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The impact of smoke from grilled fish on the hematological parameters of Indonesian grilled fish sellers Dwi Purbayanti and Rinny Ardina Department of Medical Laboratory Technology, Universitas Muhammadiyah Palangka Raya, Palangka Raya, Indonesia Syahrida Dian Ardiany Department of Pharmacy, Universitas Muhammadiyah Palangka Raya, Palangka Raya, Indonesia Rudi Gunawan Department of Medical Laboratory Technology, Universitas Muhammadiyah Palangka Raya, Palangka Raya, Indonesia, and Mohammad Rizki Fadhil Pratama Department of Pharmacy, Universitas Muhammadiyah Palangka Raya, Palangka Raya, Indonesia and Doctorate Program of Pharmaceutical Sciences, Universitas Airlangga, Surabaya, Indonesia Abstract Purpose – Fish processing by grilling can produce emissions that contain toxic compounds that can have short- and long-term effects on human health. Another study reported that exposure to air pollutants is hematotoxic.

The purpose of this paper is to determine the effect of smoke exposure on fish grill results on hematological parameters. Design/methodology/approach – The subjects of this study were 90 grilled fish sellers, with 32 processed food sellers who did not sell grill food as a control. The hematological analysis was performed using the Hematology Analyzers KX300 instrument. Findings – The results showed that the mean value of hematological parameters in the test group was higher than the control group except for the number of lymphocytes and mixed cell parameters.

Originality/value – The content of harmful compounds contained in fish grill smoke can increase hematological value in the blood of exposed individuals, which has the potential for health problems and disease progression. Keywords Carbon monoxide, Grilled fish seller, Hematology profile Paper type Research paper J o u r n a l o f H e a l t h R e s e a r c h E m e r a l d P u b l i s h i n g L i m i t e d 2586-940X D O I 1 0 . 1 1 0 8 / J H R -

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This work was supported by a grant from Direktorat Riset dan Pengabdian Masyarakat Kementerian Riset Teknologi dan Pendidikan Tinggi Republik Indonesia through the scheme Penelitian Dosen Pemula with Grant No. 3/E/KPT/2018. Impact of grilled fish smoke on hematological Introduction Grilled fish is a processed dish that is very popular in South East Asia, with Indonesia being no exception. The dish is prepared by grilling fish on charcoal or coals, which adds flavor, color and aroma to make it more enjoyable[1].

Food processing by grilling using charcoal can produce emissions such as particulate matter (PM), carbon monoxide (CO), polycyclic aromatic hydrocarbons (PAHs), nitrogen oxides (NO), sulfur dioxide, volatile organic compounds, heavy metals (fluoride, arsenic, lead, mercury and selenium) and other toxic compounds which can provide short-term and long-term effects on human health[2]. The smoke produced during the grilling process can also contribute to pollutants in the atmosphere. These pollutants are known to cause adverse health effects by interacting with molecules that are important for the biochemical or physiological processes of the human body[3, 4].

Exposure to biomass burning smoke has been shown to have a detrimental effect on cardiovascular health[5, 6]. Carbon monoxide is the most abundant emission produced via food processing using charcoal[7]. In addition, CO can also be produced from motor vehicle emissions, smoking and other household appliances that use fuel[8].

Exposure to CO can replace oxygen to bind to hemoglobin (Hb), which results in a reduced amount of oxygen in the body (hypoxia) which can affect the biochemical processes in the body[9]. This

exposure can cause tissue hypoxia and can stimulate the formation of red blood cells (RBC) and hemoglobin [10]. The second most abundant pollutant resulting from grilling fish using charcoal is PM. PM has been declared carcinogenic by the International Agency for Research on Cancer, including emissions from household fuels such as wood and charcoal [11].

PM exposure can induce the production of reactive oxygen species (ROS) which can activate pro-inflammatory and pro-thrombotic pathways, produce endothelial dysfunction, increase blood coagulation and the development of cardiovascular disease [12]. Hazardous emissions that are also produced are PAHs which are genotoxic and contain carcinogenic compounds for humans [3, 4]. PAH can further induce hemolytic anemia [13]. The content of heavy metals in grilling smoke can produce free radicals that cause oxidative stress in living cells and induce an inflammatory response [14].

A complete blood count is one of the easy screening methods to find out the hematotoxicity of pollutants in the air [15]. Research shows a relationship between hematological parameters and exposure to air pollution, although the results obtained are still not consistent. Air pollutants, especially PM, have a significantly negative correlation with Hb and RBC, and a significant positive relationship with white blood cells (WBC) and platelet counts [16].

In 2012, a study reported by the Central Pollution Control Board for healthy adult individuals in Delhi who were exposed to air pollution showed an increase in Hb, hematocrit (HCT), RBC, WBC, monocytes, basophils and platelet levels [17]. Hemoglobin and RBC levels in individuals exposed to CO have also been reported to increase [10]. The relationship between exposure to air pollutants with hematological parameters is still controversial, and there have not been many specific investigations for pollutants produced by charcoal.

This study aimed to carry out hematological analysis including Hb, RBC, HCT, mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), WBC, lymphocytes, neutrophils, mixed cells, red cell distribution width (RDW) and thrombocytes in individuals exposed to air pollutants from smoked grilled fish using charcoal. Materials and methods Research subjects and participants The subjects of the study were 90 grilled fish sellers who sold in the Pahandut and Jekan Raya sub-districts of Palangka Raya City, were male, aged between 20 and 50 years, JHR had worked as fish grillers for more than a year, were in good health, had no history of chronic diseases, were not smoking, not consuming alcohol and were subjected to smoke exposure for at least 4 h per day.

The control group selected was composed of 33 male food sellers who did not sell grilled food. The inclusion criteria were that participants had to be basically healthy, not have a history of chronic illness, not smoke, not consume alcohol, not consume drugs or vitamins that could affect the number of blood cells and not use respiratory protective equipment while working.

The test group and control group were determined based on information obtained through questionnaires, which contained participant identity, work history, medical history, smoking habits, alcohol consumption and drugs or vitamins commonly consumed. Both groups came from environments that lacked air pollution from vehicles or from industries and did not use firewood as a cooking tool. Individuals who fulfilled the criteria completed informed consent forms for blood sampling. Blood sampling Blood samples were taken in aseptic conditions of 3 ml of the cubital fossa (vein) in the morning between 8 to 10 a.m. The blood was inserted into a lavender lid vacuum tube containing EDTA anticoagulant.

All blood samples were sent to the laboratory and analyzed within 1 h from the time of sampling. Hematology examination Blood samples were taken to the Clinical Laboratory of the Universitas Muhammadiyah Palangka raya to analyze the examination parameters consisting of Hb, HCT, erythrocyte count, leukocyte count, leukocyte type (lymphocytes, neutrophils and mixed cells), platelet count, erythrocyte index (MCV, MCH and MCHC) and RDW using the Hematology Analyzers Sysmex KX300. Quality control was done using three levels of control material (low, normal and high).

Statistical analysis The results of hematological parameters were recorded in mean \pm SD. The normality of the data was analyzed using the Kolmogorov – Smirnov test. To test the differences between the study group and the control group, the independent sample t-test was used for normally distributed variables and Mann – Whitney 's U - test was used for abnormally distributed variables.

The different tests based on the duration of smoke exposure in the study group used the ANOVA analysis method. Differences were considered significant with $p < 0.05$ in 95% CI. Ethical clearance This study passed the ethical review from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Lambung Mangkurat Banjarmasin Indonesia numbered 821/KEPK.FK.UNLAM/EC/VII/2018 .

Before taking blood samples, respondents were asked to fill out informed consent as

proof that respondents were willing, without coercion to be included as research samples. Results A total of 122 food traders consisting of 90 sellers of grilled food as the test group and 33 food traders who did not sell grilled food as the control group participated in this study. The average age of study group participants was 33.3 ± 9.3 years, and the control group was 33.7 ± 8.3 years, indicating no significant difference in the age of the two groups.

We also Impact of grilled fish smoke on hematological characterized based on the working experience in the form of the duration of time (years) working as food vendors who worked with grilled food and those who did not work with grilled food. Based on result statistics, the average length of time working as a grilled food seller was 7.1 ± 5.4 years, with the control group averaging 6.6 ± 4.8 years in their food trade with no significant differences in length of work experience between the two groups. The characteristics and results of the hematological examination in the study group and control group are shown in Table I.

The hematological analysis showed that hematological parameters (hemoglobin, the number of erythrocytes, hematocrit, MCV, MCH, MCHC, leukocyte, platelet, RDW and neutrophil counts) had higher mean values in the study group than the control group. However, those who had a significant increase ($p < 0.05$) were found in the parameters of the number of erythrocytes, hematocrit, platelets, number of leukocytes and neutrophils, whereas for lymphocyte counts and mixes, these had lower mean values in the study group than the control group, and statistically, only lymphocyte count were significantly decreased. Discussion This research was conducted on sellers of grilled food exposed to air pollutants caused by smoked fish processed by grilling.

Analysis of hematological parameters showed a higher hematological value in the exposed group than controls for Hb level, RBC count, HCT, MCV, MCH, MCHC, WBC count, platelet and RDW parameters. Researchers made comparisons to determine the effect of the duration of exposure on changes in hematological parameters. The study group was divided into four sub-groups based on the duration of exposure to smoke pollution, where only hemoglobin, platelet, WBC, lymphocytes and neutrophils parameters had a significant increase in the increase in exposure duration, as shown in Table II. Values that showed positive significance were hemoglobin, platelets, WBC count and Neutrophils, whereas negative significance was in the value of lymphocytes.

These results are in line with previous studies which stated that there was an increase in

Hb, HCT, RBC, WBC and platelet levels in healthy adults exposed to air pollution[17, 18]. Characteristics Test (n ¼ 90) Control (n ¼ 33) p -value Age (years) 33.3 ± 9.3 33.7 ± 8.3 0.649 b Work experience (years) 7.1 ± 5.4 6.6 ± 4.8 0.529 b Hb (g/dL) 15.2 ± 1.1 15.0 ± 0.9 0.389 a MCV (fL) 83.3 ± 5.4 82.8 ± 3.9 0.648 a MCH (pg) 28.5 ± 2.5 28.1 ± 1.9 0.376 b MCHC (mg/dL) 34.3 ± 1.4 34.0 ± 1.3 0.400 a RBC (103/µL) 5.4 ± 0.5 5.3 ± 0.3 0.023 a * HCT (%) 45.2 ± 3.1 43.7 ± 2.4 0.008 a * RDW (fL) 41.3 ± 2.3 41.2 ± 1.9 0.551 b PLT (103/µL) 298.3 ± 62.3 277.2 ± 41.2 0.032 a * WBC (103/µL) 7.5 ± 1.3 6.5 ± 0.9 0.000 a * Lym (%) 34.5 ± 7.9 38.0 ± 5.1 0.007 a * Mxd (%) 9.4 ± 3.4 10.1 ± 3.2 0.286 a Neut (%) 56.0 ± 9.5 51.9 ± 5.1 0.006 b * Notes: Hb, hemoglobin; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RBC, red blood cell; HCT, hematocrit; RDW, red cell distribution width; PLT, platelet; WBC, white blood cell; Lym, lymphocytes; Mxd, mixed cell; Neut, neutrophils. Values for test and control represent mean ± standard deviation. a I n d e p e n d e n t s a m p l e t - t e s t. b Mann–Whitney’s. *p o 0.05 Table I.

Test and control group characteristics and hematology value JHR Other research shows that air pollution exposure can increase Hb, RBC, HCT and MCHC levels[15, 19]. Another study reported the effects of CO exposure on hematological profiles, where Hb, RBC, HCT, RDW, WBC and platelet levels were elevated in patients with CO poisoning[20]. Apart from grill smoke, smoking tobacco is also a source of CO exposure, which is reported to increase Hb, RBC, HCT, MCV, MCH, WBC, lymphocyte and platelet levels[21].

Inhalation of CO exposure can quickly enter the circulatory system and bind to Hb **200 times stronger than** oxygen to form carboxyhemoglobin, thus disrupting the oxygen transportation system to tissues, which can cause hypoxia. The condition of hypoxic tissue is a stimulus for erythropoiesis and stimulates the production of erythropoietin to produce more RBC and Hb in blood circulation[22]. RDW i s the amount of anisocytosis or variation i **n the size of** RBC. The life of R BC is shorter in conditions o f o xidative stress , which results in an increase in hemolysisand then an increase in RDW[23].

The **results of this study** indicate a h igher RDW va lue **in the test group** **than the control g**roup, in li ne wi th pr evious studies which stated that the re was an inc r ease in RDW i n CO poisoning p atients[2 4]. RDW i n smo ke rs is also re ported to increas e and c an be use d as a n i n d i c a t o r o f i n f l a m m a t i o n a c t i v i t y [25]. I n c r e a s e d R D W c a n occur due to an increase in oxi dative stress on RBC whic h results in damaged cells and d i s r u p t i o n i n t i s s u e p e r f u s i o n [26]. W B C c o u n t s c a n b e u s e d a s a b i o m a r k e r f o r endothelial damage.

We found that fish traders who were exposed to grilled fish smoke had a higher WBC and neutrophil count than those who were not exposed. Carbon monoxide intoxication

itself is associated with toxicity to WBC in the form of leukocytosis and neutrophilia[20,27,28]. The systemic inflammatory response is characterized by the release of WBC and platelets in the circulation. Several studies have shown that the WBC level is a good predictor of atherosclerosis and cardiovascular disease[29]. A high WBC amount in exposed subjects can show that they may be at higher risk of developing atherosclerosis and cardiovascular disease than traders who are not exposed to smoke[30].

Increased platelet counts in healthy adult individuals who are exposed to long-term air pollutants, especially PM, shows a detrimental effect on blood clots[31]. Other studies have shown a significant increase in platelet counts in CO poisoning patients. Excess carbon monoxide in the body can activate platelets to produce NO which can react with superoxide

| Duration of exposure (hours/day) | Parameters | A (n ¼ 34) | B (n ¼ 23) | C (n ¼ 17) | D (n ¼ 16) | p -value |
|----------------------------------|--------------|------------|------------|------------|-------------|----------|
| | Age (years) | 32.7 ± 9.9 | 34.6 ± 8.2 | 31.5 ± 7.7 | 34.5 ± 11.3 | 0.345 |
| | Hb (g/dL) | 14.8 ± 0.9 | 15.1 ± 0.9 | 15.5 ± 1.3 | 15.8 ± 1.3 | 0.021 |
| | MCV (fL) | 82.8 ± 6.4 | 82.6 ± 3.8 | 84.1 ± 4.6 | 84.5 ± 6.2 | 0.619 |
| | MCH (pg) | 28.5 ± 2.8 | 28.3 ± 1.9 | 28.4 ± 2.2 | 29.0 ± 2.9 | 0.298 |
| | MCHC (mg/dL) | 34.4 ± 1.4 | 34.2 ± 1.4 | 34.0 ± 1.4 | 34.2 | |

| | | | | | | | |
|--|----------------------------|--------------|--------------|--------------|--------------|------------|-------|
| | HCT (%) | 44.5 ± 1.5 | 44.8 ± 2.9 | 44.8 ± 2.6 | 46.0 ± 3.3 | 46.2 ± 3.3 | 0.164 |
| | RDW (fL) | 41.0 ± 1.9 | 41.2 ± 1.8 | 41.9 ± 2.0 | 42.8 ± 2.9 | 0.052 | |
| | PLT (10 ³ / µL) | 283.9 ± 55.5 | 291.1 ± 47.6 | 307.2 ± 59.8 | 310.9 ± 68.8 | 0.039 | |
| | WBC (10 ³ / µL) | 7.2 ± 1.1 | 7.3 ± 1.1 | 7.7 ± 1.6 | 8.0 ± 1.5 | 0.049 | |
| | Lym (%) | 36.4 ± 7.7 | 34.9 ± 5.9 | 34.5 ± 8.6 | 29.5 ± 7.8 | 0.033 | |
| | Mxd (%) | 10.5 ± 4.1 | 8.9 ± 2.4 | 8.5 ± 3.3 | 8.8 ± 2.2 | 0.096 | |
| | Neut (%) | 53.1 ± 9.9 | 56.2 ± 6.2 | 57.0 ± 10.9 | 61.7 ± 8.0 | 0.023 | |

* Notes: Hb, h emoglobin; MCV, mean cell v olume; MCH, me an corpuscular h em oglobin; MCHC, mean corpuscular hemoglobin concentration; RBC, r ed blood cell; HCT, hematocrit; RDW, r ed cell d istribution wid th ; PLT, platelet; WBC, whiteblood cell; Lym, lymphocy tes; Mxd, mixed cell; Neut, n eu trophils. A: 5–6h/day; B: 7 –8 h/day; C: 9–10 h/day; D:W 10 h/day. Values for A , B, C, a nd D represent mean± standard deviation. c ANOVA test. *p o 0. 05 Table II.

Hematological parameters of study groups based on exposure duration Impact of grilled fish smoke on hematological to produce peroxynitrite and other ROS[32]. ROS can affect platelet aggregation and blood flow, contributing to endothelial damage. Besides, free radicals can increase platelet adhesion and cause changes in the fibrinolytic pathway[33]. In addition, it is also known that there is a significant increase in platelet index (MPV, PDW, P-LCR and PCT) in grilled fish traders, which is a biomarker of inflammation due to increased platelet activation[34].

The results of this study show that the content of hazardous compounds in grilled fish

processing using charcoal can increase hematological values in the blood of exposed individuals, especially for parameters that lead to an inflammatory response. However, this study had some limitations. First, all test subjects were male and because there are several factors such as ranges of Hb and MCHC which are influenced by the gender of the test subjects, the results of this study could only describe conditions in male test subjects.

Conclusion This research has succeeded in showing the impact of air pollution originating from smoked fish processing by grilling. Decrease in the value of lymphocytes and mixed cells accompanied by the increase of Hb, erythrocytes count, HCT, MCV, MCH, MCHC, leukocyte count, platelets, RDW and neutrophils values is caused by exposure to hazardous chemicals contained in grilled fish smoke which have the potential to lead to health problems and disease progression.

Research similar to different observation objects must be carried out in order to ascertain the effects of exposure to air pollutants from other objects, especially with larger sample quantities. References 1. Raji MN, Karim SA, Ishak FA, Arshad MM. Past and present practices of the Malay food heritage and culture in Malaysia. *J Ethnic Foods*. 2017; 4(4): 221-31. 2. Olujimi OO, Ana GR, Ogunseye OO, Fabunmi VT. Air quality index from charcoal production sites, carboxyhemoglobin and lung function among occupationally exposed charcoal workers in South-Western Nigeria. *Springerplus*. 2016 Sep 13; 5(1): 1-18. 3. Abdel-Shafty HI, Mansour MS.

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