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# Correlation Smoking Habits To Carboxyhemoglobin (COHb) Levels Using UV-Vis Spectrophotometry Method

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**Abstract**
Smoking is one of the lifestyles of the Indonesian people which is dangerous to health because the content of cigarettes that are smoked consists of toxic chemicals, especially carbon monoxide (CO). Smoking habits are the main source of carbon monoxide (CO) exposure. Inhaled CO will bind to hemoglobin in the blood causing the blood's ability to transport oxygen to body tissues to decrease. In addition, an increase in COHb levels in the blood can cause the body's cells to become deprived of oxygen, cause narrowing of the blood vessels, and disrupt blood flow. This study aims to determine whether or not there is a relationship between smoking habit and carboxyhemoglobin (COHb) levels using the UV-Vis spectrophotometry method. This study used 30 respondents in the examination of carboxyhemoglobin (COHb) levels which were divided into 2 groups, namely 15 groups of smokers and 15 groups of non-smokers. This research is an observational-analytic study with a cross-sectional approach. Examination of carboxyhemoglobin (COHb) levels was carried out using a UV-Vis spectrophotometer at a wavelength of 420 nm. The average carboxyhemoglobin (COHb) level of smoking was 5.45% higher than that of non-smokers carboxyhemoglobin (COHb) of 5.30%. Spearmen's non-parametric correlation statistical test shows that the significance value (p-value) is 0.340 > 0.05. It was concluded that there was no relationship between smoking habit and carboxyhemoglobin (COHb) levels using the UV-Vis spectrophotometry method.

**Keywords**: *Carboxyhemoglobin Levels, Smoking Habits, Smoking impact, UV-Vis Spectrophotometer*.

## 1. Introduction

Indonesia is one of the countries with the third largest number of cigarette consumption in the world. Smokers in Indonesia in 2013 were the highest in ASEAN, namely 46.6%. There was an increase in the proportion of people who smoked every day in 2007 by 23.7% while in 2013 it increased by 24.3%.

Argues to be the main source of exposure to carbon monoxide (CO), although a small amount of exposure to CO can also come from vehicle exhaust or industrial fumes. When cigarette smoke is inhaled, carbon monoxide is absorbed through the lungs, enters the bloodstream, and then binds to hemoglobin to form carboxy-hemoglobin (COHb) whose levels in the blood can be measured as a marker of cigarette smoke absorption (Inayatillah et al., 2014 ). Cigarette smoke has chemicals consisting of 4000 chemicals in it and 200 of them are poisons found in CO, Policycyclic Aromatic Hydrocarbons (PAHs), as well as other cancer-triggering particles such as tar, benzopyrenes, vinylchorida, N-nitri-sonor nicotine (Armuni et al. al., 2022). Cigarette smoke containing chemicals enters the lungs and then flows throughout the body through the bloodstream. One liver enzyme binds to chemicals in cigarettes and can cause cancer. In addition, cigarette smoke is also a free radical originating from exogenous sources. Free radicals will damage molecules whose electrons are pulled by these free radicals, causing cell damage, impaired cell function, and even cell death (Fitria et al., 2013).

The dangers of smoking to health are caused by the fact that there are 4000 chemicals contained in each cigarette and some of them are toxic, one of which is CO gas (Hilyah et al., 2021). CO that is inhaled into the body through the respiratory tract will bind to hemoglobin which forms COHb. The presence of COHb causes the blood's ability to transport $O_2$ to body tissues to decrease. As a result, the supply of $O_2$ in the tissues and cells of the body decreases, so the higher the concentration of COHb in the blood, the possibility of health problems will increase (Dewanti, 2018).

Based on previous research by Sari (2015) stated that there is a relationship between smoking habits and COHb levels in patients with lung disease. The results of smokers' COHb levels were higher. That CO can cause a decrease in the percentage of $O_2$ carried by hemoglobin because CO bonds with hemoglobin are stronger (Sari, 2015). Based on this background, researchers wanted to know the "Correlation Smoking Habits to Carboxyhemoglobin (COHb) Levels Using the UV-Vis Spectrophotometry Method". So this research is expected to provide information about the impact of smoking on COHb levels in the body.

## 2. Materials and methods

This research is included in the observational-analytic research with quantitative research methods. Observational-analytic research is research that only makes observations and does not give behavior to the variables to be studied (Notoatmodjo, 2012).

### *2.1 Materials*

The materials to be used in this research are venous blood samples, alcohol swabs, plaster, dry cotton, sodium dithionite solution, 1% ammonium solution, and distilled water.

### *2.2 Data collection procedures*

The instruments to be used in this research were 3 cc syringes, tourniquets, vacutainer tubes (EDTA tubes), measuring pipettes (5 ml, 10 ml), yellow tips, blue tips, test tubes, tube racks, Erlenmeyer flasks, micropipette (10µl, 100 µl), spatula, cuvette, UV-Vis spectrophotometer, hand scoop, mask and cool box ice.

*2.2.1 Venous sampling procedure*

Prepare the tools and materials to be used for blood sampling. Select the vein to be stabbed, especially the median cubital vein. Apply the tourniquet about 3-4 fingers above the elbow crease. Respondents were instructed to clench their fists so that the veins could be seen. Clean the area to be stabbed using an alcohol swab in a circular motion from the inside out of the circle. Then let it dry. Hold the hand below the puncture site. Insert the needle into the selected vein at an angle of 30-45 degrees. Slowly withdraw the 37 suction syringe until it fills the required volume of blood. Remove the tourniquet, then place the dry cotton on the puncture site and slowly withdraw the syringe. Press the dry cotton to stop the blood that is still coming out and cover it with a plaster. Put the blood into the EDTA tube and make it homogeneous. Label the EDTA tube according to the respondent's number and put it in the cool box of ice (Nugraha, 2014).

*2.2.2 Procedure Determination of maximum wavelength (λ)*

Pipette 20 ml of 1% ammonium solution and put it in an Erlenmeyer flask. Then 10 µl of whole blood sample and 20 mg of sodium dithionite were added. Then homogenize it and put it in a cuvette and take absorbance readings at a wavelength (λ) of 400-700 nm. The maximum wavelength is obtained from the highest absorbance with UV-Vis Spectrophotometry at a wavelength of 420 nm (Wimpy & Harningsih, 2019).

*2.2.3 Analysis of Carboxyhemoglobin Levels by UV-Vis Spectrophotometer*

Two test tubes were coded with labels R1 (reagent) and SPL (sample reagent). A total of 20 mL of 0.1% $NH_4OH$ solution is added to Erlenmeyer and 10 L of blood sample is added and then homogenized. The solution in the Erlenmeyer has pipetted 4 mL then each inserted into the R1 and SPL tubes. A total of 20 mg $Na_2S_2O_4$ was added to the tube SPL and homogenized. The mixture was incubated at 37ºC for 8 minutes and measured absorbance R1 and SPL with a Spectrophotometer UV-Vis at a wavelength of 414.2 nm (Ohmori et al., 2019). Calculation of carboxyhemoglobin (COHb) levels using equation 1 (Wimpy & Harningsih, 2019). Equation 1:

$$\text{COHb Levels} = \frac{\Delta A}{\Delta \text{Ar Hb}} \times 6{,}08\%$$

Where A is the absorbance of the reagent, Ar Hb is the absorbance of the standard sample and 6.08 is the conversion factor for percent CO saturation in Hb. Percentage increase in COHb. Levels calculated by equation 2 where the normal value of COHb is 3.5%. Calculation the percentage increase in COHb levels is as follows (Wimpy & Harningsih, 2019) Equation 2:

$$\text{Percentage of increased levels of COHb} = \frac{\text{COHb Levels}}{\text{Normal value COHb}} \times 100\%$$

*2.3 Data analysis*

Data analysis begins with the help of calculations through the SPSS application. The first step of the normality test is to get results <0.05, so the data is not normally distributed. In the second step, the homogeneity test yields <0.05, so the data is not homogeneous and the non-parametric correlation test is continued, namely the Spearman test (Agus, 2011) (Syapitri et al., 2010).

## 3. Results and discussion

*3.1 Research Location*

The research takes place in a coffee shop in the Wonocolo area, Surabaya City. A coffee shop is a place that provides coffee or other hot drinks. This is because in the coffee

shop there are many visitors who have smoking habits so it is necessary to check the levels of carboxyhemoglobin (COHb). Cigarette smoke contributes to exposure to CO gas so that it can interfere with the body's health which is often caused by smoking habits (Muhibah & F.A.B., 2011). This research was also conducted at the Health Chemistry Laboratory, Nahdlatul Ulama of Surabaya University. The Health Chemistry Laboratory provides appropriate tools used for research from pre-analytical, analytical, post-analytical processes.

This study aims to determine the relationship between smoking habits and carboxyhemoglobin (COHb) levels using the uv-vis spectrophotometry method. The sample used was 3 ml of venous blood from smokers and non-smokers. This research uses 30 respondents who will be divided into 2 groups, namely the group of respondents who have smoking habits as many as 15 respondents and the group of respondents who do not have smoking habits as many as 15 respondents. Respondents who are willing to be respondents to this study will fill in the informed consent that will be provided by the research and fill out a questionnaire to find out the characteristics of the respondents who are willing to participate in this study.

### *3.2 Characteristics of Respondents by Age*

Based on table 1 explains that the age characteristics of smoking are obtained in the age range of 20-35 years with as many as 15 respondents with a percentage (100%) and in the age range 36-50 years there are 0 respondents with a percentage (0%) while in those who do not This smoking can be in the age range of 20-35 years as many as 15 respondents with a percentage (100%) and in the age range 36-50 years as many as 0 respondents with a percentage (0%) (Nidianti et al., 2022).

**Table 1.** Distribution of Respondents by Age

| **Age (years)** | **Smoke** | | **Not smoke** | |
|---|---|---|---|---|
| | **Number** | **Percentage** | **Number** | **Percentage** |
| 20 – 35 | 15 | 100% | 15 | 100% |
| 36 – 50 | 0 | 0% | 0 | 0% |
| Amount | 15 | 100% | 15 | 100% |

Age is one of the susceptibility factors that can affect high and low levels of carboxyhemoglobin (COHb) in the blood of respondents. In old age, the ability of organs to carry out metabolism in the body decreases. Therefore, it indicates that the elastic function of the lung tissue also decreases. At an older age, the ability of the lungs will slowly decrease lung function (Seprianto & Sainab, 2015).

### *3.3 Characteristics of Respondents According to Gender*

Based on table 2 explains that in the sex characteristics of smoking, there were 15 male respondents with a percentage (100%) and in women, there were 0 respondents with a percentage (0%) while in non-smokers there were 2 male respondents with a percentage (13%) and in women, there were 13 respondents with a percentage (87%).

**Table 2.** Distribution of Respondents According to Gender

| **Gender** | **Smoke** | | **Not smoke** | |
|---|---|---|---|---|
| | **Number** | **Percentage** | **Number** | **Percentage** |
| Male | 15 | 100% | 2 | 13% |
| Female | 0 | 0% | 13 | 87% |
| Amount | 15 | 100% | 15 | 100% |

That the smoking respondents were mostly male sex compared to female respondents (Umami et al., 2018).

*3.4 Characteristics of Respondents According to smoking habits*

Table 3 explains that for the characteristics of smoking habits per day, namely 1-10 cigarettes, there are 11 respondents with a percentage (37%). For the number of cigarettes per day, namely 11 - 20 cigarettes, there were 3 respondents with a percentage (10%) and for the number of cigarettes per day, namely more than 20 cigarettes, there was 1 respondent with a percentage (3%) and non-smokers were obtained as many as 15 respondents with a percentage ( 50%).

**Table 3.** Distribution of Respondents According to Smoking Habits

| Smoking habit | Number | Percentage |
|---|---|---|
| 1-10 cigarettes: Light Smoker | 11 | 37% |
| 11 – 20 cigarettes: Moderate Smoker | 3 | 10% |
| More than 20 cigarettes: Heavy Smoker | 1 | 3% |
| Not have the habit of smoking | 15 | 50% |
| Amount | 30 | 100% |

The number of cigarettes smoked per day will affect the expiratory CO level in smokers if the number of cigarettes is more than 20 cigarettes per day, the CO level will increase by 15-34 ppm, while if the number of cigarettes is more than 40 cigarettes per day, the CO level will increase. of 26-60 ppm (Inayatillah *et al.*, 2014).

*3.5 Characteristics of Respondents According to Duration of Consuming Cigarettes*

Table 4 it is explained that the characteristics of consuming cigarettes for a long time, namely 1 year, were obtained by 7 respondents with a percentage (47%) for a long time-consuming cigarettes for more than 1 year, namely as many as 8 respondents with a percentage (53%) of 15 respondents in the area Surabaya.

**Table 4.** Distribution of Respondents According to Duration of Consuming Cigarettes

| Smoking duration | Number | Percentage |
|---|---|---|
| 1 years | 7 | 47% |
| More than 1 year | 8 | 53% |
| Amount | **15** | **100%** |

The large number of cigarettes smoked per day and the duration of consuming cigarettes is due to the nicotine content. In accordance with the theory which states that nicotine can have addictive effects so that it can increase the number of cigarettes smoked each day (Benowitz, 2010). Cigarettes contain nicotine which can cause smokers to be unable to stop smoking continuously and so it will become a habit. The longer smoking, the longer the exposure to CO from cigarette smoke and the amount of cigarette smoke inhaled becomes more so that CO levels will increase. This is due to the more stable nature of HbCO than $HbO_2$ (Wimpy & Harningsih, 2019).

*3.6 The results of the examination of COHb levels in the blood*

Based on table 5, it is explained the results of the examination of carboxyhemoglobin (COHb) levels. Examination of carboxyhemoglobin (COHb) levels < 3.5% found 1 respondent with normal carboxyhemoglobin (COHb) levels, while examination of carboxyhemoglobin (COHb) levels > 3.5% obtained 29 respondents with abnormal carboxyhemoglobin (COHb) levels. The normal value of carboxyhemoglobin levels itself according to the Ministerial Regulation of the Republic of Indonesia number 70 of 2016 is not more than <3.5% (Rohmah, 2019).

**Table 5.** Distribution of Examination Results for Carboxyhemoglobin (COHb) Levels

| No | Levels COHb Smoke (%) | Information | Levels COHb Not smoke (%) | Information |
|---|---|---|---|---|
| 1. | 5,14% | Abnormal | 2,53% | Normal |
| 2. | 5,50% | Abnormal | 5,12% | Abnormal |
| 3. | 5,47% | Abnormal | 5,33% | Abnormal |
| 4. | 5,00% | Abnormal | 5,47% | Abnormal |
| 5. | 5,10% | Abnormal | 5,47% | Abnormal |
| 6. | 5,27% | Abnormal | 5,29% | Abnormal |
| 7. | 5,49% | Abnormal | 4,58% | Abnormal |
| 8. | 5,31% | Abnormal | 3,79% | Abnormal |
| 9. | 5,41% | Abnormal | 6,01% | Abnormal |
| 10. | 5,12% | Abnormal | 6,01% | Abnormal |
| 11. | 5,45% | Abnormal | 6,02% | Abnormal |
| 12. | 5,31% | Abnormal | 6,08% | Abnormal |
| 13. | 6,12% | Abnormal | 5,56% | Abnormal |
| 14. | 6,03% | Abnormal | 6,06% | Abnormal |
| 15. | 6,04% | Abnormal | 6,15% | Abnormal |
| Average | 5,45% | | 5,30% | |

Explains that the level of carboxyhemoglobin (COHb) in a person who does not smoke is in the range of 1 - 6% while in a person who smokes it ranges from 1 - 14%. This can show that not smoking is not free from the effects of smoke in the air. According to research (Faradilla et al., 2016), the results of carboxyhemoglobin (COHb) levels in smoking respondents were above the normal threshold, while some non-smoker respondents also had carboxyhemoglobin (COHb) levels almost the same as smoking respondents. This is possible because the lifestyle of the respondent, such as the influence of the habit of riding a motorbike, makes it possible that exposure to CO from vehicle exhaust, industrial fumes, combustion fumes, or also consuming vegetables and fruit can affect the levels of carboxyhemoglobin (COHb) in the respondent's blood. This is because the antioxidants in fruits and vegetables function as electron-donating compounds which are useful for preventing free radical chains or inhibiting the formation of new free radicals and repairing damage caused by radicals. The bond between CO and Hb which is 210 times stronger so it cannot be broken by antioxidants when consuming fruits and vegetables does not have a significant impact on reducing carboxyhemoglobin (COHb) levels in the blood.

### *3.7 Data Analysis*

Based on the results of the normality test data with the Shapiro – Wilk test, the p-value for smoking was 0.036 < 0.05, while non-smokers were 0.002 < 0.05. Then these two data means that the distribution is not normal and it is continued with the homogeneity test to find out the p-value. of 0.032 because of the p-value. less than < 0.05. It can be concluded that the two variables of the two data groups namely smoking and non-smoking are not homogeneous.

Based on the spearmen non-parametric correlation test table, the results obtained were a p-value of 0.340 or > 0.05, which means that there is no relationship between smoking habit and carboxyhemoglobin (COHb) levels using the UV-Vis spectrophotometry method.

**Table 6.** Correlation Non Parametrik *Spearmen*

| Correlation | R value (Correlation) | *p-value* | Information |
|---|---|---|---|
| Smoking Habits | 0,265 | 0,340 | No correlation |

Smoking will get the same symptoms of poisoning as not smoking because if the level of carboxyhemoglobin (COHb) has exceeded the level of toxicity, however, a smoker will experience faster elimination than a non-smoker. So this is very dangerous because the smoke released contains chemicals and one of them is CO gas (Wright, 2002). Smoking more than one pack of cigarettes per day has larger red blood cells than non-smokers. Meanwhile, smoking has higher levels of carboxyhemoglobin (COHb) than non-smokers. This is when the level of carbon monoxide in the blood increases, the body's ability to carry oxygen decreases and can cause a decrease in oxygen levels in the blood (Nidianti et al., 2023).

In the elimination of CO that occurs through the lungs, it has a half-life of about 5-6 hours at body temperature and in about 6-8 hours the blood does not contain carboxyhemoglobin (COHb) levels anymore. This inhalation of oxygen accelerates the excretion of CO so that within 30 minutes the carboxyhemoglobin (COHb) level has reduced by half from its original level (Goldstein et al., 2018) (Rodkey et al., 1979).

## 4. Conclusion

The results of carboxyhemoglobin (COHb) levels of smoker respondents in the Surabaya area with an average of 5.45%. The results of carboxyhemoglobin (COHb) levels of respondents who did not smoke in the Surabaya area with an average of 5.30%. So the conclusion is that there is no relationship between smoking habit and carboxyhemoglobin (COHb) levels using the UV-Vis spectrophotometry method.

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## Conflict of interest

No Conflict Interests

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