

## The Effect of Cigarette Smoking Duration on Hemoglobin Level Measured with Cyanmethemoglobin Method

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### Abstract

In Indonesia, cigarette consumption is quite prevalent and has been become more common over time. According to Riskeudas (Baseline Health Research from Agency of Health Research and Development) study in 2018, the national percentage of people aged who smoke increased from 2016-2018, namely from 3.39% in 2016, 3.9% in 2017 and 9.65% in 2018. Smoking habits will negatively affect human health. Cigarette smoke contains various of different chemicals that are harmful to health, including nicotine addiction, tar (carcinogen or cancer-causing), and carbon monoxide. When inhaled, they form carboxyhemoglobin with Hb (Hemoglobin). When this occurs, the amount of Hb available for oxygen transport is reduced. By way of compensation, the body will produce more red blood cells. The objective of the study was to determine the hemoglobin levels in smokers with smoking durations of 3 years, 4 years and 5 years. This study is quasi-experimental. Determine hemoglobin readings using a photometer 5010 with the Cyanmethemoglobin method. Up to 20 samples were analyzed with SPSS version 20 with Anova analysis at 95% confidence level. The results of statistical analysis showed p-value <0.05, which means that there is a significant effect between variables and vice versa. Hb level comparison between durations of smoking are of 3 years with an average value of 14.8, Hb levels of 4 years with an average value of 14.53, and Hb levels of 5 years with an average value 15.94. The results showed that there was an effect of smoking duration on the results of the examination of hemoglobin levels in smokers of 3,4 and 5 years with the greatest effect being smokers with a duration of 5 years. The duration of smoking impact on hemoglobin levels.

### Keywords

Cyanmethemoglobin, Hemoglobin, Photometer, Smoking.



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## INTRODUCTION

Cigarettes are one of the products of tobacco processing, using or without additives. Cigarettes contain additives and their use can pose health risks to individuals and society (1). Cigarettes are one of the tobacco products that are burned, smoked and/or inhaled, including kretek cigarettes, white cigarettes, cigars or other forms produced from the plants *Nicotiana tabacum*, *Nicotiana rustica*, and other types or synthetic products whose smoke contains nicotine and tar with or without additives (2, 9).

Smoking is practiced repeatedly and on a continuous basis. Smoking activity occurs in all environments, children, teens, adults, and the elderly. That is because the transaction process to purchase and sell cigarettes is easy and affordable for youth and students (2). Smoking provides pleasure and fulfillment for smokers. Apart from that, several factors behind someone to smoke include the desire to try the taste (menthol, cappuccino, black tea, etc.), the price is cheap and easy to obtain. It is believed that tobacco use relieves stress; relieves boredom, loneliness and confusion (3).

Smoking habits have become part of people's lifestyle. Smoking is considered the leading preventable cause of death worldwide and around 6 million people die each year from causes related to exposure to secondhand smoke (4). According to the

Risikesdas ((Baseline Health Research from Agency of Health Research and Development) survey (2018) the percentage of smoking in the population aged 18 years at the national level is increased. The survey indicated that from 2016 to 2018, tobacco use increased from 3.39% in 2016, 3.9% in 2017 and 9.65% in 2018 (5). Smoked cigarettes have a negative impact on the human body, especially on the respiratory organs. Various lung diseases that arise due to smoking are lung cancer and chronic obstructive pulmonary disease (1). Cigarettes also greatly affect the hemoglobin in the body. Smoking is also known to have a significant relationship with a person's immune system, because it can weaken the immune system, which affects the innate and adaptive immune systems and plays a dual role in regulating immunity by exacerbating the body's immune response (6).

According to Paskaria et al., (7) study findings from 2018, there was a substantial difference in CO levels between students who smoked (12.21 ppm) and nonsmokers (5.11 ppm). The consensus recommendations of European Respiratory Society (ERS) suggest that the expiratory concentration of CO level in non-smokers is < 4 ppm. Additionally, Waseem and Alvi (8) noted that Hb levels rise as smoking intensity rises.

Smoking can affect blood components such as platelets, hemoglobin, erythrocyte sedimentation rate (21, 14). Hemoglobin is a

protein pigment that makes red blood cells red. Each hemoglobin is made up of a protein called hemoglobin. The Hemoglobin in the blood, which is a protein in red blood cells that contains iron atoms and binds oxygen in the lungs and carries it to the tissues (9). Cigarette smoke contains more than 4000 toxic compounds, including free radicals, nicotine, and carbon monoxide that have a negative impact on human health. Nicotine induces clotting of the coronary arteries, reduces vascular activity, and increases endothelial dysfunction (11, 9). CO (carbon monoxide) gas inhaled during smoking cigarette smoke, concentrations ranging from 400-5000 ppm (parts per million) (10).

Continued exposure to carbon monoxide gas induces the formation of carboxyhemoglobin. It is an inactive form of Hb and does not carry oxygen. Carboxyhemoglobin causes the dissociation curve Hb to move to the left. Thus, reducing Hb's capacity to supply oxygen to tissues (11, 9). The results of the study from Malenica et al., (11) showed that continuous smoking had a severe adverse effect on hematological parameters (e.g., hemoglobin, white blood cell count, mean cell volume, mean corpuscular hemoglobin concentration, red blood cell count, hematocrit). Results from the Qadir (9) study also stated that smoking had an effect on hemoglobin concentration and PCV. Based on this description, the researcher wanted to know whether there was

an effect of smoking duration on blood hemoglobin levels. The objective of this study was to determine the impact of smoking duration on hemoglobin levels in smokers.

## MATERIALS AND METHODS

The type of research was experimental laboratory research with a descriptive approach. The hemoglobin level measured with cyanmethemoglobin method. Five mL of venous blood was collected per patient using a venous puncture technique. Then, 5 mL of the Drabkin's reagent was put into a test tube and 20  $\mu$ L of blood was mixed and then allowed to stand for 5 minutes at room temperature. The absorbance of the solution was then measured in a photometer at a wavelength of 540 nm (12). The absorbance of pure parental Hb standard was also recorded at each run using a 5010 photometer. The population in this study was all students at the University of East Indonesia. A total 20 subjects who were smokers were included in this study. Male chronic smokers with smoking duration of 3,4, and 5 years. The sampling technique used a purposive sampling method. The exclusion criteria for this study were (1) patients with severe hypertension, endocrine disorders, liver disease, heart and respiratory illness; (2) patients taking medication; and (3) patients with habits such as alcohol and tobacco. The results obtained were subsequently analyzed in a descriptive

manner. Further analysis is a two-variable statistic statistical using the ANOVA with a 95% degree of confidence. Therefore, if the p-value <0.05, it means that statistically there is a significant effect between the variables and vice versa.

## RESULTS

Table 1 Results of hemoglobin examination using the cyanmethemoglobin

method in students who smoked with the lowest Hb level of 14.10 g/dL and the highest level of 18.48 g/dL.

The results of the study revealed an effect between 3, 4 and 5 years of smoking duration. On the examination of Hb levels, and 5 years of smokers gave the greatest effect or increase on the results of the examination of hemoglobin levels using the Cyanmethemoglobin method (Table 1).

**Table 1.** Results of Hemoglobin Examination Using Cyanmethemoglobin Method in Students Who Smoke

| Sample Code | Smoking Duration | Number of cigarettes smoked / days | Hemoglobin (gr/dL) |
|-------------|------------------|------------------------------------|--------------------|
| A           | 4 years          | 10                                 | 15.40              |
| B           | 5 years          | 10                                 | 18.34              |
| C           | 5 years          | 12                                 | 15.55              |
| D           | 5 years          | 10                                 | 14.32              |
| E           | 5 years          | 10                                 | 18.48              |
| F           | 3 years          | 8                                  | 14.95              |
| G           | 5 years          | 12                                 | 15.06              |
| H           | 5 years          | 12                                 | 15.22              |
| I           | 4 years          | 8                                  | 15.02              |
| J           | 3 years          | 8                                  | 15.49              |
| K           | 3 years          | 12                                 | 14.40              |
| L           | 3 years          | 11                                 | 14.48              |
| M           | 4 years          | 8                                  | 14.12              |
| N           | 5 years          | 10                                 | 16.45              |
| O           | 4 years          | 10                                 | 14.12              |
| P           | 3 years          | 9                                  | 14.12              |
| Q           | 5 years          | 10                                 | 14.12              |
| R           | 4 years          | 12                                 | 14.45              |
| S           | 4 years          | 8                                  | 14.10              |
| T           | 3 years          | 10                                 | 15.49              |

The results of the study, the duration of smoking with hemoglobin levels, the results of the examination of Hb levels are presented in the Table 2.

**Table 2.** Duration of Smoking with Hb levels

| No    | Duration of Smoking | Total | Mean    |
|-------|---------------------|-------|---------|
| 1     | 3 years             | 6     | 14.8217 |
| 2     | 4 years             | 6     | 14.5350 |
| 3     | 5 years             | 8     | 15.9425 |
| Total |                     | 20    | 15.1840 |

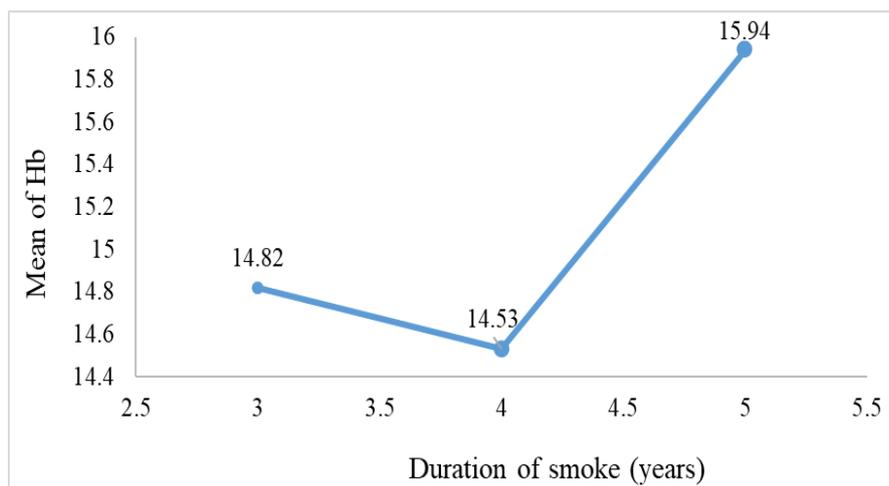
Table 2 shows the findings of a descriptive analysis of smokers, namely 6 smokers with a duration of smoking of 3 years with an average value of 14.8217, Hb levels of 6 smokers who have smoked for 4 years with an average value of 14.5350, and

8 smokers with a duration of smoking of 5 years with an average value 15.942.

**Table 3.** ANOVA Table (One Way)

| Total variation | dK | Sum of Squares | average squared | F     |
|-----------------|----|----------------|-----------------|-------|
| Inter-group     | 2  | 7.917          | 3.959           | 2.917 |
| In group        | 17 | 23.074         | 1.357           |       |
| Total           | 19 | 30.992         |                 |       |

Table 3 shows the results of ANOVA test to determine the effects of smoking on hemoglobin levels with  $dK = 19$  and  $F = 2.917$  and  $F$ . The table obtained is 2.05 ( $F$  count  $>$   $F$  table), indicating that smoking duration has an impact on the outcomes of hemoglobin examination.



**Figure 1.** Result of ANOVA according to Means Plots on Smokers

Figure 1 shows that the means plots from the ANOVA analysis (versus smokers) are not parallel. It may be inferred that there is an interaction of effect between groups. Table 2 displays the results for smokers who have

been smoking for three years or less. The average score for these smokers was 14.8217, for smokers who have been smoking for four years or more, it was of 14.5350, and for smokers who have been smoking for 5-year

as many as 8 people with an average score of 15.1840. Table 3 shows the results of the ANOVA test to determine the effect of smoking duration on hemoglobin levels with  $dK = 19$  and  $F = 2.917$ , and the  $F$  table obtained is 2.05 ( $F_{\text{count}} > F_{\text{table}}$ ) means that there is an effect of smoking duration on the results of hemoglobin testing. In the subsequent test, namely the *Post Hoc* test, the highest effect was seen on smokers who were 5 years old. Figure 1 depicts Means Plots. The influence in each group is depicted in the image of the Smokers Anova Analysis (one way).

## DISCUSSION

In the cyanmethemoglobin method, a certain volume of blood is diluted with a reagent and the hemoglobin concentration is calculated using a precise and well-calibrated photometer after a specific amount of time. Cyanmethemoglobin determination is a reference test method for quantitatively measuring hemoglobin and is used to compare and standardize other methods. Hb is converted into methemoglobin, which is converted into cyanmethemoglobin (HiCN) by potassium cyanide.

The absorbance of the solution is then measured in a spectrophotometer at a wavelength of 540 nm (12, 14). Hb has the main physiological function of transporting oxygen to tissues and plays a role in the carrying of carbon dioxide.

According to the analysis of 20 samples, it was discovered that there was an increase in Hb levels in smokers. This results in an increase in hemoglobin levels is influenced by smoking habits. Furthermore, CO levels in the blood increase which greatly results in the formation of carboxyhemoglobin. The blood's level of hemoglobin will rise as a result of the synthesis of CO. Sulfo-hemoglobin, which is a component of carboxyhemoglobin and can impact high levels of Hb in male smokers, cannot be measured by the cyanmethemoglobin method, which is utilized in this study (7).

In addition, high levels of Hb in the blood in male smokers are influenced by  $\text{CO}_2$  in large quantities entering the body. As a result, it can stimulate the production of the hormone erythropoietin in the kidneys and the synthesis of Hb in the blood can be further increased. This is because the hormone erythropoietin stimulates the coenzyme pyridoxal phosphate (Vitamin B) in the process of Hb synthesis in the bone marrow. Heme synthesis occurs in the mitochondria through a series of biochemical reactions starting with the condensation of glycine and succinic coenzyme Pyridoxal phosphate (Vitamin B6) is the coenzyme for this process, which is limited by the activity of the important Delta-aminolevulinic acid (AL) enzyme, which is increased by erythropoietin and inhibited by hem. Protoporphyrin IX and ferrous iron unite to produce Heme, each of

which molecules is connected to a chain of globin comprised of polyribosomes. The Hb molecule is then held together by the four globin chains, each of which has a separate haem group in a "pocket." Its primary effect are on the sympathetic nervous system and the carbon monoxide-induced desaturation of hemoglobin (Hb).

Cigarettes greatly affect hemoglobin (Hb) in the body. Carbon monoxide (CO) is a colorless, odorless, flammable and highly toxic gas. The effect of Carbon monoxide gas is harmful to the body because the binding capacity of Carbon monoxide gas to hemoglobin is 240 times that of Oxygen (O<sub>2</sub>) to hemoglobin. If carbon monoxide is inhaled by humans, the molecule will enter the respiratory tract and then enter the lungs and then attach to blood hemoglobin to form carboxy hemoglobin (15). Mariani and Kartini (16) reported that there was a significant relationship between the degree of smoking and hemoglobin levels.

The process of synthesis of Hb in the body begins with the process of CO<sub>2</sub> from the tissues to the lungs. To carry out this gas exchange, blood cells contain the special protein hemoglobin. Each red blood cell's main function is to transport O<sub>2</sub> to the tissues and restore it. Red blood cells contain approximately 640 million hemoglobin molecules and each normal adult hemoglobin (HbA) molecule consists of 4 a<sub>2</sub>b<sub>2</sub> polypeptide chains, each with its own Hb

group. The molecular weight of Hb A is 68,000. Normal adult blood also contains small amounts of 2 other hemoglobin and Hb A<sub>2</sub> which also contains a chain but y chain and s chain respectively instead of B 65% of hemoglobin b is synthesized in erythroblasts and 35% of hemoglobin is synthesized in the erythroblast stage reticulosis. Most of the synthesis occurs in the mitochondria by a series of biochemical reactions initiated by the condensation of glycine succinyl coenzyme A under the action of the key AL enzyme. Finally, Protoporphyrin combines with Fe to form Heme, each molecule of which is joined by a globin chain made up of polyribosomes, then a 4 meter of globin chain with each of its own Heme groups is formed in the hemoglobin molecule (17).

Table 3 displays the findings of the ANOVA test to determine the impact of smoking duration on smokers' hemoglobin levels. With  $dK = 19$  and  $F = 2,917$ , and  $F$  table obtaining a value of 2.05 ( $F$  count  $>$   $F$  table), it is clear that smoking duration has an impact on the test's outcomes hemoglobin. This result is supported by Putri (18) with the Wilcoxon test, there is a relationship between the number of cigarettes smoked and CO with a  $P$  value of 0.000. Hemoglobin At concentrations as small as 0.1%, carbon monoxide will bind to half of the total hemoglobin in the blood and reduce the oxygen carrying capacity of the blood by 50%. In an effort to boost the amount of

oxygen delivered to the tissues, the body will launch a compensation mechanism if this persists, which will result in an increase in the erythropoiesis process.

Hemoglobin levels will rise and surpass those in normal circumstances as a result. Smoking is one of the factors that contribute to hypoxia brought on by high amounts of carbon monoxide. The body's levels of monoxide (CO) are extremely harmful. One of the health issues brought on by excessive levels of carbon monoxide (CO) in the body is an interruption in the heart's rhythm and blood circulation. (18).

Table 1 demonstrates a trend for smokers who have been smoking for of 3, 4 and 5 years to have higher hemoglobin levels. According to Riskesdas (Baseline Health Research from Agency of Health Research and Development) in 2018, smokers were categorized into three groups according to cigarette smoking intensity, namely light, moderate and heavy smokers.

Light smokers are individuals who smoke 1 to 10 cigarettes a day, while smokers in the moderate category consume 11 to 20 cigarettes a day. Individuals who smoke more than 20 cigarettes a day are categorized as heavy smokers. Numerous factors, including smoking behaviors, affect normal hematological results. Smoking 10 cigarettes or more in a day will cause an increase in hemoglobin and hematocrit (Packed Cell Volume) (5).

In addition to nicotine, tar, 3,4-benzopyrene, carbon monoxide, carbon dioxide, nitrogen oxides, ammonia, and sulfur, cigarette smoke also contains over 4000 other substances. About 210–300 times more so than its affinity for oxygen, carbon monoxide has a strong affinity for hemoglobin (19).

The content of substances in cigarettes, especially nicotine, also affects psychological conditions, the nervous system, as well as brain activity and function, both in active and passive smokers. Given the many dangers of nicotine in cigarettes, the organs in the body do not operate properly, especially in the main organs in the body, one of which is the heart pumping blood that will run abnormally because of disturbances in the heart work system due to nicotine and carbon monoxide. Smoking habits affect cardiovascular endurance because carbon monoxide is released by smoke by 4% and binds Hb levels faster than oxygen (20).

Hemoglobin is a tetrameric protein in erythrocytes that binds to oxygen and is responsible for releasing oxygen into tissues. Carbon dioxide will bind to Hemoglobin and be transported back to the lungs. Carbon monoxide contained in cigarettes has a high affinity for hemoglobin, making it easier for the two to bind to each other to form carboxyhemoglobin, an inactive form of hemoglobin. Due to hemoglobin's inability to bind oxygen, oxygen is released to different

tissues, leading to tissue hypoxia. By raising hemoglobin levels, the body will attempt to make up for the drop in oxygen levels (21).

The results of this study are consistent with studies done by Makawekas et al., (1), namely a significant difference between hemoglobin levels in active smokers and passive smokers in seventh semester students of the Faculty of Medicine, Sam Ratulangi University, Manado. According to this study, active smokers had hemoglobin levels that were higher than passive smokers. This is because the carbon monoxide content in cigarettes has a stronger affinity for hemoglobin than oxygen does. The findings of a study done in Makassar also indicated a connection between active smokers' hemoglobin and hematocrit levels. Hematocrit and hemoglobin have a positive relationship (1).

Different results were found by Katari et al., (15) which showed that there was no significant effect between the length of exposure to cigarette smoke on hemoglobin (Hb) levels in the Wistar strain of white rats. This can occur due to several factors, namely the time and dose of exposure to cigarette smoke, the type of cigarette used, the laboratory environment and the food consumed (15).

The findings of Katari et al., (15) are confirmed by Ramadhanti et al., (22), who report that the hemoglobin levels in active smokers in 31 respondents who were active

smokers are still within normal limits. Because the body's response to obtaining oxygen and maintaining homeostasis and metabolism is still functioning normally. However, if this condition persists for a long time, the body will lose its ability to maintain homeostasis, which will allow smoking-related illnesses like lung cancer to develop. Additionally, study from Medan revealed that smokers' hemoglobin levels were both normal and abnormal, though not by a great margin (15).

## CONCLUSIONS

Based on the study's findings, it was discovered that the length of smoking 3, 4, and 5 years had an impact on the results of the Cyanmethemoglobin method used to measure hemoglobin levels. Smokers who had smoked for 5 years or longer had the biggest impact. Your Hb level will increase the longer you smoke. Due to Hb's affinity for the carbon monoxide in cigarettes, this is one example of how the body compensates for a lack of oxygen. Therefore, it is advised that smokers cut back on their cigarette intake and try to quit because doing so will prevent health issues including a rise in hemoglobin levels that have an effect on lung conditions like. Therefore, it is advised that smokers cut back on their cigarette consumption and try to quit because smoking has a negative impact on their health by raising their hemoglobin levels, which can lead to lung

conditions like pulmonary fibrosis, congenital heart disease, cor pulmonale, and polycythemia. By using masks, engaging in sufficient exercise, and establishing a healthy lifestyle, passive smokers can avoid being directly exposed to cigarette smoke. The findings of this study are anticipated to serve as a guide for future investigations into the many factors that can impact adult hemoglobin levels. To verify the findings of this study, additional research utilizing sizable samples from a range of age groups is required.

## AUTHOR CONTRIBUTIONS

All authors have contributed equally to a study.

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## CONFLICT OF INTEREST

This research has no conflict of interest.

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