Comparison of Co Levels in The Blood of Students of Electric Smokers and Conventional Smokers at Poltekkes Surabaya

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Abstract. Smoking has been a part of human life for a long time, including teenagers. Cigarette smoke produced from burning tobacco contains a toxic gas, namely carbon monoxide (CO), which can cause dangerous diseases to the body. The danger posed makes innovations continue to emerge to seek other alternatives to conventional cigarettes, namely electric cigarettes. E-cigarettes are devices designed to heat liquids using battery power to create aerosols which are then inhaled by the user. The research aimed to find out the comparison of carbon monoxide (CO) levels in the blood of student electric smokers and conventional smokers at the Poltekkes Kemenkes Surabaya. The research design used is quantitative research, with comparative methods. The method for examining CO levels in the blood uses Conway diffusion with UV-Vis Spectrophotometry. The study used the analysis technique of the two-difference test on the average of the independent sample T-test. Total of 30 people were the subjects of this study consisting of 14 electric smokers and 16 conventional smokers, samples were taken based on purposive sampling technique with certain required criteria. Blood COHb levels examined from all samples had results that were still within normal limits, less than 3.5%. The independent sample T-test analysis showed that there was no significant difference between blood levels of carbon monoxide (CO) in electric smokers and conventional smokers.

Keywords: Carbon monoxide, E-cigarettes, Conventional cigarettes, Conway diffusion.

1 Introduction

Smoking has been a part of human life for a long time. Most of the Indonesian people also have smoking habits. The increase in smoking habits in Indonesia continues to occur over the years, even though the dangers of smoking are well-known to many people and can cause health problems. The results of a survey in 2021 conducted by the Ministry of Health (GATS-Global Adult Tobacco Survey), stated that in Indonesia, the total number of smokers of the entire adult population reached 33.5%, with the highest number of smokers by men at 64.7%, while the number of female smokers is 2.3% [1].

Cigarettes are rolled tobacco wrapped in paper, usually 8-10 cm in size. Tobacco in cigarettes contains hidden poisons and works slowly, but can be dangerous. A cigarette

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contains more than 4000 types of chemical compounds, 400 harmful substances, and 43 other substances that can cause cancer (carcinogenic) [2]. The burning of tobacco contained in cigarettes produces smoke that contains a poisonous gas, namely carbon monoxide (CO). Called the "silent killer", carbon monoxide is a substance that has no color, no smell, and no taste [3]. Carbon monoxide can cause hypoxic toxicity when inhaled because carbon monoxide (CO) in the blood can bind oxygen up to 200 times to hemoglobin. so that it can reduce the ability to transport oxygen in cells [4] [5]. In addition, carbon monoxide (CO) can also cause various dangerous diseases such as heart disease and stroke (cardiovascular), and various cancers, and can cause fetal death [5] [6]. Besides endangering the smoker himself, smoking also harms the people around him who also inhale the smoke [7]. WHO (World Health Organization) states that about 8 million more people die each year due to smoking. As many as 7 million people more than these deaths are caused by direct use of cigarettes, while around 1.2 million people die as a result of non-smokers who inhale too much cigarette smoke [8].

The dangers caused by conventional cigarettes do not deter smokers deterred and stop consuming cigarettes. Breakthroughs keep popping up to find other alternatives to conventional cigarettes [9]. One of the new types of cigarettes being pursued is the electric cigarette. An electric cigarette is a device designed to heat liquid (liquid) using battery power to create an aerosol which is then inhaled by the user [10]. Unlike conventional cigarettes which produce smoke due to combustion, electric cigarettes produce moisture due to heating, thus giving a sensation like smoking in general. Research shows that the carbon monoxide (CO) emissions contained in the vapor produced by electric cigarettes are lower than those contained in conventional cigarette smoke [11]. Public Health England also informs that electric cigarettes are around 95% less harmful than conventional cigarettes [12]. But even though it is considered harmless, the liquid heated in an electric cigarette consists mostly of organic compounds, especially propylene glycol, vegetable glycerin, and carcinogenic substances. When heated, the chemicals in electric cigarettes have the potential to be oxidized to carbon monoxide (CO) and other harmful substances [13] [14]. Many people who smoke also use both, which causes the possibility of carbon monoxide levels in the blood being higher than those who use only one type of cigarette [15]. Based on the description above, electric cigarettes have the same risk of side effects as dangerous as conventional cigarettes because they both contain carbon monoxide (CO), therefore this will be investigated to compare CO (carbon monoxide) levels in the blood of e-cigarette users and conventional cigarettes.

2 Research Methods

The research design used is quantitative research, with comparative methods. Quantitative research is the process of collecting and analyzing numerical data. This can be used to find the average as in this study, while the comparative method is research with the aim of finding out the differences between a variable in two different groups. The population in this study were students of the Poltekkes Surabaya who smoked using electric cigarettes or conventional cigarettes, with a total sample of 30. The sample was

taken using a purposive sampling technique, with the criteria of male students aged 18 to 22 years, smoking only using electric cigarettes and smoking only using conventional cigarettes for at least 1 year. CO levels were measured using the Conway diffusion cell method, with a UV-Vis spectrophotometer. The data analysis technique uses the data normality test with the Shapiro-Wilk test. If the data is normally distributed, it will be followed by a two-free sample T-test (independent sample T-test). The code of ethics for this research is No. EA/1549/KEPK-Poltekkes_Sby/V/2023, published by the Health Research Ethics Committee of the Ministry of Health Surabaya Polytechnic on April 10, 2023.

3 Procedure

The tools and materials that will be used are a micropipette (10 µL – 1000 µL), measuring flask, Conway cup, and UV-Vis spectrophotometer, whole blood blood samples, 70% alcohol, 5% KI, 0.005 N $PdCl_2$, 5N H_2SO_4.

CO levels were measured using the Conway diffusion method with a UV Vis spectrophotometer, which offers sufficient precision and accuracy for measuring the percentage of carboxyhemoglobin (%COHb) in blood [16]. Before measuring CO levels on a spectrophotometer, the first thing to do is determine the maximum wavelength. The maximum wavelength is determined so that the absorbance of the sample is at the maximum wavelength, then maximum results will be obtained. Put 10 mL of distilled water into a 25 mL volumetric flask, add 1 mL of a 5% KI solution, add 0.20 mL of 0.005 N $PdCI_2$, then add distilled water up to the mark, and read the absorbance at a wavelength of 350-550 nm. Next, the operating time is determined which aims to determine the perfect time and stability of the reaction as evidenced by the highest absorbance. Put 10 mL of distilled water into a 25 mL measuring flask, add 0.25 mL of blood, add 1 mL of a 5% KI, add 0.20 mL of 0.005 N $PdCl_2$, then add distilled water up to the mark. Solution testing (read absorbance) was carried out from 60 to 120 minutes at the maximum wavelength. After the wavelength and operating time are determined, the next step is to create a standard curve. A standard curve is created to find the linear regression equation, the results obtained can be used to calculate the levels whose absorbance has been measured. Making a standard curve is done by prepare 6 volumetric flasks of 25 mL. Fill each volumetric flask with 10 mL of distilled water and 1 mL of KI. Into volumetric flasks 1-6 each added a 0.005 N $PdCl_2$ solution as follows: 1 = 0.35 mL; 2 = 0.40 mL; 3 = 0.45 mL; 4 = 0.50 mL; 5 = 0.55 mL; 6 = 0.60 mL. Add distilled water to the limit, and measure the absorbance of the solution at the maximum wavelength. In this research, the equation $y = 0.7004x + 0.0178$ was obtained with a regression value of 0.991. A good regression value is higher or equal to 0.99, because it is almost close to 1. The regression value obtained is considered good because it is more than 0.99.

Measurements of CO levels in the blood were carried out using a Conway cup. Fill the Conway cup with the solution A = 1.0 mL of $PdCl_2$ solution; B = 0.2 mL H_2SO_4 5N; C = 1.5 mL distilled water. Add 0.25 mL of blood in section C which contains distilled water. The Conway cup was closed and left for the operating time. After the

operating time is complete, pipette 0.25 $PdCl_2$ solution, the tip of the pipette must touch the bottom so that the thin layer of Pd metal is not sucked in. Pour the contents of the pipette into a 25 mL volumetric flask which has previously been filled with 10 mL of distilled water and 1 mL of 5% KI. Add distilled water up to the mark, then homogenize. Measure the absorbance of the solution using a UV-Vis spectrophotometer at the maximum wavelength. Using distilled water as an absorbent blank (absorbent = 0). Calculation of COHb levels was carried out using the linear regression equation formula obtained from making a standard curve.

4 Result

4.1 CO Levels in Blood of Electric Smokers

Table 1 is test results of CO levels in blood from electric smokers. From table 1, it can be seen that from 14 students who smoked electric, the highest blood COHb level was 1.42% and the lowest blood COHb level was 0.26%, with an average of 0.68%. The normal value for blood Co levels is less than 3.5%, the test results show that blood CO levels in electric smokers are normal.

Table 1. Blood CO Levels of Electric Smokers.

No.	Sample Code	CO Levels (%)
1.	SAE01	0,57
2.	SAE02	0,99
3.	SAE03	0,33
4.	SAE04	1,42
5.	SAE05	0,31
6.	SAE06	0,47
7.	SAE07	1,17
8.	SAE08	1,02
9.	SAE09	0,26
10.	SAE10	0,66
11.	SAE11	0,39
12.	SAE12	0,35
13.	SAE13	0,69
14.	SAE14	1,02
Average		0,6893

4.2 CO Levels in Blood of Conventional Smokers

Table 2 is test results of CO levels in blood from conventional smokers. Table 2 shows that of the 16 conventional smoking students the highest blood CO level was 1.38% and the lowest blood CO level was 0.17%, with an average of 0.78%. From the test results, blood CO levels of student conventional smokers is normal, less than 3.5%.

Table 2. Blood CO Levels of Conventional Smokers.

No.	Sample Code	CO Levels (%)
1.	SAK01	1,04
2.	SAK02	0,17
3.	SAK03	0,24
4.	SAK04	1,38
5.	SAK05	0,51
6.	SAK06	0,41
7.	SAK07	0,49
8.	SAK08	0,85
9.	SAK09	1,08
10.	SAK10	0,41
11.	SAK11	1,38
12.	SAK12	0,74
13.	SAK13	1,35
14.	SAK14	0,68
15.	SAK15	1,00
16.	SAK16	0,85
Average		0,7862

4.3 Data Analysis

Table 3 is the result of the data normality test and the independent sample T-test statistical test. Data normality test in this research used Shapiro Wilk, with the results in accordance with the table. Based on the table, it is known that the data is normally distributed because it has a significant value of >0.05, so it can be continued with parametric statistical tests. Statistical analysis uses independent sample t-test which aims to compare two sample means from unrelated groups [17]. The significant value is 0.498 ($p = 0.498 > 0.05$), which indicates that there is no significant difference between students who smoke electric cigarettes and conventional smokers.

Table 3. Shapiro Wilk normality test and Independent Sample t-Test test results.

Type of Cigarette	Sig.
E-cigarette	0.143
Conventional cigarette	0.237
	Sig.
CO Levels	0.498

5 Discussion

Examination of blood COHb levels was carried out using the Conway diffusion cell method using a UV-Vis spectrophotometer. The principle of this method is that the carbon monoxide present in the hemoglobin molecule is released by sulfuric acid. The carbon monoxide then diffuses with the $PdCl_2$ solution so that it can reduce Pd^{2+} ions to Pd metal, which appears as a palladium mirror on the surface of the PdCl2 solution [18]. Before taking a reading on the device, three reagents were added to each part of the Conway cup. This study used a modified Conway cup made of two Petri dishes of different sizes due to the limitations of existing tools, so the pipetting process was done carefully to avoid the risk of mixing the solutions. Then incubation was carried out during the operating time on the Conway cup, which was 60 minutes with the aim that the CO molecule diffused in the $PdCl_2$ solution. Several blood samples in this study were not analyzed immediately after the blood collection process but were stored in a refrigerator with a temperature of ±2-4°C for 10-12 hours. One study stated that the concentration of COHb remained stable in blood samples stored in the refrigerator for up to 3 years, so it did not affect the analysis to be carried out [19] [20]. Low COHb levels are indicated by the absence of a silver mirror in the center of the Conway dish, or no visible color change (clear yellow-gold color from $PdCl_2$ solution reagent). The intensity of the silver mirror is proportional to the concentration of carbon monoxide in the blood. After incubation, the $PdCl_2$ solution reacted with 5% KI solution and was read for its absorbance using a UV-Vis spectrophotometer at the maximum wavelength. This research was carried out in duplicate measurements, from all the samples worked no palladium mirrors were formed on the surface of the well of the dish. This can be used as a reference, that if the sample has COHb levels more than normal a palladium mirror will form. Conversely, samples with normal COHb levels will not form a silver mirror on the surface of the Conway dish. So it can be concluded that from 30 blood samples of electric smokers and conventional smokers examined in this study, COHb levels were within normal limits.

The results of COHb levels were tested with the T-test of two independent samples (independent sample T-test) which aims to determine whether there is a difference between the two types of smoker samples taken [17]. The results obtained from the T-test of two independent samples were that there was no significant difference in blood carbon monoxide levels between student electric smokers and conventional smokers. If the results of the two types of smokers are compared on average, conventional smokers have an average COHb level of 0.78%, higher than electric smokers who have an average COHb level of 0.68%. However, these two results have no difference. large, so it is considered that there is no difference in COHb levels between the two types of cigarettes.

Carbon monoxide (CO) in conventional cigarettes is produced from the burning tobacco smoke contained in cigarettes. Meanwhile, carbon monoxide in electric cigarettes

can be formed through the thermal decomposition of e-liquid base ingredients (propylene glycol and vegetable glycerin) and several flavoring compounds [21]. Carbon monoxide (CO) emissions from e-cigarettes also differ depending on the device and flavor used [22]. A study also states that flavored e-liquids produce CO_2 up to 5 to 7 times higher than unflavored e-liquids [11] [23]. Although electric cigarettes are considered to have lower CO (carbon monoxide) emissions than conventional cigarettes, they do not reduce the dangers that can be caused by electric cigarettes [11] [24]. This is also supported by a statement that electric cigarettes still leave CO gas residue in rooms that have been used for smoking [25]. Daily carbonyl exposure from e-cigarette use can still exceed acute exposure limits, with upper coil devices potentially being more harmful than conventional cigarettes [11].

Another factor that can influence COHb levels is age. Someone older will more easily experience a decrease in cardiovascular capacity and reduce the elastic function of lung tissue, which causes a person to become more susceptible to CO exposure so that CO levels in the blood will be higher [25]. Low cigarette consumption can also affect carbon monoxide (CO) levels in the blood because not too much cigarette smoke is inhaled [26]. Several other factors can also be caused by the social environment between electric smokers students and conventional smokers students. Many students still use conventional cigarettes instead of electric cigarettes, due to the low levels of nicotine intake in e-cigarettes [27]. Student electric smokers still often inhale smoke from conventional cigarettes originating from friends and the environment where they live. Likewise with students who smoke conventionally, also often consume e-cigarettes. Thus, e-cigarettes and conventional cigarettes have the same effect on blood carbon monoxide (CO) levels.

6 Conclusion

From this research it can be concluded that the level of carbon monoxide (CO) in the blood of electric smokers and conventional smokers students at the Poltekkes Surabaya is within normal limits with an average of less than 3.5%. There is no difference in blood levels of carbon monoxide (CO) between electric smokers students and conventional smokers students at the Poltekkes Kemenkes Surabaya. For future researchers, they can carry out a similar examination by developing or adding other examination parameters or different methods. It is better for the community and students to reduce smoking habits to avoid the long-term effects arising from exposure to cigarette smoke, by maintaining a healthy lifestyle.

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