ORIGINAL ARTICLE

Comparative Study of Examination of LED Value Using the Wintrobe Method and Hemoglobin Examination Using the Sahli Method on *Rattus norvegicus* Wistar Exposed to Cigarette Smoke

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ABSTRACT

Introduction: Smoking can cause the risk of various diseases or health problems such as non-communicable diseases, both to smokers themselves and to other people around who do not smoke. There are many substances released by a cigarette and are harmful to human health. The purpose of this study was to determine the levels of LED and Hb in rats exposed to cigarette smoke. **Methods:** The research method used was laboratory observation; the sample used was wistar rats exposed to cigarette smoke. The results showed a decrease in Hb levels and LED levels in rats exposed to cigarette smoke. **Results:** This decrease in levels is caused by differences in the metabolic rate of each test animal for recovery. The immunity of the test animals to one another also has a role in this recovery. These results indicate that the mean hemoglobin (Hb) levels of white rats (Rattus norvegicus) decreased after being exposed to cigarette smoke. **Conclusion:** So that sometimes even though both are exposed to the same cigarette smoke for the same length of time, it will cause different recovery responses between the two.

Keywords: Cigarettes, carbon monoxide (CO), Hemoglobin (HB), Mice

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INTRODUCTION

Smoking is a risk factor for various heart diseases, hypertension, inflammation, stroke, blood clotting disorders, and respiratory diseases. Smoking also accelerates the pathogenesis of various malignant diseases, such as lung, pancreas, breast, liver, and kidney malignancies. In recent years of research, it has been suggested that smoking can also affect blood components. For example, the effect of smoking on white blood cell count indicates that there is an increase in white blood cells in smokers than in nonsmokers. Smoking is also thought to affect other blood components, for example, erythrocytes, platelets, hemoglobin, and so on.

In Indonesia, abusing smoking is also a serious health problem. The emergence of news about the number of alcohol abuse. At least, this is an indication that the community consumes a lot of liquor. According to WHO in 2000 it was informed that in Indonesia there were more than 13,000 patients suffering from alcohol-related illnesses, and in 2001 there were 50% of the total 65 smoking cases that caused death (1).

Smoking can cause the risk of various diseases or health problems such as non-communicable diseases, both to the smoker itself and to other people around who do not smoke.There are many substances released by a cigarette and are harmful to human health.However, some of them are CO (carbon monoxide) gas and nicotine. The CO gas produced by a cigarette can reach 3-6%, this gas can be inhaled by anyone, either the smoker himself, people who are near smokers, or people who are in the same room with smokers.CO gas has the ability to bind hemoglobin (Hb) in red blood cells (erythrocytes) stronger than oxygen. Therefore, there will be a lack of oxygen in the air. Body cells that suffer from lack of oxygen will try to increase, namely by compensating the blood vessels by shrinking (spasm) (2).

In essence, the results of laboratory examinations provide an overview that will be used as information material and is very necessary for one's health. Especially for

patients or sufferers, so that the diagnosis and treatment therapy decisions can be made appropriately. The results of these laboratory examinations really depend on the accuracy, skills, and knowledge of health laboratory technicians, especially health analysts in preparing examination materials and the examination process (3). A person's health is often checked based on their blood health. Some measure blood pressure, hemoglobin level, or sedimentation rate. Erythrocyte sedimentation rate is usually done routinely to detect acute illness or just a regular check-up.A high sedimentation rate can also indicate the presence of certain infections in the body such as influenza or viral syndrome, sore throat, or skin infections. A higher sedimentation rate (> 100 mm/hour) can accompany symptoms of heart infection (endocarditis) or infection. joints (septic arthritis).

Latif R, describes association of exposure to environmental tobacco smoke on mother during pregnancy with Low Birth Weight infants (4). Prevalence of CYP1A1 and GST polymorphisms in the population results in cancer (5).The health laboratory as one of the health service units that play a role in the diagnosis of a disease is divided into more specific laboratories, one of which is the hematology laboratory which analyzes blood samples from patients. Among the types of hematological examination is the determination of hemoglobin levels in the blood (6).

The study aims to evaluate the comparative study of examination of led value with the wintrobe method and hemoglobin examination using the sahli method on rattus novergicus wistar exposed to cigarette smoke.

MATERIALS AND METHODS

This research is a laboratory observation to determine the image of hemoglobin (Hb) levels using the Sahli method and the sedimentation rate (LED) using the Wintrobe method on Rattus novergicus Wistar exposed to cigarette smoke. This research was conducted in the Laboratory of Medical laboratory technology IKesTMP and BBLK Palembang. The sample used in this study was the blood sample of *Rattus novergicus* Wistar. Tools and materials for haemometer were prepared. Diluent tube was filled with 0.1 N HCL up to mark 2 into the haemometer. Capillary blood or EDTA venous blood was absorbed with a hemoglobin pipette up to the 20 or 0.02 ml mark. Any blood that sticks to the outside of the tip was removed. Next, immediately the blood flow from the pipette into the bottom of the diluting tube containing 0.1 N HCl. The pipette raised a little, then the HCl was sucked into the pipette 2-3 times to clean the remaining blood in the pipette. The contents of the tube were mixed so that the blood and acid were compound (homogeneous), let it 2 - 3 minutes until dark brown. Distilled water was added dropwise, stirring each time with a stirring rod. The mixed color equation and the Sahli standard are read in bright lightThe hemoglobin level was read in grams / 100 ml of blood or g / dl or g /%.

The sedimentation rate was determined using the Wintrobe Method. EDTA blood or oxalate blood was obtained. Using a wintrobe pipette enters the blood into the wintrobe tube at the level of the o mm mark. Do not cause air bubbles or foam. The wintrobe tube was left in an upright position in a windless place for 60 minutes. The height of the plasma layer was read in mm and report the number as the sedimentation rate.

The present research study has been approved by Research Ethics comittee Institut Ilmu Kesehatan dan Teknologi Muhammadiyah Palembang, Indonesia , No.793/A.I/IKESTMP/XII/2020 dated 1 December 2020

Data analysis is presented in tabular form and descriptive analysis based on the percentage and description of hemoglobin levels and sedimentation rates on the results of the examination.

RESULTS

Hemoglobin levels using the Sahli Method on Rattus Novergicus Wistar exposed to cigarette smoke

Examination of hemoglobin (Hb) levels by the Sahli method is carried out in stages, namely the initial hemoglobin (Hb) level before cigarette smoke exposure and the hemoglobin (Hb) level after cigarette smoke exposure. The results of hemoglobin (Hb) levels can be seen in Table I.

It can be seen that the average initial hemoglobin (Hb) level is 22.1 g / dl with a maximum hemoglobin (Hb) level of 24.9 g / dl and the lowest hemoglobin (Hb) level of 19.5 g / dl. The average hemoglobin (Hb) level after exposure was 21.7g / dl with a maximum hemoglobin (Hb) level of 24.2 g / dl and the lowest hemoglobin (Hb) level of 19.8 g / dl. The average percentage of initial hemoglobin (Hb) levels with hemoglobin (Hb) levels after exposure to cigarette smoke was 2.75%.

LED Value using the Wintrobe Method on Rattus Novergicus Wistar Exposed to Cigarette Smoke

Examination of the sedimentation rate using the

Table I: Hemoglobin (Hb) Levels in White Rats (*Rattus norvegicus*) Wistar Strain

Replication	Hb levels (g / dl)	
	Baseline	After exposure
1	19.5	21.7
2	22.6	20.9
3	24.9	24.2
4	21.4	22.0
5	21.9	19.8
Highest grade	24.9	24.2
Lowest grade	19.5	19.8
Average	22.1	21.7

Table II. LED Level in White Rats (*Rattus norvegicus*) Wistar Strain

No	Replication	Baseline	finale
1	1	0.4	0.5
2	2	0.5	0.5
3	3	0. 3	0.6
4	4	0. 3	0.6
5	5	0.6	0.5
Highest grade		0.6	0.6
Lowest grade		0.3	0.5
Average		0.575	0.525

Wintrobe method on Rattus Novergicus Wistar exposed to cigarette smoke using tubes and racks from Wintrobe. The total research results and the average value of the LEDs in each group are shown in Table II.

The average initial sedimentation rate (LED) is 0.575 with a maximum LED level of 0.6 and the lowest LED level is 0.3. The average LED level after exposure was 0.525 with a maximum LED level of 0.6 and the lowest LED level of 0.5.

DISCUSSION

The Cigarette Smoking affect the hemoglobin level has been demonstrated previously (8). The present study has been conducted to determine the effect of exposure to cigarette smoke on hemoglobin (Hb) levels using the Sahli method in white rats (*Rattus norvegicus*).

From the results of an examination of hemoglobin (Hb) levels in the group of white rats (*Rattus norvegicus*) exposed to cigarette smoke, it was found that the average result on day 0 was 22.1 g / dl. After being exposed to cigarette smoke, the hemoglobin (Hb) levels of white rats (*Rattus norvegicus*) became 21.7 g / dl. These results indicate that the mean hemoglobin (Hb) levels of white rats (*Rattus norvegicus*) decreased after being exposed to cigarette smoke.

The decrease in Hb levels, which was not significantly different, was probably due to differences in the metabolic rate of each test animal for recovery. The immunity of the test animals to one another also has a role in this recovery. So that sometimes even though both are exposed to the same cigarette smoke for the same length of time, it will cause a different recovery response between the two.

The whole process that occurs in the body, in the presence of CO gas that is inhaled during treatment causes the seizure of the active side of blood Hb by O2 and CO gas. This struggle causes the formation of carboxyhemoglobin (COHb) which results in a little dissolved O2 in the blood plasma and the partial pressure of oxygen (PO2) in the blood decreases. This condition operates the O2 chemoreceptors in the form of catecholamines to convey impulses to nerves to the inspiratory area so that there is an increase in the respiratory rate.

This increase is higher in the presence of nicotine which has a nicotinic effect on acetylcholine so that it interferes with the nervous system to develop impulses. In this case, nicotine, which has a molecular group similar to acetylcholine, seizes the active side of the enzyme acting on the synaptic area so that this can disrupt the transmission of electrical signals to the nervous system, especially in the synapse area. Besides this nicotinic effect can also cause depolarization of muscle cells followed by muscle spasms.

The increased rate of respiration by these two factors is followed by an increase in the frequency of inspiration and expiration, which causes the respiratory muscles to work faster than usual and triggers the release of corticosteroid hormones to reduce spasm in the respiratory muscles. If this muscle spasm continues, it can cause muscle paralysis which results in respiratory failure (abnormal exchange of O2 and CO2). This condition causes hypoxia at the tissue level which can stimulate the central nervous system so that the adrenal glands of the medulla are contacted to release the hormone aldosterone to the kidneys so that junkstaglomerulosa cells in the kidneys are stimulated and will secrete erythropoietin which can stimulate the red bone marrow to run the erythropoiesis process faster, resulting in erythrocytes. production increases as well. With the increase in erythrocyte production, the blood Hb level and the hematocrit percentage will also increase. Increased levels of Hb will cause the viscosity (thickness) of blood to change, this condition will trigger the liver to produce protein components of blood plasma such as albumin, globulin, fibrinogen, and prothrombin in excess.

But because liver cells and Kupffer cells are disrupted by the presence of carbon residues from CO, the central nervous system contacts the adrenal glands of the medulla to release the hormone ACTH (adrenocorticotropic) to the liver to help stimulate the production of proteins such as ß and γ-globulin. So that plasma protein orders are fulfilled so that blood viscosity is maintained and blood osmosis pressure remains stable. But if this hypoxic condition lasts for a long time, the amount of plasma protein levels will also decrease.

This research was conducted to determine the value of the sedimentation rate (LED) using the wintrobe method of Rattus Novergicus Wistar experimental animals exposed to cigarette smoke. A normal LED examination can give the doctor reassurance that there is no serious organic disease. In infectious diseases, the LED is useful for monitoring the course of the disease and to determine whether there are organic abnormalities in patients who show vague symptoms and show no abnormalities on

physical examination.

Obtained observations indicate that there are differences when viewed based on the average value. This occurs because fibrinogen in the blood remains negatively charged. The existing erythrocyte cells will repel each other in the blood so that they will not form rouleaux.

LED is an indicator that is sensitive to a change that occurs in the immune system. LED examination is usually used to monitor a suspected acute and chronic severe disease. When an infection occurs, the LED value will increase, whereas if there is no severe infection the LED value will decrease / normal. The LED describes the plasma composition and the ratio between erythrocytes to plasma. Anticoagulant blood that is inserted into a small luminal tube and placed perpendicular, will show the deposition of erythrocytes at a rate determined by the surface ratio.

The volume of erythrocyte deposition of this cell which is called the sedimentation rate increases faster when the cell increases, but the speed decreases when the cell surface is wider. Small cells settle more slowly than clumping cells because when cells clump, the increase in clot weight is greater than the increase in surface area. In normal blood, the value of the sedimentation rate is relatively small due to the deposition of erythrocytes due to the pull of gravity.

CONCLUSION

The mean hemoglobin (Hb) level in the experimental animal of Wistar white rats (*Rattus norvegicus*) in the control group was 19.8 g / dl and the treatment group was 22.1 g / dl. The average hemoglobin (Hb) levels in the experimental white rat (*Rattus norvegicus*) Wistar strain in the treatment group after being exposed to cigarette smoke for 15 days was 20.1 g / dl. c. The average hemoglobin (Hb) level in the experimental animal of Wistar white mouse (Rattusnorvegicus) control group on day 15 was 21.7 g / dl. The mean of hemoglobin (Hb) levels in the experimental white rat (*Rattus norvegicus*) Wistar strain in the treatment group after being exposed to cigarette smoke for 30 days was 18.7 g / dl. e. The average hemoglobin (Hb) level in the experimental animal of the Wistar white mouse (*Rattus norvegicus*) control group on day 30 was 19.7 g / dl. The results of the examination and the mean value of LEDs in white mice (*Rattus norvegicus*) before exposure to 0.575 and after exposure to cigarette smoke 0.525. In this study, the results are different but not significant because when an infection occurs, the ESR value will increase, whereas if there is no severe infection, the ESR value will decrease / normal.

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