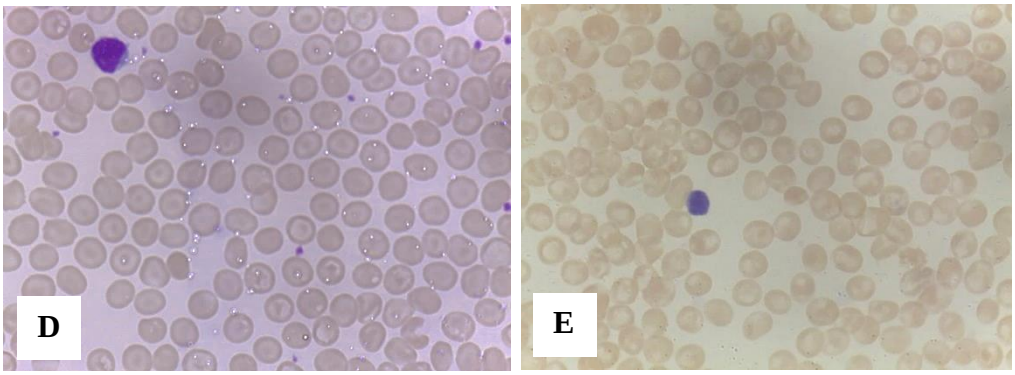


Figure 4.4: Light microscope images of microcytic and hypochromic (D) and macrocytic and hypochromic (E) of the RBC with 1000× magnification

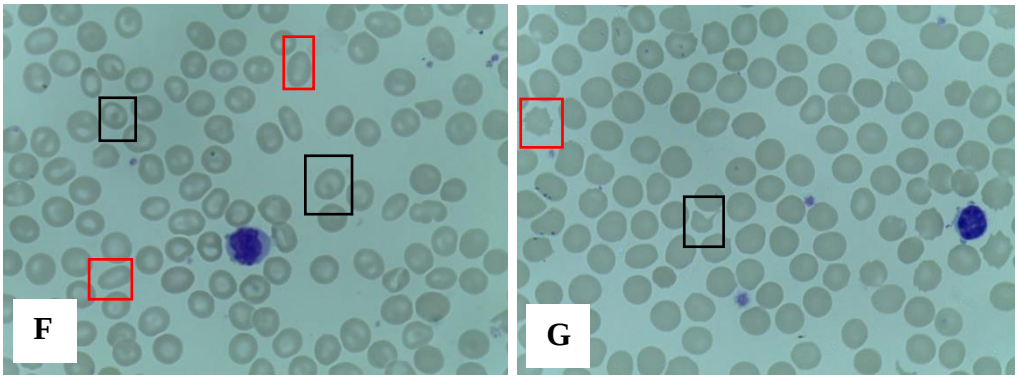


Figure 4.5: Light microscope images of ovalocytes (red boxes) and target cells (black boxes) (F), echinocytes (red boxes) and schistocytes (black boxes) (G) with 1000× magnification

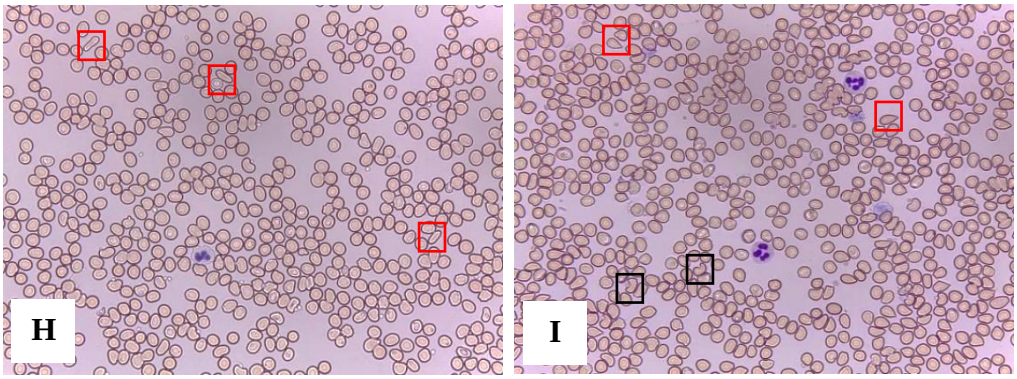


Figure 4.6: Light microscope images of elliptocytes (red boxes) (H), tear drop cells (red boxes) and bite cells (black boxes) (I) with 400× magnification

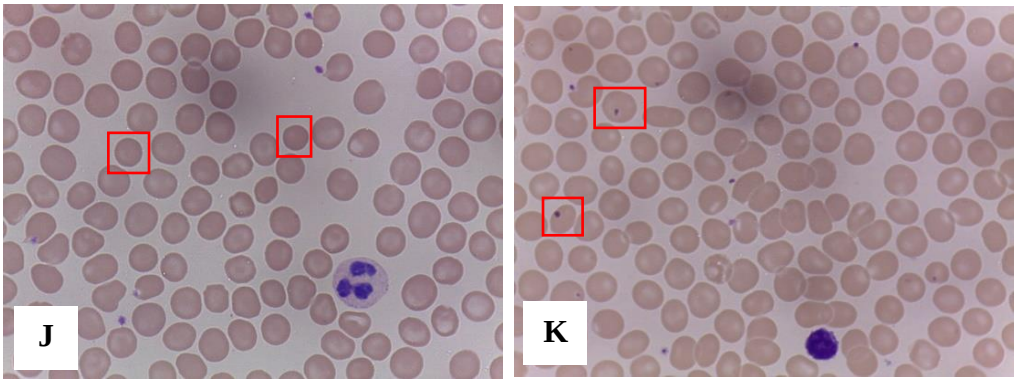


Figure 4.7: Light microscope images of spherocytes (red boxes) (J), heinz bodies (red boxes) (K) with 1000× magnification

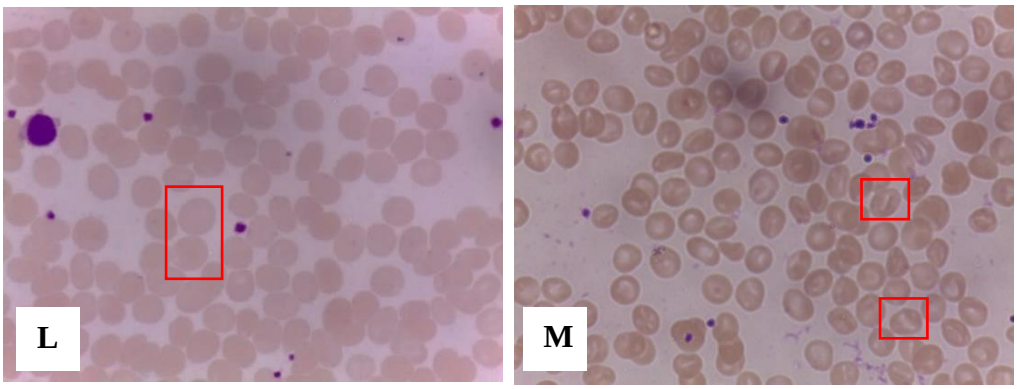


Figure 4.8: Light microscope images of polychromasia (red boxes) (L), and stomatocytes (red boxes) (M) with 1000× magnification

4.8 Association of Blood Morphology Abnormalities with Sociodemographic Characteristics

Chi-square analysis was used to identify the association between sociodemographic characteristics and blood morphology abnormalities. Blood morphology abnormalities was analysed based on the variation in size (anisocytosis), colour and shape (poikilocytosis).

Based on the Table 4.8, there was no statistically significant associations between the sociodemographic characteristics with RBC size.

Table 4.8: Association of RBC Size with Sociodemographic Characteristics

Variable	RBC Size				Chi-square; *p*-value
	n (%)				
	Total	Normocytic	Microcytic	Macrocytic	
Age					
18	53 (16.5)	26 (8.1)	24 (7.5)	3 (0.9)	4.188; 0.980
19	71 (22.1)	34 (10.6)	33 (10.3)	4 (1.2)	
20	68 (21.2)	27 (8.4)	39 (12.1)	2 (0.6)	
21	80 (24.9)	36 (11.2)	40 (12.5)	4 (1.2)	
22	26 (8.1)	14 (4.4)	11 (3.4)	1 (0.3)	
23	16 (5.00)	6 (1.9)	9 (2.8)	1 (0.3)	
>23	7 (2.2)	3 (0.9)	4 (1.2)	0 (0.0)	
Gender					
Female	191 (59.5)	79.9 (24.6)	103 (32.1)	9 (2.8)	3.340; 0.188
Male	130 (40.5)	67 (20.9)	57 (17.8)	6 (1.9)	

Table 4.8 continued: Association of RBC Size with Sociodemographic Characteristics

Year of Study					
Foundation	80 (24.9)	42 (13.1)	35 (10.9)	3 (0.9)	5.692; 0.682
Year 1	76 (23.7)	32 (10.0)	41 (12.8)	3 (0.9)	
Year 2	51 (15.9)	23 (7.2)	27 (8.4)	1 (0.3)	
Year 3	108 (33.6)	47 (14.6)	53 (16.5)	8 (2.5)	
Financial Status					
Monthly Income (RM)					
0-700	249 (77.6)	113 (35.2)	124 (38.6)	12 (3.7)	8.944; 0.177
701-1400	57 (17.8)	25 (7.8)	31 (9.7)	1 (0.3)	
1401-2100	12 (3.7)	6 (1.9)	5 (1.6)	1 (0.3)	
>2100	3 (0.9)	2 (0.6)	0 (0.0)	1 (0.3)	

Table 4.9 concluded the association between the sociodemographic characteristics with variation in RBC colour. It was found that only gender (χ^2=6.576; p=0.010) had significantly associated with the RBC colour. In addition, according to the percentage, participants with females (35.2%) were more likely to have the blood with hypochromic than males (18.1%).

Table 4.9: Association of RBC Colour with Sociodemographic Characteristics

Variable	RBC Colour			Chi-square; *p*-value
	n (%)			
	Total	**Normochromic**	**Hypochromic**	
Age				
18	53 (16.5)	26 (8.1)	27 (8.4)	1.442; 0.963
19	71 (22.1)	34 (10.6)	37 (11.5)	
20	68 (21.2)	28 (8.7)	40 (12.5)	
21	80 (24.9)	40 (12.5)	40 (12.5)	
22	26 (8.1)	12 (3.7)	22	
23	16 (5.0)	7 (2.2)	23	
>23	7 (2.2)	3 (0.9)	4 (1.2)	
Gender				
Female	191 (59.5)	78 (24.3)	113 (35.2)	6.576; 0.010*
Male	130 (40.5)	72 (22.4)	58 (18.1)	
Year of Study				
Foundation	80 (24.9)	44 (13.7)	36 (11.2)	5.970; 0.201
Year 1	76 (23.7)	29 (9.0)	47 (14.6)	
Year 2	51 (15.9)	21 (6.5)	30 (9.3)	
Year 3	108 (33.6)	54 (16.8)	54 (16.8)	
Year 4	6 (1.9)	2 (0.6)	4 (1.2)	
Financial Status				
Monthly Income (RM)				
0-700	249 (77.6)	113 (35.2)	136 (42.4)	5.546; 0.136

Table 4.9 continued: Association of RBC Colour with Sociodemographic Characteristics

701-1400	57 (17.8)	26 (8.1)	31 (9.7)
1401-2100	12 (3.7)	8 (2.5)	4 (1.2)
>2100	3 (0.9)	3 (0.9)	0 (0.0)

*p value less than 0.05

As for results in Table 4.10, Chi-square test was performed to associate the poikilocytosis with sociodemographic characteristics. However, there were no significantly was found in this category.

Table 4.10: Association of Poikilocytosis with Sociodemographic Characteristics

Variable	**Poikilocytosis**			**Chi-square; *p*-value**
	n (%)			
	Total	**Yes**	**No**	
Age				
18	53 (16.5)	27 (8.4)	26 (8.1)	6.568; 0.363
19	71 (22.1)	28 (8.7)	43 (13.4)	
20	68 (21.2)	32 (10.0)	36 (11.2)	
21	80 (24.9)	40 (12.5)	40 (12.5)	
22	26 (8.1)	15 (4.7)	11 (3.4)	
23	16 (5.0)	5 (1.6)	11 (3.4)	
>23	7 (2.2)	2 (0.6)	5 (1.6)	

Table 4.10 continued: Association of Poikilocytosis with Sociodemographic Characteristics

Gender				
Female	191 (59.5)	90 (28.0)	101 (31.5)	0.195; 0.659
Male	130 (40.5)	58 (18.0)	72 (22.4)	
Year of Study				
Foundation	80 (24.9)	36 (11.2)	44 (13.7)	4.764; 0.312
Year 1	76 (23.7)	31 (9.6)	45 (14.0)	
Year 2	51 (15.9)	30 (9.3)	21 (6.5)	
Year 3	108 (33.6)	49 (15.2)	59 (18.3)	
Financial Status				
Monthly Income (RM)				
0-700	249 (77.6)	119 (37.0)	130 (40.4)	2.519; 0.472
701-1400	57 (17.8)	26 (8.1)	31 (9.6)	
1401-2100	12 (3.7)	3 (0.9)	9 (2.8)	
>2100	3 (0.9)	1 (0.3)	2 (0.6)	

4.9 Association of Blood Morphology Abnormalities with Exercise Status of The Participants

Table 4.11 concluded the association between exercise status of the participants with the RBC size. No significant associations were identified for the lifestyle factor among the participants.

Table 4.11: Association of RBC Size with Exercise Status

Variable	RBC Size				Chi-square; *p*-value
	n (%)				
	Total	Normocytic	Microcytic	Macrocytic	
Exercise in past 1 hour					
1 - Yes	21 (6.5)	11 (3.4)	9 (2.8)	1 (0.3)	0.456; 0.796
2 - No	300 (93.5)	135 (42.1)	151 (47.0)	14 (4.4)	
Frequency of exercise in a week					
1-<30 minutes	95 (29.6)	43 (13.4)	45 (14.0)	7 (2.2)	4.107 0.392
2- >30minutes	145 (45.2)	70 (21.8)	69 (21.5)	6 (1.9)	
3 - None	81 (25.2)	33 (10.3)	46 (14.3)	2 (0.6)	

Table 4.12 shows the association between lifestyle factors of the participants and the RBC colour. No significant associations were identified for the lifestyle factor among the participants.

Table 4.12: Association of RBC Colour with Exercise Status

Variable	RBC Colour			Chi-square; *p*-value
	n (%)			
	Total	Normochromic	Hypochromic	
Exercise in past 1 hour				
1 - Yes	21 (6.5)	10 (3.1)	11 (3.4)	0.007; 0.933

Table 4.12 continued: Association of RBC Colour with Exercise Status

2 - No	300 (93.5)	140 (43.6)	160 (49.8)	
Frequency of exercise in a week				
1 - <30 minutes	95 (29.6)	46 (14.3)	49 (15.3)	2.306; 0.316
2 - >30minutes	145 (45.2)	72 (22.4)	73 (22.70)	
3 - None	81 (25.2)	32 (10.0)	49 (15.3)	

Furthermore, Table 4.13 summarized the association between exercise status of the participants with the poikilocytosis. No significant associations were identified for the exercise status among the participants.

Table 4.13: Association of Poikilocytosis with Exercise Status

Variable	**Poikilocytosis**			**Chi-square; *p*-value**
	n (%)			
	Total	**Yes**	**No**	
Exercise in past 1 hour				
1 - Yes	21 (6.5)	11 (3.4)	10 (3.1)	0.356; 0.551
2 - No	300 (93.5)	137 (42.7)	163 (50.8)	
Frequency of exercise in a week				
1 - <30 minutes	95 (29.6)	44 (13.7)	51 (15.9)	0.008; 0.996
2 - >30minutes	145 (45.2)	67 (20.9)	78 (24.3)	

Table 4.13 continued: Association of Poikilocytosis with Exercise Status

3 - None	81 (25.2)	37 (11.5)	44 (13.7)

4.10 Association of Blood Morphology Abnormalities with Smoking Status

Table 4.14 demonstrates the association between the blood morphology abnormalities with smoking status. It was found that secondary smoker (χ^2=7.182, *p*=0.028) was significantly associated with the RBC size. Secondary smoker had significantly showed the macrocytic RBC (2.8%) compared with the non-smokers (1.9%). Thus, it can be said that the blood morphology abnormalities were influenced by expose to the secondary smoke.

Table 4.14: Association of RBC Size with Smoking Status

Variable	**RBC Size**				**Chi-square; *p*-value**
	n (%)				
	Total	**Normocytic**	**Microcytic**	**Macrocytic**	
Secondary smoker					
1 - Yes	94 (29.3)	41 (12.8)	44 (13.7)	9 (2.8)	7.182; 0.028*
2 - No	227 (70.7)	105 (32.7)	116 (36.1)	6 (1.9)	

Table 4.14 continued: Association of RBC Size with Smoking Status

Tobacco or vape users					
1 - Tobacco smoking	5 (1.6)	1 (0.30)	4 (1.2)	0 (0.0)	4.653; 0.325
2 - Vape	15 (4.7)	7 (2.2)	6 (1.9)	2 (0.6)	

*p value less than 0.05

In addition, Table 4.15 summarizes the association between RBC colour with smoking status. There was no significant association between the RBC colour among the participants.

Table 4.15: Association of RBC Colour with Smoking Status

Variable	**RBC Colour**			**Chi-square; p-value**
	n (%)			
	Total	**Normochromic**	**Hypochromic**	
Secondary smoker				
1 - Yes	94 (29.3)	41 (12.8)	53 (16.5)	0.517; 0.472
2 - No	227 (70.7)	109 (34.0)	118 (36.8)	
Tobacco or vape users				
1 - Tobacco smoking	5 (1.6)	2 (0.6)	3 (0.9)	0.388; 0.824
2 - Vape	15 (4.7)	6 (1.9)	9 (2.8)	

Table 4.16 displays the association between poikilocytosis with smoking status. No significant associations were identified for the poikilocytosis among the participants.

Table 4.16: Association of Poikilocytosis with Smoking Status

Variable	Poikilocytosis			Chi-square; *p*-value
	n (%)			
	Total	**Yes**	**No**	
Secondary smoker				
1 - Yes	94 (29.3)	46 (14.3)	48 (15.0)	1.315; 0.252
2 - No	227 (70.7)	127 (38.6)	100 (31.2)	
Tobacco or vape users				
1 – Tobacco smoking	5 (1.6)	3 (0.9)	2 (0.6)	0.398; 0.819
2 - Vape	15 (4.7)	7 (2.2)	8 (2.5)	

4.11 Association of Blood Morphology Abnormalities with Anthropometry Measurement

Table 4.17 summarizes the relationship between the blood morphology abnormalities with anthropometry measurement. It was found that only height group (χ^2=32.841, p=0.000) was found significantly associated with the RBC size.

Table 4.17: Association of RBC Size with Anthropometry Measurement

Variable	RBC Size				Chi-square; *p*-value
	n (%)				
	Total	**Normocytic**	**Microcytic**	**Macrocytic**	
Height (m)					
Mean±SD	1.639±0.086				
1.40-1.49	11 (3.4)	4 (1.2)	4 (1.2)	3 (0.9)	32.841; 0.000*
1.50-1.59	101 (31.5)	39 (12.1)	57 (17.8)	5 (1.6)	
1.60-1.69	119 (37.1)	47 914.6)	70 (21.8)	2 (0.6)	
1.70-1.79	80 (24.9)	51 (15.9)	24 (7.5)	5 (1.6)	
1.80-1.89	10 93.1)	5 (1.6)	5 (1.6)	0 (0.0)	
Weight (kg)					
Mean±SD	57.454±12.607				
<40.0	4 (1.2)	2 (0.6)	2 (0.6)	0 (0.0)	17.812; 0.122
40.0-49.9	95 (29.6)	39 (12.1)	52 (16.2)	4 (1.2)	
50.0-59.9	109 (34.0)	46 (14.3)	58 (18.1)	5 (1.6)	
60.0-69.9	64 (19.9)	32 (10.0)	31 (9.7)	1 (0.3)	
70.0-79.9	27 (8.4)	16 (5.0)	8 (2.5)	3 (0.9)	
80.0-89.9	14 (4.4)	7 (2.2)	7 (2.2)	0 (0.0)	
>90.0	8 (2.5)	4 (1.2)	2 (0.6)	2 (0.6)	
Body Mass Index					
Mean±SD	21.268±3.713				
Underweight (<18.5 kg/m^2)	77 (24.0)	35 (10.9)	41 (12.8)	1 (0.3)	10.33; 0.111
Normal (18.5-24.9 kg/m^2)	200 (62.3)	87 (27.1)	102 (31.8)	11 (3.4)	

Table 4.17 continued: Association of RBC Size with Anthropometry Measurement

Overweight (25-29.9 kg/m^2)	34 (10.6)	20 (6.2)	13 (4.0)	1 (0.3)
Obese (>30 kg/m^2)	10 (3.1)	4 (1.2)	4 (1.2)	2 (0.6)

*p value less than 0.05

As presented by Table 4.18, it was found that height (χ^2=21.040, p=0.000) and weight (χ^2=13.310, p=0.038) was significantly associated with the RBC colour.

Table 4.18: Association of RBC Colour with Anthropometry Measurement

Variable	**RBC Colour**			**Chi-square; *p*-value**
	n (%)			
	Total	**Normochromic**	**Hypochromic**	
Height (m)				
Mean±SD	1.639±0.086			
1.40-1.49	11 (3.4)	5 (1.6)	6 (1.9)	21.040; 0.000*
1.50-1.59	101 (31.5)	36 (11.2)	65 (20.2)	
1.60-1.69	119 (37.1)	49 (15.3)	70 (21.8)	
1.70-1.79	80 (24.9)	54 (16.8)	26 (8.1)	
1.80-1.89	10 93.1)	6 (1.9)	4 (1.2)	
Weight (kg)				
Mean±SD	57.454±12.607			

<40.0	4 (1.2)	2 (0.6)	2 (0.6)	13.310; 0.038*
40.0-49.9	95 (29.6)	39 (12.10)	56 (17.4)	
50.0-59.9	109 (34.0)	43 (13.4)	66 (20.6)	
60.0-69.9	64 (19.9)	34 (10.6)	30 (9.3)	
70.0-79.9	27 (8.4)	19 (5.9)	8 (2.5)	
80.0-89.9	14 (4.4)	7 (2.2)	7 (2.2)	
>90.0	8 (2.5)	6 (1.9)	2 (0.6)	
Body Mass Index				
Mean±SD	21.268±3.713			
Underweight (<18.5 kg/m^2)	77 (24.0)	33 (10.3)	44 (13.7)	4.499; 0.212
Normal (18.5-24.9 kg/m^2)	200 (62.3)	90 (28.0)	110 (34.3)	
Overweight (25-29.9 kg/m^2)	34 (10.6)	21 (6.5)	13 (4.0)	
Obese (>30 kg/m^2)	10 (3.1)	6 (1.9)	4 (1.2)	

*p value less than 0.05

Based on the Table 4.19, no significant association can be found between the poikilocytosis and anthropometry measurement.

Table 4.19: Association of Poikilocytosis with Anthropometry Measurement

Variable	Poikilocytosis			Chi-square; *p*-value
	n (%)			
	Total	Yes	No	
Height (m)				
Mean±SD	1.639±0.086			
1.40-1.49	11 (3.4)	6 (1.9)	5 (1.6)	5.781; 0.216
1.50-1.59	101 (31.5)	47 (14.6)	54 (16.8)	
1.60-1.69	119 (37.1)	74 (23.1)	45 (14.0)	
1.70-1.79	80 (24.9)	41 (12.8)	39 (12.1)	
1.80-1.89	10 93.1)	5 (1.6)	5 (1.6)	
Weight (kg)				
Mean±SD	57.454±12.607			
<40.0	4 (1.2)	1 (0.3)	3 (0.9)	3.748; 0.711
40.0-49.9	95 (29.6)	50 (15.6)	45 (14.0)	
50.0-59.9	109 (34.0)	57 (17.8)	52 (16.2)	
60.0-69.9	64 (19.9)	36 (11.2)	28 (8.7)	
70.0-79.9	27 (8.4)	14 (4.4)	13 (4.0)	
80.0-89.9	14 (4.4)	9 (2.8)	5 (1.6)	
>90.0	8 (2.5)	6 (1.9)	2 (0.6)	
Body Mass Index				
Mean±SD	21.268±3.713			
Underweight (<18.5 kg/m^2)	77 (24.0)	44 (13.7)	33 (10.3)	1.672; 0.653
Normal (18.5-24.9 kg/m^2)	200 (62.3)	104 (32.4)	96 (29.9)	
Overweight (25-29.9 kg/m^2)	34 (10.6)	18 (5.6)	16 (5.0)	
Obese (>30 kg/m^2)	10 (3.1)	7 (2.2)	3 (0.9)	

4.12 Association of Blood Morphology Abnormalities with Hemoglobin Level

Table 4.20 shows the association of RBC size with Hb level among the female and male. There was no significant association between the RBC size with the Hb level. However, based on the percentage the low hemoglobin tends to have a microcytic RBC.

Table 4.20: Association of RBC Size with Hemoglobin Level

Variable	**RBC Size**				**Chi-square; *p*-value**
	n (%)				
	Total	**Normocytic**	**Microcytic**	**Macrocytic**	
Hemoglobin					
Female					
Mean±SD	12.867±1.474				
Low (<11.6g/dL)	39 (20.4)	16 (8.4)	21 (11.0)	2 (1.0)	1.013; 0.908
Normal (11.6 - 15g/dL)	142 (74.3)	58 (30.4)	78 (40.8)	6 (3.1)	
High (>15g/dL)	10 (5.2)	3 (1.6)	6 (3.1)	1 (0.5)	
Male					
Mean±SD	14.861±1.449				
Low (<13.2g/dL)	17 (13.1)	7 (5.4)	9 (6.9)	1 (0.8)	2.782; 0.595

Table 4.20 continued: Association of RBC Size with Hemoglobin Level

Normal (13.2-16.6g/dL)	102 (78.5)	42 (32.3)	54 (41.5)	6 (4.6)
High (>16.6g/dL)	11 (8.4)	5 (3.8)	4 (3.1)	2 (1.5)

Table 4.21 shows the association of RBC colour with Hb level. There was no significant association can be found in this category. However, based on the percentage that showed below, the low Hb level tends to have a hypochromic RBC.

Table 4.21: Association of RBC Colour with Hemoglobin Level

Variable	**RBC Colour**			**Chi-square; *p*-value**
	n (%)			
	Total	**Normochromic**	**Hypochromic**	
Hemoglobin				
Female				
Mean±SD	12.867±1.474			
Low (<11.6g/dL)	39 (20.4)	14 (7.3)	25 (13.1)	0.689; 0.709
Normal (11.6-15g/dL)	142 (74.3)	57 (29.8)	85 (44.5)	
High (>15g/dL)	10 (5.2)	5 (2.6)	5 (2.6)	

Table 4.21 continued: Association of RBC Colour with Hemoglobin Level

Male				
Mean±SD	14.861±1.449			
Low (<13.2g/dL)	17 (13.1)	8 (6.2)	9 (6.9)	1.414; 0.493
Normal (13.2-16.6g/dL)	102 (78.5)	39 (30.0)	63 (48.5)	
High (>16.6g/dL)	11 (8.4)	6 (4.6)	5 (3.8)	

Table 4.21 displays the association of poikilocytosis with Hb level. There was no significant association can be found between the poikilocytosis and Hb level.

Table 4.22: Association of Poikilocytosis with Hemoglobin Level

Variable	**Poikilocytosis**			**Chi-square; *p*-value**
	n (%)			
	Total	**Yes**	**No**	
<u>Hemoglobin</u>				
Female				
Mean±SD	12.867±1.474			
Low (<11.6 g/dL)	39 (20.4)	23 (12.0)	16 (8.4)	1.430; 0.489
Normal (11.6 – 15 g/dL)	142 (74.3)	70 (36.6)	72 (37.7)	
High (>15 g/dL)	10 (5.2)	6 (3.1)	4 (2.1)	

Table 4.22 continued: Association of Poikilocytosis with Hemoglobin Level

Male				
Mean±SD	14.861±1.449			
Low (<13.2 g/dL)	17 (13.1)	5 (3.8)	12 (9.2)	3.326; 0.190
Normal (13.2 - 16.6 g/dL)	102 (78.5)	54 (41.5)	48 (36.9)	
High (>16.6 g/dL)	11 (8.4)	6 (4.6)	5 (3.8)	

CHAPTER 5

DISCUSSION

5.1 Sociodemographic Characteristics

Most of the respondents in this study were female students (59.5%) compared with male (40.5%). This may be because UTAR is composed of more female students than male students. Besides that, the students majoring in Faculty of Science (34.3%) with year 3 students (33.6%) have exceptionally high proportion. Students in faculty of science responded more frequently than students in other programs or faculties due to more connection and contacts since the students experience of having the same subjects together. Furthermore, the students mainly populated from Perak (27.7%). This may be because UTAR is located in Perak, Kampar. The majority of the Perak students will select this university as their first choice. Lastly, most of the students have their monthly income, ranging from RM0 to RM700 (77.9%). The majority of the respondents are full-time students, the monthly income always supplies by their families to support their living expenses.

5.2 Smoking Status

Based on the analysis, 94 students (29.3%) were belonging to secondary smokers. The students always exposed to the secondary smoke in restaurants or at their homes if their family member are smokers. Centers for Disease Control and Prevention, (2022c) also found that homes, places of work and public places are the places where most of the people exposed to the secondary smoke.

Besides that, there are 20 cigarette smokers (6.2%) were recruited from UTAR. In the total of 20 smokers, there have 5 smokers (1.6%) were tobacco user while there have 15 smokers (4.7%) were vaping user. There are only a few smokers could be recruited in this study. This is mainly because smoking and vaping is strictly prohibited in UTAR campus. However, among the smoker recruited, more of the smokers tend to be a vape user. This scenario showed that vaping is more likely to perceive by young adults compare with tobacco smoking. This can be proved by Thatcher, (2015) where 20% of the young adults at 15 to 19 of aged were vape users compared to 11% who have tried tobacco smoking. Cooper, Harrell and Perry, (2016) also agreed that the vaping is higher used among young adults compare with older adults because of increased accessibility, visibility, advertising, lower prices and perception that vaping is safer than tobacco smoking.

5.3 Anthropometry Measurement

Based on the Centers for Disease Control and Prevention, (2022d), BMI of all the participants were analyzed and grouped into 4 groups, which including underweight (<18.5 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (>30 kg/m^2). The majority of the students (63.2%) have a normal BMI. However, there are large number of students were grouped into underweight (22.7%). This finding was consistent with a previous study conducted by Hong, et al. (2018) where the young adult with 18 to 29 years old had the highest prevalence of underweight (20.2%). Family history, high metabolism, excess physical activity or tobacco are some of the factors that can cause underweight among the young adults (Bubnis, 2018). According to the

National Institute of Mental Health, (2021) the percentage of mental illness was highest (33.7%) in young adults with 18 to 25 years old. In addition, academic stress was commonly found in the university students (84.4%). Stress can come from the test or exam burden and also poor performance in academic (Asif, et al., 2020). Poor mental health can cause the students loss of appetite. For example, depression, anxiety, obsessive-compulsive disorder (OCD) and eating disorder will cause a person continued weight loss and eventually became underweight (Bubnis, 2018).

Besides that, there are small population of students were grouped into overweight (10.9%) and obese (3.1%). World Health Organization (2021) have reported that 39% of adults with 18 years old and above were accounted overweight and 13% of the young adults were obese. Thus, a low prevalence of overweight and obesity was observed in this study. Mahaletchumy, Rampal, and Sharif, (2019) have reported that lack of exercising, insufficient sleeping, unhealthy lifestyle and poor eating habits are the main factors that increase the prevalence of overweight and obesity among the young adults. Young adults usually prefer to consume fast food, sugary snacks and puffed food that also contributed to the overweight and obesity condition.

5.4 Hemoglobin Level

According to the Hb range stated by Mayo Clinic, (2022a) the healthy Hb range for men is 13.2 to 16.6 g/dL and for women is 11.6 to 15 g/dL. Based on the analysis result, both female and male participants have a normal range of Hb

level. This may be due to the inclusion of this study, only healthy students were recruited in this study.

However, there were small population of students have a low or high level of Hb level. Several factors that can cause the low level of Hb, which including blood loss with frequent blood donation or menorrhagia in female during menstrual cycle, even normal menstrual bleeding may lead to the female have a slightly drop in Hb level (Mayo Clinic, 2022b).

Furthermore, a high Hb concentration usually occurs when the body tissue needed more oxygen-carrying capacity. High Hb level can cause by smoking exercise and living in high altitude. This is also proved by the previous studies conducted by Çiftçiler, et al. (2019), Mairbäurl, (2013) and Zhong, et al. (2015), where they stated that smoking habit, regular exercise and living living in high altitude in long-term period have increased the level of Hb.

5.5 Blood Morphology Abnormalities

The blood morphology abnormalities were analysed based on the size, colour and shape of the RBC. There have a large population of the participants had their blood size and colour with normocytic (45.5%) and normochromic (46.7%). This may be because the majority the participants recruited was healthy students and their healthy blood samples contributed to a normal result. However, more than half of the participants (50.8%) showed the anisocytosis in their blood morphology. The prevalence of microcytic and hypochromic was high (49.8%; 53.3%) in the blood of the participants. This could be caused by

iron deficiency in the diet or poor absorption of iron from the gut (Chaudhry, and Kasarla, 2023). Meanwhile, female have a higher respond rate in this study. During the menstrual cycle, the menstrual blood loss will cause iron loss from the blood and thus showed the RBC with microcytic and hypochromic (Berry, 2017).

In this study, macrocytic have a lowest prevalence (4.7%) among the size variation of blood. This is because the there was only 20 smokers was be recruited in this study. Smokers always have a macrocytic RBC. This is also agreed by Çiftçiler, et al. (2019) and Aldosari, et al. 2020), where they stated the smokers have an enlargement RBC compare with non-smokers.

In addition, even all the participants recruited were a healthy person but there were several types of poikilocytosis can be observed in this study. This may due to the lifestyle factors, such as smoking or insufficient nutrient intake. Firstly, both elliptocytes and ovalocytes was found higher in this study. According to the previous study conducted by Ford, (2013) and Bandaru, Killeen, and Gupta, (2023) elliptocytes and ovalocytes were the oval shaped of RBC. This appearance can be resulted by the iron deficiency, megaloblastic anemia due to folate and vitamin B12 deficiency, or hereditary elliptocytosis. Besides that, target cell is the RBC with centrally red area surrounded by the zone of central pallor. Target cell can be cause by iron deficiency, lack of enzyme called lecithin cholesterol acyltransferase, or hemoglobinopathies, which is an inherited disorder where the abnormal structure of Hb produced (Ford, 2013; David, 2022). Furthermore, tear drop cells have sharply tapered ends,

resembling the shape of a water drop. The presence of the tear drop cells can indication by folate or vitamin B12 deficiency and severe iron deficiency. Tear drop cells also present due to the artifact during blood smear preparation (Scordino, 2016; David, 2022). Moreover, echinocytes also known as burr cell, which is the RBC with regularly distributed, equally size of projections on the membrane surface. The occurrence of the echinocytes can be due to the artifact when the blood smear was prepared improperly, slow drying process or prolonged of blood storage before blood smearing. It can also cause by severe burn due to decreased level of plasma lipoprotein (Ford, 2013; John, 2008). However, echinocytes can cause by uremia, which is abnormal elevated of nitrogen waste product in the bloodstream or pyruvate kinase deficiency (David, 2022; Bandaru, Killeen, and Gupta, 2023). In addition, schistocyte is fragmented RBC that lack of spherical round shape (Ford, 2013). The present of schistocyte may de due to disseminated intravascular coagulation, which is a disorder where the overactive of the proteins that control blood clotting. Besides that, infection or inflammation in the digestive system that releasing toxic substances will damage RBC and caused the RBC became fragmented (David, 2022). Acanthocytes also referred to spur cells is the RBC with irregular distributed, unequal size and projections on the surface of RBC membrane (Ford, 2013). The present of acanthocytes can cause by liver disease due to excessive alcohol assumption, pyruvate kinase deficiency, and the intestines unable to fully absorb the dietary fats (David, 2022).

5.6 Association Factor That Cause Blood Morphology Abnormalities

5.6.1 Sociodemographic Characteristics

In terms of sociodemographic characteristic, there was only a significant association between gender, with female participants (35.2%) contributing higher percentage of hypochromic than male (18.1%). Meanwhile, based on the percentage, RBC with microcytic is significantly increased in female (32.1%) compared with male (17.8). Findings from Berry (2017) and Perla Health (2020) supported this statement where they indicated that female will loss the iron though menstrual blood during the menstrual cycle. If the iron loss during the menstrual cycle does not replace by the dietary iron intake, it will increase the risk to develop iron deficiency anemia. Iron deficiency caused the insufficient numbers of Hb produced and give the RBC with less colour than normal (Gersten, 2022).

5.6.2 Exercise Status

In this study, there was no significant association with blood morphology abnormalities. In contrast, several studies revealed that Hb levels can temporarily decreased during exercise because of exercise-induced hemolysis. Hb level decreased due to Hb was hemolyzed when RBC are destroyed during physical activity (Lippi and Gomar, 2019; Mairbäurl, 2013). Saat, et al. (2005) also proposed that the level of Hb and RBC were significantly decreased after the 40 minutes of exercise. Besides that, a decreased MCV will make the RBC become slightly smaller after exercise due to the loss of fluid and electrolytes while sweating. The Hb level and the size of RBC will return normal within 30 minutes after exercise (Rocker, and Kiesewetter, 2022).

Besides that, Hb levels in the blood might rise due to the regular physical activity or exercise. The body requires more oxygen during exercise or physical activity compared with the resting state. Increased level of Hb improves oxygen-carrying capacity and this will be able to fulfil the increased demand of oxygen during activity (Sepriadi, Jannah and Eldawaty, 2020). In addition, finding from Mairbäurl, (2013) assumed that regular exercise will triggering the erythropoietin (EPO) release and increasing the bone marrow activity. This can cause an increased Hb level in blood.

5.6.3 Smoking Status

In this section, there was only a significant association between secondary smokers. Secondary smokers demonstrating higher prevalence of macrocytic RBC (2.9%) compare with non-smokers (1.9%). However, there was no significant associated between tobacco or vape user in this study, this may be because the smokers recruited in this study were young adults. The body cells are still intact, haven't shown the obvious alteration in RBC. Even there was no significant associated between tobacco or vape user in this study, but there are several studies conducted by Reilly, et al. (2013), Aldosari, et al. (2020) and Nargish, et al. (2022) where they assumed that smokers or secondary smokers who exposure to tobacco smoke have shown the RBCs with macrocytic compared with of non-smokers. The macrocytic RBC of the smokers and secondary smokers always caused by the direct toxic effect on RBCs of acetaldehyde in tobacco smoke and the response to decrease oxygen-carrying capacity. Thus, tissue hypoxia was result in increased level of Hb for the compensatory mechanism and enlargement of RBC size is to improve the

oxygen-carrying capacity (Çiftçiler, et al., 2019; Malenica, et al., 2017). Besides, Shatha, (2017) have revealed that hypochromic RBC can be found in smoker due to deficiency of iron, folic acid or vitamin B12. Iron is an importance substance for RBC production. Lack of iron or other nutrients, hypochromic RBC may be produced by bone marrow instead of healthy RBC.

5.6.4 Anthropometry Measurement

Significant associations were reported between the height and weight. According the study conduction by Chmielewski, et al. (2017) taller and heavier people had higher Hb level compared with people who shorter and lighter. Growth hormone (GH) or insulin-like growth factor 1 (IGF-1) will give the difference the rate of erythropoiesis between shorter and taller individual. Taller and heavier individual required more oxygen to support their extra body mass (Robert, 2007). Thus, the increasing oxygen required of tissues will cause the growth hormone to trigger erythropoiesis to compensate the oxygen consumption of tissues (Chmielewski, et al., 2017).

Furthermore, the individual with high BMI tends to have increased level of steroid hormones. High level of steroid hormone will stimulate the bone marrow activity with increased the Hb production and RBC tissues (Chmielewski, et al., 2017). Findings from Tanaka, et al. (2021) revealed that obesity is associated with an increase in oxidative stress and caused an increased in inflammation. Oxidation stress will result in RBC shrinkage and leading to microcytosis.

5.6.5 Hemoglobin Level

In this study, there was no significant association between the Hb level with blood morphology abnormalities. In contrast, the study conducted by (Mayo Clinic, 2022b) had stated that normal menstrual bleeding may lead to the female have a slightly drop in Hb level. In addition, nutrient or iron deficiency due to insufficient iron intake from the diet or malabsorption from intestines can results in decreased Hb production (Moawad, 2022). Therefore, low level of Hb production will results in RBC smaller and paler in colour (Berry, 2017). On the other hand, Reilly, et al. (2013), Aldosari, et al. (2020) and Nargish, et al. (2022) have reported that high level of Hb caused by tissue hypoxia can lead to macrocytic RBC.

5.7 Limitation and Future Recommendation

This study had a number of limitations. Firstly, the respondents rate of the smokers is very low and nearly 90% of respondents are non-smoker. The small population of the smoker will make it harder to determine the particular results is a true finding (Clancy, 2019). This may contribute to the inaccuracy in result analysis. It is advised to increase the sample size in the future study. This is because increasing the sample size for smokers can make sure that the result reported is representative of the population being studied. This can help to increase the significant results by improve the its accuracy and precision (Biau, Kernéis and Porcher, 2008).

Secondly, some of the methodological limitations had included in this study. The high humidity of the environment was affecting the quality of the blood

smear. High humidity was prolonged the drying process, this will increase the risk of cell to shrink and would change the morphology of RBC (Kazuo, 2018). For example, since the blood smear was performed at the high humidity environment, the possibility of the blood to absorb the moisture was causing a lot of echinocytes can be observed under the light microscope. Therefore, the blood smear quality can be improved in the future study by reduced the blood film exposure to moisture and speed up the drying process by using a fan or hair dryer with cool setting (Centers for Disease Control and Prevention, 2020b).

Thirdly, the improper protocol was accidentally used in the blood staining procedure. At the beginning of the study, the use of time to perform the blood staining is too long caused the blood smear was heavily stained with Leishman stain. The overstaining of the blood film may be difficult to identify the morphology and the features of the RBC. The heavily stained blood film can cause the artifacts and increased the background staining. It causes the misidentification of the changes or structures of the RBC, resulting in false-positive or false-negative results and also reduced the accuracy of result analysis. Thus, the time of blood staining should be 10 minutes instead of 20 minutes. In future study, the protocol of blood staining should be well study before the research started.

CHAPTER 6

CONCLUSION

6.1 Conclusion

Assessment of blood morphology in this study can reveal healthy status of an individuals. The prevalence of anisocytosis, hypochromic and poikilocytosis are 50.8%, 53.3% and 47.0% respectively among the young adults. This study showed that the gender, secondary smokers, height and weight were significantly associated with the blood morphology abnormalities, provides the alternative hypothesis of the study is correct. Besides, macrocytic and hypochromic RBC can be seen in cigarette smoker, vape and secondary smoker whereas the microcytic and hypochromic RBC can be observed after exercise among the young adults. These results offer valuable insight into how the lifestyle factor can contributed to the blood morphology abnormalities. All the undergraduates might improve their lifestyle habit by exercise regularly to maintain their healthy weight range. Overweight and obesity due poor diet habit, such as food or snacks with high calories, high sugar will result in abnormal size, colour or shape of RBC produced from the bone marrow. Having a healthy and balanced diet is important to improve the normal and healthy RBC production. Deficiency of nutrients, iron and vitamin intake from the diet can also lead to the production of unhealthy RBC. Female students were encouraged to intake the sufficient iron from the diet in order to replace the iron loss by menstrual cycle. This study also reveals that smoking is also a lifestyle factor that influenced the blood morphology by altered the size and colour of RBC. Therefore, smokers are encouraged to quit smoking and also the non-smokers

should stay away from exposed to secondhand smoke. This is because the toxic substances in tobacco smoke can contribute to the microcytic and hypochromic of RBC and lead to tissue hypoxia.

6.2 Implications

Blood smear is a critical step to evaluate the morphology of the RBC (Kurec, 2022). Assessment of RBC morphology is a useful laboratory tool to examine the normocytic, microcytic, and macrocytic of the RBC (Ford, 2013). From this study, we found that smoking both tobacco and vape have adverse effect on altering the blood morphology. These alterations might be associated with a higher chance to developing chronic diseases to long-term of smoking expose among young adults (Herath, et al., 2021). This is a serious problem since vaping and secondary smoke expose is become more popular among public (AlQahtany, et al., 2020). Therefore, greater emphasis should be placed in raising the awareness of seriousness of smoking's effect on the blood. This improvement can be made by campaign, educational programs or seminars, especially among young adults (World Health Organization, 2019). Besides that, the young generation awareness towards healthy lifestyle should not be ignored. Students are encouraged to participate these education programs to gain more structured experienced and apply useful knowledge, which improves their understanding of how the lifestyle factor can contributed to the health concern and eventually develop into the diseases. In short, giving adequate education and knowledge to the young adult may be a critical step to boost their awareness toward the healthy lifestyle and lowering the smoking rate in the future.

REFERENCES

Aldosari, K. H., Ahmad, G., Ghamdi, S. A. and Alsharif, M., H., 2020. The Influence and Impact of Smoking on Red Blood Cell Morphology and Buccal Microflora. *Journal of Clinical Laboratory Analysis,* 34(6).

AlQahtany, F. S., Algahtani, F. H., Alshebly, M. M. and Madkhaly, F. M., 2020. Association between Cigarette & Shisha Smoking and The Severity of Polycythemia. *Saudi Journal of Biological Sciences,* 27(1), pp. 460-464.

American Lung Association, 2022. *Why Kids Start Smoking.* [online]. Available at: <https://www.lung.org/quit-smoking/helping-teens-quit/why-kids-start-smoking> [Accessed by 6 April 2023].

Appn, 2021. *HemoCue Hb 201+ Method and Sample Collection.* [online] Available at: <https://www.appn.net.au/Data/Sites/1/appn/02implementation/technicalresources/haematology/hemocuehb201methodandsamplecollection.pdf> [Accessed by 25 March 2023].

Asif, S., Mudassar, A., Shahzad, T.Z. and Raouf, M., 2020. Frequency of Depression, Anxiety and Stress Among University Students. *Pakistan Journal of Medical Science*, 36(5), pp. 971-976.

Ballard, H. S., 1997. The Hematological Complications of Alcoholism. *Alcohol Health & Research World,* 21(1), pp. 42-52.

Bandaru, S. S., Killeen, R. B. and Gupta, V., 2023. *Poikilocytosis.* [e-book] Treasure Island (FL): StatPearls Publishing. Available at: Google Books <https://www.ncbi.nlm.nih.gov/books/NBK562141/> [Accessed by 14 April 2023].

Berry, J., 2017. *What is Microcytic Anemia?* [online] Available at: <https://www.medicalnewstoday.com/articles/319050> [Accessed by 12 April 2023].

Biau, D. J., Kernéis, S. and Porcher, R., 2008. Statistics In Brief: The Importance of Sample Size in The Planning and Interpretation of Medical Research. *Clinical Orthopaedics Related Research,* 466(9), pp. 2282-2288.

Bigger, A., 2021. *Megaloblastic Anemia.* [online] Available at: <https://rarediseases.org/rare-diseases/anemia-megaloblastic/> [Accessed by 8 April 2023].

Bogdanova, A., Kaestner, L., Simionato, G. and Wickrema, A., 2020. Heterogeneity of Red Blood Cells: Causes and Consequences. *Frontiers in Physiology,* 11, pp. 392.

Bubnis, D., 2018. *What are The Risks of Being Underweight?* [online] Available at: <https://www.medicalnewstoday.com/articles/321612> [Accessed by 11 April 2023].

Cancer Research, 2021. *How Does Smoking Cause Cancer?* [online] Available at: <https://www.cancerresearchuk.org/about-cancer/causes-of-cancer/smoking-and-cancer/how-does-smoking-cause-cancer> [Accessed 28 August 2022].

Centers for Disease Control and Prevention, 2014. *Smoking and Cardiovascular Disease.* [online] Available at: <https://www.cdc.gov/tobacco/data_statistics/sgr/50th-anniversary/pdfs/fs_smoking_CVD_508.pdf> [Accessed 29 August 2022].

Centers for Disease Control and Prevention, 2020a. *Blood Specimens - Specimen Processing.* [online] Available at: <https://www.cdc.gov/dpdx/diagnosticprocedures/blood/specimenproc.html. [Accessed 25 March 2023].

Centers for Disease Control and Prevention, 2020b. *Specimen Processing.* [online] Available at: <https://www.cdc.gov/dpdx/diagnosticprocedures/blood/specimenproc.html> [Accessed by 12 April 2023].

Centers for Disease Control and Prevention, 2021. *Chemical in Tobacco Smoke.* [online] Available at: <https://www.cdc.gov/tobacco/data_statistics/sgr/2010/consumer_booklet/chemicals_smoke/index.htm> [Accessed 21 March 2023].

Centers for Disease Control and Prevention, 2022a. *Youth and Tobacco Use.* [online] Available at: <https://www.cdc.gov/tobacco/data_statistics/fact_sheets/youth_data/tobacco_use/index.htm> [Accessed 11 October 2022].

Centers for Disease Control and Prevention, 2022b. *Quick Facts on the Risks of E-cigarettes for Kids, Teens, and Young Adults.* [online] Available at: <https://www.cdc.gov/tobacco/basic_information/e-cigarettes/Quick-Facts-on-the-Risks-of-E-cigarettes-for-Kids-Teens-and-Young-Adults.html#> [Accessed 11 October 2022].

Centers for Disease Control and Prevention, 2022c. *General Information About Secondhand Smoke.* [online] Available at: <https://www.cdc.gov/tobacco/secondhand-smoke/about.html#:~:text=Where%20are%20most%20people%20exposed,bars%2C%20restaurants%2C%20and%20casinos> [Accessed by 9 April 2023].

Centers for Disease Control and Prevention, 2022d. *Adult BMI Calculator.* [online] Available at: <https://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/english_bmi_calculator/bmi_calculator.html> [Accessed by 9 April 2023].

Chaudhry, H.S. and Kasarla, M. R., 2023. *Microcytic Hypochromic Anemia.* [e-book] Treasure Island (FL): StatPearls Publishing. Available at: Google Books <https://www.ncbi.nlm.nih.gov/books/NBK470252/> [Accessed by 12 April 2023].

Chmielowiec, P., Strzelec, B., Chmielowiec, J. and Chmielowiec, K., 2017. Association Between Body Size and Selected Hematological Parameters in Men and Women Aged 45 and Above from A Hospitalized Population of Older Adults: An Insight from The Polish Longitudinal Study of Aging (1960–2000). *Anthropological Review,* 80(2), pp. 171-190.

Çiftçiler, R., Güven, A., Haznedaroğlu, İ. C. and Aksu, S., 2019. Effects of Smoking on Hematological Parameters and Ferritin Levels. *The Medical Bulletin of Haseki,* 57, pp. 372-376.

Clancy, L., 2019. *Discussing your Study's Limitations*. [online] Available at: <https://www.internationalscienceediting.com/study-limitations/#:~:text=A%20small%20sample%20size%20may,the%20study%20groups%20is%20reported> [Accessed by 12 April 2023].

Cooper, M., Harrell, M.B. and Perry, C. L., 2016. Comparing Young Adults to Older Adults in E-Cigarette Perceptions and Motivations for Use: Implications For Health Communication. *Health Education Research,* 31(4), pp. 429-438.

Cylwik, B. and Chrostek, L., 2011. Disturbances of Folic Acid and Homocysteine Metabolism in Alcohol Abuse. *Polish Medical Journal*, 30(178), pp. 295-299.

David, C., 2022. *Blood Smear.* [online] Available at: <https://www.mountsinai.org/health-library/tests/blood-smear#:~:text=The%20presence%20of%20teardrop%2Dshaped,Severe%20iron%20deficiency> [Accessed by 13 April 2023].

Davos, N., 2023, *The Short and Long Terms Effects of Smoking on Your Body.* [online] Available at: <https://cbqmethod.com/effects-of-smoking-on-your-body/> [Accessed 12 September 2022].

Duntoye, S. J., 2015. *Getting The Sample Size Right!* [online] Available at: <https://www.linkedin.com/pulse/getting-sample-size-right-sunday-j-duntoye> [Accessed by 6 April 2023].

Ford, J., 2013. Red Blood Cell Morphology. *International Journal of Laboratory Hematology,* 35(3), pp. 351-357.

Frederick Health, 2021. *Dangers of Smoking at a Young Age.* [online] Available at: <https://www.frederickhealth.org/news/2021/august/dangers-of-smoking-at-a-young-age/> [Accessed 11 October 2022].

Gallucci, G., Tartarone, A., Lerose, R. and Lalinga, A. V., 2020. Cardiovascular Risk of Smoking and Benefits of Smoking Cessation. *Journal of Thoracic Disease.* 12(7), pp. 3866-3876.

Gedde, M. M., Davis D.K. and Huestis, W. H., 1997. Cytoplasmic pH and Human Erythrocyte Shape. *Biophysical Journal,* 72(3), pp. 1234-1246.

Gersten, T., 2022. *Hypochromia.* [online] Available at: <https://www.mountsinai.org/health-library/tests/hypochromia> [Accessed by 14 April 2023].

Glen**,** S., 2013. *Sample Size in Statistics (How to Find it): Excel, Cochran's Formula, General Tips.* [online] Available at: <https://www.statisticshowto.com/probability-and-statistics/find-sample-size/> [Accessed by 6 April 2023].

Herath, P., Wimalasekera, S. W., Amarasekara, T. D. and Fernando, M. S., 2021. Adverse Effects of Cigarette Smoking on Exhaled Breath Carbon Monoxide, Blood Carboxyhemoglobin, and Hematological Parameters amongst Sri Lankan Adult Tobacco Smokers: A Descriptive Study. *Journal of Population Medicine,* 3(10), pp.27.

Hong, S. A., Peltzer, K., Lwin, K. T. and Aung, L. S., 2018. The Prevalence of Underweight, Overweight and Obesity and Their Related Socio-Demographic and Lifestyle Factors Among Adult Women in Myanmar, *PLoS ONE,* 13(3).

Hospice, 2020. *Why Do People Smoke Cigarettes?* [online] Available at: <https://myallamericanhospice.com/people-smoke-cigarettes/> [Accessed by 2 April 2023].

John, W. H., 2008., Chapter 7 - The Erythrocyte: Physiology, Metabolism, and Biochemical Disorders. *Clinical Biochemistry of Domestic Animals,* pp. 173-240.

Kandel, D., 2015. *Teens More Likely to Smoke if Parents are Smokers.* [online] Available at: <https://www.publichealth.columbia.edu/news/if-mom-or-dad-smoker-their-teenager-more-likely-be-smoker-too> [Accessed by 2 April 2023].

Kazuo, O., 2018. Blood Lymphocyte Morphology: It Depends on How Blood Smears are Dried. *Biomedical Journal of Science and Technical Research,* 2(3).

Kurec, A., 2022. *Blood Smear*. [online] Available at: <https://www.testing.com/tests/blood-smear/#:~:text=A%20blood%20smear%20is%20typically,that%20affect%20blood%20cell%20populations> [Accessed by: 15 April 2023].

Latvala, J., 2004. *Effect of Alcohol Consumption and Acetaldehyde on Blood Cells and Molecules.* [online] Available at: <https://trepo.tuni.fi/bitstream/handle/10024/67269/951-44-6095-2.pdf?sequence=1&isAllowed=y> [Accessed by 26 April 2023].

Lim, K. H., The, C. H., Pan, S. and Ling, M.Y., 2018. Prevalence and factors associated with smoking among adults in Malaysia. *Prevention of Tobacco Induced Diseases,* 16, (1).

Lippi, G. and Gomar, S. F., 2019. Epidemiological, Biological and Clinical Update on Exercise-Induced Hemolysis. *Annalis of Translational Medicine,* 7(12), pp. 270.

Mahaletchumy, A., Rampal, L. and Sharif, Z. M., 2019. Prevalence of Overweight/Obesity and its Associated Factors Among Secondary School Students in Semi Urban Area in Malaysia. *Medical Journal Malaysia,* 74(6), pp. 513-520.

Mairbäurl, H., 2013. Red Blood Cells in Sports: Effects of Exercise and Training on Oxygen Supply by Red Blood Cells. *Frontiers in Physiology,* 12(4).

Malenica, M., Prnjavorac, B., Bego, T. and Dujic, T., 2017. Effect of Cigarette Smoking on Haematological Parameters in Healthy Population. *Medical Archives,* 71(2), pp. 132-136.

Mayo Clinic, 2022a. *Hemoglobin Test.* [online] Available at: <https://www.mayoclinic.org/tests-procedures/hemoglobin-test/about/pac-20385075> [Accessed by 11 April 2023].

Mayo Clinic, 2022b. *Low Hemoglobin Count.* [online] Available at: <https://www.mayoclinic.org/symptoms/low-hemoglobin/basics/causes/sym-20050760> [Accessed by 11 April 2023].

Miwa, T, Hatano, Y, Kochi, T. and Aiba, M., 2020. Spur Cell Anemia Related to Alcoholic Liver Cirrhosis Managed Without Liver Transplantation: A Case Report and Literature Review. *Clinical Journal of Gastroenterology,* 13(5), pp. 882-890.

Moawad, H., 2022. *Macrocytosis: What You Need to Know.* [online] Available at: <https://www.verywellhealth.com/macrocytosis-5198380> [Accessed by 14 April 2023].

Mohan, N., 2021. *Sideroblastic Anemias.* [online] Available at: <https://emedicine.medscape.com/article/1389794-overview> [Accessed by 8 April 2023].

Nargish, S., Kabir, M. H., Akhter, K. and Nahid, K., 2022. Effect of Smoking on the Red Blood Cell Count, Hemoglobin Concentration, Hematocrit and Red Cell Indices in Adult Male Smokers. *Eastern Medical College Journal*, 7(1).

Natalie, 2018. *Hematology Case Study on Low Platelets.* [online] Available at: <https://commons.bcit.ca/news/2018/12/hematology-case-study-on-low-platelets/> [Accessed by 25 March 2023].

National Institute of Mental Health, 2021. *Mental Illness.* [online] Available at: <https://www.nimh.nih.gov/health/statistics/mental-illness> [Accessed by 11 April 2023].

National Library of Medicine, 2022. *MCV (Mean Corpuscular Volume).* [online] Available at: <https://medlineplus.gov/lab-tests/mcv-mean-corpuscular-volume/> [Accessed 29 August 2022].

OMRON Healthcare, 2008. *Full Body Sensor Body Composition Monitor and Scale.* [online] Available at: <https://omronhealthcare.com/wp-content/uploads/hbf-514c-instruction-manual.pdf> [Accessed 25 March 2023].

Perla Health, 2020. *How Your Period Can Lead to Iron Deficiency.* [online] Available at: <https://perlahealth.com/how-your-period-can-lead-to-iron-deficiency/#:~:text=When%20you%20lose%20blood%20during,may%20develop%20iron%20deficiency%20anemia> [Accessed by 12 April 2023].

Reilly, M. A., Buckley, C. M., Harrington, J. M. and Shea, S. O., 2013. Cigarette Smoking is An Under Recognised Cause of Macrocytosis. *The American Society of Hematology*, 122(21), pp. 4660.

Robalino, J. D. and Macy, M., 2018. Peer effects on adolescent smoking: Are popular teens more influential? *PLoS One*, 13(7).

Robert, M., 2007. *Obesity and Oxygen Therapy: Super-Sizing Your Oxygen Program.* [online] Available at: <https://rtmagazine.com/disorders-diseases/chronic-pulmonary-disorders/copd/obesity-and-oxygen-therapy-super-sizing-your-oxygen-program/#:~:text=The%20additional%20weight%20around%20the,just%20to%20maintain%20proper%20oxygenation> [Accessed by 14 April 2023].

Rocker, L. and Kiesewetter, H., 2022. *Effect of Physical Exercise on Laboratory Test Results.* [online] Available at: <https://www.clinical-laboratory-diagnostics.com/k51.html> [Accessed by 14 April 2023].

Ruhi, R., 2018. *Procedure of Leishman Staining.* [online] Available at: <https://paramedicsworld.com/hematology-practicals/leishman-staining-principle-procedure-interpretation/medical-paramedical-studynotes> [Accessed by 25 March 2023].

Saat, M., Sirisinghe, R. G., Singh, R. and Tochihara, Y., 2005. Effects of Short-Term Exercise in The Heat on Thermoregulation, Blood Parameters, Sweat Secretion and Sweat Composition of Tropic-Dwelling Subjects. *Journal of Physiological Anthropology and Applied Human Science,* 24(5), pp. 541-549.

Scordino, T., 2016. Teardrop Cells or Dacrocytes. [online] Available at: <https://imagebank.hematology.org/image/60311/teardrop-cells-or-dacrocytes?type=atlas> [Accessed by 13 April 2023].

Seca, 2015. *How can I Accurately Measure The Height of A Person Standing?* [online] Available at: <https://us.secashop.com/knowledge/application-tips/how-can-i-accurately-measure-the-height-of-a-person-standing-1> [Accessed 25 March 2023].

Sepriadi, Jannah, K. and Eldawaty, 2020. The Effect of Jogging Exercise to Improve Hemoglobin Levels. *Journal of Physics: Conference Series,* 1481(1).

Shadel, W. G., Tharp, T. S. and Fryer, C. S., 2008. Exposure to cigarette advertising and adolescents' intentions to smoke: the moderating role of the developing self-concept. *Journal of Pediatric Psychology,* 33(7), pp. 751-760.

Shamsuddin, K. and Haris, M. A., 2000. Family Influence on Current Smoking Habits Among Secondary School Children in Kota Bharu, Kelantan. *Singapore Medical Journal,* 41(4), pp. 167-171.

Shatha, Q., 2017. The Effect of Cigarette Smoking on Some Blood Parameters, Blood Pressure and Renal Function Test. *Journal University of Kerbala,* 15(1).

Susan, A., 2016. *Steps of Blood Smear Preparation.* [online] Available at: <https://www.researchgate.net/figure/Figure-1-Steps-of-Blood-Smear-Preparation_fig5_311877838> [Accessed by 25 March 2023].

Tanaka, M., Okada, H., Hashimoto, Y. and Kumagai, M., 2021. Association of Mean Corpuscular Volume with Sarcopenia and Visceral Obesity in Individuals Without Anemia. *Journal of Diabetes Investigation.* 12(7), pp. 1287-1292.

Thatcher, A., 2015. E-cigarettes more popular than tobacco among youth. *Canadian Medical Association Journal,* 187(6).

Thomas, L., 2020. *Cross-Sectional Study | Definition, Uses & Examples.* [online] Available at: <https://www.scribbr.com/methodology/cross-sectional-study/> [Accessed 23 March 2023].

Universiti Tunku Abdul Rahman, 2020. *Universiti Tunku Abdul Rahman (UTAR).* [online] Available at: <https://www.mypacer.com/routes/218445/tunku-abdul-rahman-university-utar-walking-and-running-trail-kampar-perak-malaysia> [Accessed 21 March 2023].

Universiti Tunku Abdul Rahman, 2023. *Introduction.* [online] Available at: <https://utar.edu.my/Introduction.php> [Accessed 21 March 2023].

Windsor J. S. and Rodway G. W., 2007. Heights and Haematology: The Story of Haemoglobin at Altitude. *Postgraduate Medical Journal*, 83(977), pp.148-151.

Wood, L., Greenhalgh, EM., Vittiglia, A. and Jones, H., 2020. *The Smoking Behaviour of Peers, and Peer Attitudes and Norms.* [online] Available at: <https://www.tobaccoinaustralia.org.au/chapter-5-uptake/5-8-the-smoking-behaviour-of-peers-and-peer-attitu> [Accessed by 6 April 2023].

World Health Organization, 2010. *WHO Guidelines on Drawing Blood.* [online] Available at: <https://www.ncbi.nlm.nih.gov/books/NBK138650/> [Accessed by 25 March 2023].

World Health Organization, 2016. *Collection of Finger-Prick Blood and Preparation of Thick and Thin Blood Films.* [online] Available at: <https://www.who.int/docs/default-source/wprodocuments/toolkit/malaria-sop/gmp-sop-05a.pdf?sfvrsn=d50716d8_2> [Accessed 25 March 2023].

World Health Organization, 2019. *World No Tobacco Day 2019 - Tobacco and Lung Health.* [online] Available at: <https://www.who.int/news-room/events/detail/2019/05/31/default-calendar/world-no-tobacco-

day#:~:text=Every%20year%2C%20on%2031%20May,of%20tobacco%20in%20any%20form> [Accessed by: 15 April 2023].

World Health Organization, 2021. *Obesity and Overweight.* [online] Available at: <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight> [Accessed by 11 April 2023].

World Health Organization, 2022. *Tobacco.* [online] Available at: <https://www.who.int/news-room/fact-sheets/detail/tobacco> [Accessed 29 August 2022].

Zhong, R., Liu, H., Wang, H. and He, Z., 2015. Adaption to High Altitude: An Evaluation of the Storage Quality of Suspended Red Blood Cells Prepared from the Whole Blood of Tibetan Plateau Migrants. *PLoS ONE,* 10(12).

APPENDICES

ADDENDIX A – Ethical Approval Form

UNIVERSITI TUNKU ABDUL RAHMAN DU012(A)
Wholly owned by UTAR Education Foundation Co. No. 578227-M

Re: U/SERC/242/2022

17 November 2022

Dr Teh Lai Kuan
Head, Department of Allied Health Sciences
Faculty of Science
Universiti Tunku Abdul Rahman
Jalan Universiti, Bandar Baru Barat
31900 Kampar, Perak.

Dear Dr Teh,

Ethical Approval For Research Project/Protocol

We refer to the application for ethical approval for your students' research projects from Bachelor of Science (Honours) Biomedical Science programme enrolled in course UDDN3108. We are pleased to inform you that the application has been approved under Expedited Review.

The details of the research projects are as follows:

No	Research Title	Student's Name	Supervisor's Name	Approval Validity
1.	Differences of the Effects of Smoking Cigarette on Health Factors, Hemoglobin Concentration, and Red Blood Cell Morphology Among Young Adults Who Use Cigarette, Electronic Devices, and Passive Smokers	1. Ng Zhen Yong 2. Khoo Sin Ye 3. Yeong Shue Rou 4. Wendy Khor 5. Liow Zhe Ying	Dr Annaletchumy a/p Loganathan	17 November 2022 – 16 November 2023

The conduct of this research is subject to the following:

(1) The participants' informed consent be obtained prior to the commencement of the research;

(2) Confidentiality of participants' personal data must be maintained; and

(3) Compliance with procedures set out in related policies of UTAR such as the UTAR Research Ethics and Code of Conduct, Code of Practice for Research Involving Humans and other related policies/guidelines.

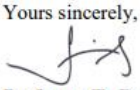
(4) Written consent be obtained from the institution(s)/company(ies) in which the physical or/and online survey will be carried out, prior to the commencement of the research.

Kampar Campus : Jalan Universiti, Bandar Barat, 31900 Kampar, Perak Darul Ridzuan, Malaysia
Tel: (605) 468 8888 Fax: (605) 466 1313
Sungai Long Campus : Jalan Sungai Long, Bandar Sungai Long, Cheras, 43000 Kajang, Selangor Darul Ehsan, Malaysia
Tel: (603) 9086 0288 Fax: (603) 9019 8868
Website: www.utar.edu.my

Should the students collect personal data of participants in their studies, please have the participants sign the attached Personal Data Protection Statement for records.

Thank you.

Yours sincerely,



Professor Ts Dr Faidz bin Abd Rahman
Chairman
UTAR Scientific and Ethical Review Committee

c.c Dean, Faculty of Science
Director, Institute of Postgraduate Studies and Research

Kampar Campus : Jalan Universiti, Bandar Barat, 31900 Kampar, Perak Darul Ridzuan, Malaysia
Tel: (605) 468 8888 Fax: (605) 466 1313
Sungai Long Campus : Jalan Sungai Long, Bandar Sungai Long, Cheras, 43000 Kajang, Selangor Darul Ehsan, Malaysia
Tel: (603) 9086 0288 Fax: (603) 9019 8868
Website: www.utar.edu.my

APPENDIX B – Questionnaire

Assessment on hemoglobin concentration, red blood cell morphology among smokers and non-smokers among UTAR students in Kampar, Perak

Dear participants,

We are year 3 students from Bachelor of Science (Honours) Biomedical Science, Universiti Tunku Abdul Rahman (UTAR) Kampar Campus. We are conducting our Final Year Project entitled "Assessment on hemoglobin concentration, red blood cell morphology and smoking among UTAR students in Kampar, Perak".
We would like to invite you to participate in this research study by helping us to complete this questionnaire.

This questionnaire consists of 4 sections:
Section A: Sociodemographic analysis
Section B: Individual health's status
Section C: Cigarette, e-cigarette, secondary smokers screening
Section D: Smoker's knowledge, attitude and perception on smoking

Objective: To study the association between hemoglobin concentration, red blood cell morphology and smoking among UTAR students in Kampar, Perak.

Participants MUST meet all the criteria below:
a) UTAR (Kampar) Chinese students
b) Age: 18 – 30 yrs old
c) No blood-related-diseases

Date: 14th November 2022 - 10th December 2022
Time:
Monday 10am - 12pm
Tuesday 10am - 2pm
Wednesday 10am - 3pm
Thursday 10am - 3pm
Friday 10am - 3pm
Venue: Block D, ground floor

Screening includes:
Anthropometry measurements
Hemoglobin concentration level
Microscopic examination of red blood cell morphology

Note: You will spend around 5 minutes to complete all assessments.

Your participation is truly appreciated. Thank you very much.
If you have any inquires, please do not hesitate to contact us

Khoo Sin Ye, 011 1897 4432
Liow Zhe Ying, 018 918 4063
Ng Zhen Yong, 010 218 7200
Wendy Khor, 010 371 8336
Yeong Shue Rou, 012 494 4227

*Required

1. Acknowledgment of Notice *

Mark only one oval.

- ◯ I have been notified and that I hereby understood, consented and agreed per UTAR above notice
- ◯ I disagree, my personal data will not be processed

Part A: Socio-demographic analysis

A combination of social and demographic characteristics that characterize members of a given group or population are referred to as socio-demographics.

2. Your name *

_____

3. Gender *

Mark only one oval.

- ◯ Male
- ◯ Female

4. Date of birth *

_____
Example: 7 January 2019

PERSONAL DATA PROTECTION STATEMENT (INFORMED CONSENT FORM)

Please be informed that in accordance with Personal Data Protection Act 2010 ("PDPA") which came into force on 15 November 2013, Universiti Tunku Abdul Rahman ("UTAR") is hereby bound to make notice and require consent in relation to collection, recording, storage, usage and retention of personal information.

1. Personal data refers to any information which may directly or indirectly identify a person which could include sensitive personal data and expression of opinion. Among others it includes:
a) Name
b) Identity card
c) Place of Birth
d) Address
e) Education History
f) Employment History
g) Medical History
h) Blood type
i) Race
j) Religion
k) Photo
l) Personal Information and Associated Research Data

2. The purposes for which your personal data may be used are inclusive but not limited to:

a) For assessment of any application to UTAR
b) For processing any benefits and services
c) For communication purposes
d) For advertorial and news
e) For general administration and record purposes
f) For enhancing the value of education
g) For educational and related purposes consequential to UTAR
h) For replying any responds to complaints and enquiries
i) For the purpose of our corporate governance
j) For the purposes of conducting research/ collaboration

3. Your personal data may be transferred and/or disclosed to third party and/or UTAR collaborative partners including but not limited to the respective and appointed outsourcing agents for purpose of fulfilling our obligations to you in respect of the purposes and all such other purposes that are related to the purposes and also in providing integrated services, maintaining and storing records. Your data may be shared when required by laws and when disclosure is necessary to comply with applicable laws.

4. Any personal information retained by UTAR shall be destroyed and/or deleted in accordance with our retention policy applicable for us in the event such information is no longer required.

5. UTAR is committed in ensuring the confidentiality, protection, security and accuracy of your personal information made available to us and it has been our ongoing strict

5. Marital status * *Mark only one oval.*

- Married
- Divorced
- Single

policy to ensure that your personal information is accurate, complete, not misleading and updated. UTAR would also ensure that your personal data shall not be used for political and commercial purposes.

Consent:

6. By submitting or providing your personal data to UTAR, you had consented and agreed for your personal data to be used in accordance to the terms and conditions in the Notice and our relevant policy.

7. If you do not consent or subsequently withdraw your consent to the processing and disclosure of your personal data, UTAR will not be able to fulfill our obligations or to contact you or to assist you in respect of the purposes and/or for any other purposes related to the purpose.

8. You may access and update your personal data by writing to us at zyyxxo@1utar.my

6. Contact number *

7. Faculty *

Mark only one oval.

- ◯ Faculty of Science (FSc)
- ◯ Faculty of Arts and Social Science (FAS)
- ◯ Faculty of Business and Finance (FBF)
- ◯ Faculty of Engineering and Green Technology (FEGT)
- ◯ Faculty of Information and Communication Technology (FICT)
- ◯ Centre for Extension Education (CEE)
- ◯ Centre for Foundation Studies (CFS)
- ◯ Institute of Chinese Studies (ICS)
- ◯ Institute of Postgraduate Studies and Research (IPSR)
- ◯ Other: _____

8. Your course (e.g. Biomedical Science) *

_____

9. Year of Study *

Mark only one oval.

- ◯ Year 1
- ◯ Year 2
- ◯ Year 3
- ◯ Year 4
- ◯ Other: _____

10. Which state are you from? (eg: KL, penang..etc) *

_____

11. Monthly income (RM) or pocket money? *

Mark only one oval.

- 0-700
- 701-1400
- 1401-2100
- >2100

12. Appointment time *

Mark only one oval.

- Monday 10am - 12pm
- Tuesday 10am - 2pm
- Wednesday 10am - 3pm
- Thursday 10am - 3pm
- Friday 10am - 3pm

Part B: Individual Health's status

Health status referred to individual's mental and physical condition by using following factors: medical condition, claims experience, medical history, and health service utilization.

13. Do you have any blood-related diseases? *

Mark only one oval per row.

	Yes	No	Not sure
Anemia	◯	◯	◯
Haemophilia	◯	◯	◯
Venous thromboembolism (Blood clots)	◯	◯	◯
Thalassemia	◯	◯	◯
Von Willebrand disease	◯	◯	◯
Sickle Cell Disease	◯	◯	◯

14. Others please state

_____

15. Besides blood-related disease, were you diagnosed with any other diseases? *

Tick all that apply.

	Yes	No	Not sure
Cardiovascular disease (e.g. Heart disease)	☐	☐	☐
Diabetes mellitus (High blood glucose)	☐	☐	☐
Dyslipidemia (Abnormal amount of lipids(cholesterol/fat) in blood)	☐	☐	☐
Others	☐	☐	☐

16. Others please state

17. Do you exercise for the past 1 hour? *

Mark only one oval.

- Yes
- No

18. How frequent do you exercise in a week? *

Mark only one oval.

- None
- <30 minutes (per week)
- >30 minutes (per week)

Part C: Smokers and non-smokers screening

19. Are you a smoker? *

Mark only one oval.

- Yes
- No

20. If YES, how long you have been smoking? (In years or month); for non-smokers '0' *

Secondary smokers screening

* Secondhand smoke refer to the people who do not smoke breathe in smoke exhaled by people who smoke or from burning tobacco products.

21. Are you a secondary smoker? *

Mark only one oval.

- ◯ Yes
- ◯ No
- ◯ Not sure

22. How long per day you have expose to the smoke? *

Mark only one oval.

- ◯ less than 30 minutes
- ◯ 30 minutes to 1 hour
- ◯ more than 1 hour
- ◯ Not sure

23. Where do you always exposure to the smoke? *

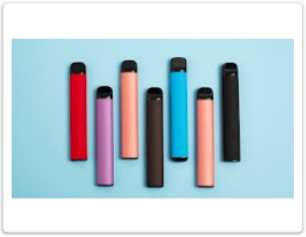
Mark only one oval.

- ◯ Closed environment
- ◯ Open environment
- ◯ Not sure

24. Are you a tobacco users or vape users? (For non-smokers please select N/A) *

Mark only one oval.

Tobacco smoking
Skip to question 25



Vape (e-cigarette)
Skip to question 30

N/A *Skip to question 50*

Tobacco (Cigarette smokers) screening

25. Have you smoked at least 100 cigarettes in your entire life? *

Mark only one oval.

Yes
No
Don't know/ Not sure

26. Do you NOW smoke cigarettes every day, some days or not at all? *

Mark only one oval.

- ◯ Everyday
- ◯ Some days
- ◯ Not at all

27. On the average, how many cigarette you have smoked PER DAY? *

Mark only one oval.

- ◯ 1 to 5
- ◯ 6 to 10
- ◯ Above 10

28. Do you vape (e-cigarette) as well? *

Mark only one oval.

- ◯ Yes
- ◯ No

29. Do you now use e-cigarettes or other electronic vaping products everyday, some days or not at all? *

Mark only one oval.

- ◯ Daily *Skip to question 36*
- ◯ Weekly *Skip to question 36*
- ◯ Monthly *Skip to question 36*
- ◯ Never used vaping products (e-cigarette) *Skip to question 36*
- ◯ Don't know/ Not sure *Skip to question 36*

Vape (E-cigarette) user screening

30. How frequent do you smoke? *

Mark only one oval.

- Daily
- Weekly
- Monthly
- N/A

31. During the **past 30 days**, on how many days did you use vape (e-cigarette)? *

32. Which of the following best describes the **type of e-cigarette** you have used in the **past 30 days**? If you have used more than one type, please think about the one you use most often. No *

Mark only one oval.

A disposable e-cigarette

An e-cigarette that uses pre-filled pods or cartridges

An e-cigarette with a tank that you refill with liquids

A mod system (an e-cigarette can be customized by the user with their own combination of batteries or other parts)

I don't know the type

33. Do you frequently use the e-cigarette in places where tobacco smoking is banned? *

Mark only one oval.

- ◯ Yes
- ◯ No

34. Do you smoke tobacco as well? *

Mark only one oval.

- ◯ Yes *Skip to question 36*
- ◯ No *Skip to question 36*

35. If YES, on the average, how many cigarette you have smoked PER DAY? *

Mark only one oval.

- ◯ 1-5
- ◯ 6-10
- ◯ above 10
- ◯ I did not smoke tobacco

Part D: Smoker's knowledge, perceptions and factors on smoking (Smokers)

Health knowledge

36. How much do you know about smoking ? *

Mark only one oval.

Poor

1 ◯

2 ◯

3 ◯

4 ◯

5 ◯

Excellent

37. Do you know about the health effects of smoking ? *

Mark only one oval.

◯ Yes

◯ No

Smoking initiation

38. How old were you when you **first used vaping device** (an e-cigarette) or **tobacco smoking** even once or twice? *

_____

39. Do you start smoking because of... *

Tick all that apply.

- [ ] Friends
- [ ] Family or relatives
- [ ] Peers
- [ ] Advertisement
- [ ] Other: _____

40. Do you continue smoke because of...? (Others: please state)

Tick all that apply.

- [ ] Mental depression
- [ ] Bad family relations
- [ ] Educational problems
- [ ] Difficulties in relationship
- [ ] Curiosity
- [ ] Other: _____

Smoking perceptions

Do you think that...

41. E-cigarettes feel healthier than smoking *

Mark only one oval.

- Yes
- No

42. E-cigarette use is as satisfying as tobacco smoking *

Mark only one oval.

- Yes
- No

43. I get definite nicotine hit from the e-cigarette *

Mark only one oval.

- Yes
- No

44. Tobacco/e-cigarette brings mental tranquility (relax) *

Mark only one oval.

- Yes
- No

45. Smoking helps smokers to stay slim

Mark only one oval.

- Yes
- No

46. I have an urge for a cigarette/e-cigarette *

Mark only one oval.

Strongly Disagree

1 ◯

2 ◯

3 ◯

4 ◯

5 ◯

Strongly Agree

47. Alcohol consumption *

Mark only one oval.

◯ Yes

◯ No

48. Do you attempt to quit smoking? *

Mark only one oval.

◯ Yes

◯ No

49. What are the most RELEVENT barriers that stop you to quit? *

Mark only one oval.

- Lack of self-control
- Nicotine withdrawal
- Peer pressure
- High stress level

Thank you for your participation

Smoker's knowledge, perceptions and factors on smoking (Non-smokers)

50. How much do you know about smoking ?

Mark only one oval.

Poor	
1	
2	
3	
4	
5	
Excellent	

51. Do you know about the health effects of smoking ? *

Mark only one oval.

- ◯ Yes
- ◯ No

52. Student should not smoke *

Mark only one oval.

- ◯ Agree
- ◯ Disagree

53. Tobacco smoke is harmful *

Mark only one oval.

- ◯ Yes
- ◯ No