Differences in Haemoglobin and Erythrocytes Levels in Smokers and Non-smokers

Kumboyono Kumboyono(✉), Kadek Meindra Abdi Anggara, Risa Agustina, Mifetika Lukitasari, and Titin Andri Wihastuti

School of Nursing, Faculty of Health Sciences, Universitas Brawijaya, Malang, Indonesia
abu_hilmi.fk@ub.ac.id

Abstract. Smoking is the cause of various health problems. The prevalence of smokers continues to increase due to the content of addictive substances in cigarettes, such as tobacco, tar, and nicotine. This addictive substance in nicotine causes adverse effects on various organ systems. Several studies have stated a link between blood components and smokers, as evidenced by the relationship between nicotine levels and blood components. This study aims to determine the relationship between nicotine and its metabolite levels and smokers' Haemoglobin and erythrocytes levels. The study used an analytic observational design, and the approach used was cross-sectional. Samples were taken using the total sampling technique and considering the research criteria. The research sample was 57 people, consisting of 34 smokers and 23 non-smokers in Malang City. The level of nicotine and its metabolites is a significant variable; the dependent variable is the level of Haemoglobin and erythrocytes. Demographic data collection using a google form questionnaire. Variable data on nicotine and its metabolites were measured using the Human Cotinine ELISA Kit instrument, and varying levels of Haemoglobin and erythrocytes using the Cobas Micros tool. Pearson Correlation test was used on the results of data analysis. The correlation between cotinine and Haemoglobin (p-value) is 0.981, and cotinine and erythrocytes (p-value) are 0.313. Each has a p-value > 0.05; therefore, it is concluded that smokers have no association between nicotine levels and its metabolites with Haemoglobin and erythrocytes levels. Based on the results of this study, people better understand the dangers of smoking and prevent smoking behaviour.

Keywords: Smokers · nicotine levels · haemoglobin levels · erythrocytes levels

1 Introduction

Smoking is risky behaviour that can cause harm to both the smoker and the environment. Some diseases caused by smoking include increased incidence of cancer, cardiovascular, respiratory, pregnancy complications, and fetus abnormalities [1]. The World Health Organization stated that 7.2 million deaths from exposure to second-hand smoke are caused by tobacco and will increase in the following year [2]. The negative impact of smoking on psychosocial as a gateway to drug abuse and free sex [3, 4]. Economic losses in the form of substantial funds that the government must provide to overcome

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smoking-related diseases amounted to 157 billion US dollars per year from 1995 to 1999 in the United States [5]. Meanwhile, smokers in Indonesia use waste funds of more than 100 trillion rupiahs per year to buy cigarettes that can endanger themselves and others [6].

The 2018 Basic Health Research stated that the number of smokers in the population aged 10–18 years was 9.1%, an increase from 2013 to 7.2% [7]. In Malang City, there are three age ranges, with the first most significant percentage being aged 15–24 years, as much as 29.2%, then aged 25–34 years, as much as 36.9%, and age 35–44 years as much as 35.1% [6, 7]. Smokers in Indonesia have characteristics, especially among early adolescents, due to various risk factors related to public health efforts and community responses to overcoming smoking initiation in early adolescents [8]. Smoking prevalence is increasing due to the content of addictive substances in cigarettes, such as tobacco, tar, and nicotine.

Nicotine found in tobacco which is the raw material for making cigarettes. Smoking behaviour is related to the cause of health problems related to nicotine dependence on smokers. Another study stated that the addictive effect of nicotine is also influenced by different brain systems, such as cholinergic, serotonergic, GABAergic, and noradrenergic [9]. Smokers affected by the addictive substance of nicotine cause adverse effects on various organ systems. Chronic effects of nicotine consumption in adults are changes in cognitive function that will decrease with long-term use of tobacco products [10]. Another impact is that nicotine increases bradykinin vascular metabolism in smokers' blood vessels and the vascular renin-angiotensin system, thereby triggering endothelial dysfunction [11, 12]. Many organs and other organ systems are still affected by nicotine consumption, ranging from neurology to haematology.

Harmful effects on health will arise from smoking habits. Various organ systems will experience health problems due to smoking behaviour. Diseases such as COPD and lung cancer of the respiratory system arise from smoking [13]. In the cardiovascular system, smoking could cause the risk of coronary heart disease and atherosclerosis to stroke [14, 15]. In adolescents, smoking becomes a severe problem when they start smoking in their teens, which will cause nicotine addiction and be difficult to quit [4]. Smoking behaviour hurts blood vessels in the form of inflammation with endothelial activation that forms a pattern of pathological adaptation [16]. Another influence on endothelial cells in smokers, namely smoking behaviour, causes endothelial progenitor cells to age by showing dysfunction in the EPC, which results in a decrease in angiogenic cell function impact on the haematological system [17]. Several studies have stated a link between blood components and smokers, as evidenced by the relationship between nicotine levels and blood components. Research conducted in 2019 on the relationship between various types of cigarettes with increased Ig-E, oxidative stress, and Haemoglobin concentration found an increase in Haemoglobin concentration. Shisha smokers experience an increase in Haemoglobin more than active and passive kretek smokers [18]. In a study conducted in 2017 on the effect of smoking on haematological parameters, it was found that smoking behaviour continuously had an increasing impact on the haematological component. Blood components that have increased are Haemoglobin, white blood cell count, mean corpuscular volume, mean corpuscular Haemoglobin concentration, red blood cell count, and haematocrit. This increase also indicated a risk factor for

the development of atherosclerosis, polycythaemia vera, chronic obstructive pulmonary disease, and cardiovascular disease [19].

Previous studies showed a correlation between nicotine levels and haematological components, but with different age ranges and various characteristics. An initial investigation showed the characteristics of smokers in Malang City at a productive age with a background of consuming cigarettes to help their work. Therefore, with different features, age ranges, and samples, researchers are interested in examining the relationship between nicotine levels and their metabolites with Haemoglobin and erythrocytes levels in smokers in Malang City.

2 Methods

The study used a cross-sectional design and purposive sampling at Saiful Anwar General Hospital from September to November 2021. The smokers and non-smokers as participants in this study have to meet inclusion criteria: age over 18 years, working at the Faculty of Medicine, Universitas Brawijaya, and Saiful Anwar General Hospital is willing to become research participants by filling out a google form questionnaire.

The sample of this study amounted to 57 people, with details of 34 smokers and 23 non-smokers. Variable data of nicotine and its metabolites were measured using the Human Cotinine ELISA Kit instrument, and varying levels of Haemoglobin and erythrocytes using the Cobas Micros Tool. Pearson Correlation test on SPSS version 25 for Windows was used to analyse the data.

3 Results and Discussion

3.1 Participants Characteristics

Based on the Table 1, it can be concluded that the average age of smokers who participated in this study was 29 years old, while the average age of non-smokers was 45 years. All participants who were smokers were male and preferred mild cigarettes over kretek and electric cigarettes. In the smoking group, several participants had diseases such as hypertension, bronchitis, heart disease, and so on, while all non-smoker participants had no history of chronic disease.

Table 2 shows that the average cotinine, haemoglobin, and erythrocyte levels in the smoker's group are higher than in non-smokers.

3.2 The Association Between Nicotine Levels and Cotinine with Haemoglobin and Erythrocytes Levels in Smokers

Table 3 shows that data on cotinine levels, Haemoglobin levels, and erythrocyte levels in both the smoker and non-smoker groups have a p-value < 0.05, so it can be concluded that all variables are significantly different.

The Table 4 shows the results of the SPSS calculation obtained a significance value or p-value > 0.05, so it can be concluded that there is no significant relationship between cotinine levels with Haemoglobin levels and erythrocyte levels.

Table 1. Demographic Characteristics of the Participants

Demographic Characteristics			**f**	**%**	**mean**	**Std. dev.**
Age	Smokers	34 years			29.06	7.06
	Non-smokers	12 years			45.17	7.48
Sex	Smokers	Male	34	100		
	Non-smokers	Male	11	47.8		
		Female	12	52.2		
Smoking Duration (years)		≤5 years	14	41.2		
		6–10 years	12	35.3		
		11–15 years	3	8.8		
		16–20 years	4	11.8		
Type of Cigarettes		Mild	21	61.8		
		Kretek	9	26.5		
		E-Cigarettes	4	11.8		
Sum of Mild Cigarettes Consumption		1–6 cigarettes/day	7	33.3		
		7–12 cigarettes/day	12	57.1		
		13–18 cigarettes/day	1	4.8		
		19–24 cigarettes/day	1	4.8		
Sum of Kretek Cigarettes Consumption		2–6 cigarettes/day	3	33.3		
		7–11 cigarettes/day	2	22.2		
		12–16 cigarettes/day	3	33.3		
		27–30 cigarettes/day	1	11.1		
Sum of E-cigarettes Consumption		3 mg/day	3	75		
		12 mg/day	1	25		
Previous medical history	Smokers	Healthy	25	73.5		
		Hypertension	1	2.9		
		Bronchitis	2	5.9		
		Asthma	3	8.8		
		Jantung	1	2.9		
		Gastritis	1	2.9		
		Dyslipidemia	1	2.9		
	Non-smokers	Healthy	23	100		

Table 2. Comparison of Cotinine, Haemoglobin, and Erythrocytes in Smokers and Non-smokers

Variables		n	Mean	Std. Deviation
Cotinine (pg/mL)	Smokers	34	12.01	3.03
	Non-smokers	23	9.09	4.22
Haemoglobin (g/dL)	Smokers	34	16.09	1.08
	Non-smokers	23	13.83	1.53
Erythrocytes ($10^6/\mu L$)	Smokers	34	5.46	0.45
	Non-smokers	23	4.88	0.51

Table 3. Differences Cotinine, Haemoglobin and Erythrocytes Levels among Smokers and Non-smokers

Variable	Smokers	Non-smokers	*p-Value*	Interpretation
Cotinine (pg/mL)	12.01 ± 3,03	9.09 ± 4,21	0.004	Significant
Haemoglobin (g/dL)	16.10 ± 1,08	13.83 ± 1,53	<0.001	Significant
Erythrocyte ($10^6/\mu L$)	5.46 ± 0,45	4.88 ± 0,51	<0.001	Significant

Table 4. Correlation of Cotinine with Haemoglobin and Erythrocytes in Smokers

Variables		*p-Value*	*R*	Interpretation
Cotinine (pg/dL)	Haemoglobin (g/dl)	0.98	0.004	No Correlation
	Erytrosit (10^6/µl)	0.31	0.178	No Correlation

3.3 Identification of Differences in Nicotine Levels and Cotinine in Smokers and Non-smokers

The results of this study are levels of nicotine and its metabolite, called cotinine, found in both smokers and non-smokers. Nicotine and cotinine in smokers come from exposure to cigarette smoke that is consumed directly. Nicotine levels in a person's body are influenced by several things, including the number of cigarettes, the type of cigarette consumed, and the duration of smoking. This research is in line with a study conducted by Shinta & Marisa in 2021, which suggested that nicotine was found in active and passive smokers and nicotine levels would increase if the duration of smoking consumption and the number of cigarettes consumed remained constant in the following year [20].

Cotinine levels in the group of smokers were higher than in non-smokers. This finding strengthens the research of Mahrous, which compared cotinine levels in two groups: smokers and non-smokers. Active smokers have higher cotinine levels than non-smokers [21]. Both of these studies used the same method but with a slightly different version of the instrument to measure cotinine levels. Research conducted by Mahrous

used the Human Cotinine ELISA Kit (Cat. No. BYEK1111), while this study used the Human Nicotine ELISA Kit (Cat. No. E2043Hu).

3.4 Identification of Differences in Haemoglobin Levels in Smokers and Non-smokers

In this study, the results of Haemoglobin levels had different values in smokers and non-smokers. Haemoglobin levels are higher in smokers than non-smokers. This finding is in line with the research by Malenica, which concluded that smokers have much greater Haemoglobin levels than non-smokers [19]. The study also noted that male smokers have higher Haemoglobin levels than female smokers.

Harmful cigarette substances, including nicotine and cotinine, affect differences in Haemoglobin levels in both active and passive smokers. Previous research conducted by Elisia showed that smokers have higher Haemoglobin levels than non-smokers [15]. The study by Makawekes also found significant differences in Haemoglobin levels between smokers and non-smokers [22].

The increase in Haemoglobin levels in smokers is caused by inhaled carbon monoxide and interferes with the partial pressure of oxygen. Carbon monoxide will also affect the binding of oxygen to Haemoglobin because it has a stronger affinity for binding to Haemoglobin than oxygen. When it binds, it causes a low partial pressure of oxygen, triggering hematopoiesis. Then in the acute period, a compensatory mechanism will occur, which causes an increase in Haemoglobin levels [22].

Haemoglobin levels in the body are not only influenced by smoking behaviour. Abnormal Haemoglobin levels are also affected by living in the highlands, consumption of drugs, dehydration, and history of illness [15, 22]. Thus, to obtain more specific results, it is necessary to add research on other factors that can affect Haemoglobin levels. This study has recorded these factors but has not been used as a factor influencing the study's results.

3.5 Identification of Differences in Erythrocytes Levels in Smokers and Non-smokers

This study found that the levels of erythrocytes in smokers and non-smokers have different values. Erythrocyte levels were found to be higher in smokers than in non-smokers. This finding is in line with the research of Alkhedaide, which stated that the levels of erythrocytes were higher in tobacco smokers/tobacco smokers compared to the standard/non-smokers group [23].

The difference in the erythrocyte levels of smokers and non-smokers is influenced by the consumption of cigarettes and the substances in them; cigarettes make the formation of carboxyHaemoglobin and then increased secretion of erythropoietin thereby increasing erythropoiesis which leads to the increasing number of Haemoglobin and erythrocyte [19]. Other research showed an increase in several erythrocyte indices based on the theory that this increase occurs due to carbon dioxide in cigarettes that enters the body and then binds to oxygen, causing tissue hypoxemia which stimulates the release of the hormone erythropoietin resulting in erythropoiesis. Secretion increases the level of erythrocytes in the body [24].

3.6 Association Between Nicotine Levels and Its Metabolites with Haemoglobin and Erythrocytes Levels in Smokers

This study showed no significant relationship between nicotine and its metabolite levels and Haemoglobin and erythrocyte levels in smokers. Not many studies discuss nicotine and its correlation with Haemoglobin or erythrocyte levels, but several studies examine the correlation of smoking behaviour with Haemoglobin and erythrocyte levels. Research by Elisia et al. states that smoking correlates with Haemoglobin levels and smokers have higher Haemoglobin levels than non-smokers [15]. Alkheidaide found a relationship between smoking and erythrocyte levels, where smokers have higher levels than normal individuals [23].

However, several studies are in line with the results of this study which state that there is no relationship between smoking and Haemoglobin and erythrocyte levels in smokers. Among them is Wibowo's study, which said there was no relationship between smoking and Haemoglobin levels [25]. Several factors underlie the differences in the results of research on Haemoglobin levels in smokers. These factors determine the level of Haemoglobin in the body, such as lifestyle, drug consumption, nutritional intake, medical history, place of residence, and duration of smoking. All these things need to be studied further so that the results are more particular about the relationship between smoking behaviour and Haemoglobin levels in smokers.

Pedersen et al., in their research, concluded that there was no significant difference between erythrocyte levels in smokers and non-smokers [26]. This may be caused by other factors that affect the levels of erythrocytes in the smoker's body—decreased erythrocyte survival due to increased haemolysis in smokers or a history of other diseases that can affect erythrocyte levels and their relationship to smoking. Therefore, it is necessary to carry out further research that pays attention to the selected research subjects and looks at the history of the disease so that later the results you want to get are appropriate.

4 Conclusion

There is no significant relationship between nicotine and its metabolite (cotinine) levels with Haemoglobin and erythrocyte levels. Still, there are substantial differences where smokers' cotinine levels, Haemoglobin levels, and erythrocyte levels tend to be higher than non-smokers.

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