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CORRELATION BETWEEN LEAD LEVELS AND PERCENTAGE OF BASOPHILIC STIPPLING IN THE BLOOD OF SILVER MEN

Ratu Azzahra*, Kurnia Ritma Dhanti, Asmiyenti Djaliasrin Djalil, Retno Sulistiyowati
Universitas Muhammadiyah Purwokerto, Jl. KH. Ahmad Dahlan, Dusun III, Dukuhwaluh, Kembaran, Banyumas, Jawa Tengah 53182 Indonesia,
*ratuazzahra.tlm@gmail.com

ABSTRACT
The phenomenon of silver men has become increasingly prevalent in recent years. The direct application of metallic paint to the body and the nature of their activities are major factors contributing to potential lead expsure. Elevated blood lead levels can disrupt hematopoiesis, leading to various blood abnormalities, including the presence of basophilic stippling. Basophilic stippling is a distinctive marker indicative of heavy metal toxicity, particularly lead poisoning. This study aims to examine the correlation between blood lead levels and the presence of basophilic stippling in the blood of silver men in Purbalingga Regency and Purwokerto Sub-district. This study employed an observational analytical design with a cross-sectional approach. The research sample consisted of blood specimens from 14 silver men, selected using accidental sampling. Blood samples (3 mL) were collected from the respondents arms into EDTA tubes. Blood lead levels were analyzed using Atomic Absorption Spectrophotometry (AAS), while basophilic stippling was examined through Giemsa-stained blood smears observed under a microscope. The collected data were analyzed using Pearsons correlation test. The Pearson correlation test yielded a p-value of 0.045 (p 0.05) and a correlation coefficient of 0.542, indicating a strong positive correlation. There is a strong correlation between blood lead levels andthe presence of basophilic stippling in silver men. An increase in blood lead levels is accompanied by the occurrence of basophilic stippling, highlighting the potential toxic effects of lead exposure in this population.

Keywords: basophilic stippling; blood lead level; lead; silver men

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INTRODUCTION

The phenomenon of the silver men has been increasingly encountered in recent years, especially after the COVID-19 pandemic, which has made it difficult for people to find jobs. Silver men is a term used for an individual who deliberately dyes his body with silver paint. Silver men are usually found in public places such as road junctions, shopping centers, or tourist areas. The primary purpose of this action is to attract public attention and make money. However, individuals often put their health aside in practice, including using paint to color the body. Silver men use paint for the house, and even enamel paint has a high gloss and good for resisting weather and scratches.Silver men is a busker who mostly sells pantomime art by coloring his entire body in silver to attract the attention of people on the street (Stiawati and Maisaroh 2023). The silver man performs his actions by standing on the side of the road where there are traffic lights on, while carrying a money box hoping to get money from people who see the action. The silver man volunteers his body to be smeared with silver paint, so that the silver color is seen all over the body. no matter the impact of the paint on the health of the body, the silver man continues to carry out his profession in order to survive (Radindasyah 2023).

Research to determine the lead level in household enamel paint in Indonesia was conducted in 2012-2013 to analyze the lead contained in the paint. The results showed that as many as 73 paints from 43 brands of decorative enamel paints or 77% of the paints analyzed distributed in the Indonesian market were shown to contain lead concentrations higher than 90 ppm. The

second study was conducted in the same study in 2014-2015 with a sample of 121 solvent-based decorative enamel paints around Bogor, Tangerang, Denpasar, Depok and Jakarta from various stores and outlets. Based on the analysis as many as 83% of paints contain lead of more than 90 ppm. This value exceeds the maximum allowable lead content in many industrialized countries (Ismawati et al. 2015)Lead, also known as Pb metal, is a heavy metal that occurs naturally in the earth's crust and is dispersed into nature in small quantities through natural processes. However, lead can also come from human activities and can reach 300 times more than natural Pb (Syam and Irnawati 2021). Heavy metals enter the human body through the gastrointestinal tract, skin, or inhalation. Toxic metals have been shown to pose a significant threat to human health, primarily due to their ability to cause damage to membranes and DNA and disrupt protein function and enzyme activity (Witkowska, Słowik, and Chilicka 2021). Lead can come from the smoke of industrial plants, motor vehicles, mining, coal burning, smelting metal grains, burning garbage, and wall paint that dissolves with rainwater, resulting from the disposal of industrial waste from factories. Exposure to lead will affect the functioning of the body's systems (Rahmawati et al. 2022).

Lead can enter the human body in three ways: through absorption in the skin, the respiratory tract, and the digestive tract. If this is limited only to the contact area, it is referred to as a local effect, but if the substance is absorbed into blood circulation, it will be brought to the various organs contained in the body and cause systemic effects (Ervianti et al. 2021). The nature of Pb compounds that are able to dissolve in oils and fats allows the absorption of Pb through the skin. Diffusion of lead through the epidermis is the first phase of percutaneous absorption, particularly of the stratum corneum, which is composed of thin and dense layers of dead cells. The second phase is the diffusion of toxicants through the dermis which is porous, nonselective and liquid. Then the lead will enter the bloodstream and be distributed throughout the body (Rosidah and Pranoto 2023). Poisoning due to lead metal contamination (Pb) can cause various things, including shortening the life of red blood cells, reducing the number of red blood cells and levels of young red blood cells (reticulocytes), and increasing the iron content (Fe) in blood plasma (Kustiningsih, Thomas, and Nurlailah 2017).

The hematotoxicity effect of Pb is that it inhibits most of the enzymes that play a role in heme biosynthesis. The pathogenesis of hemolysis in Pb poisoning is thought to be related to the inhibition of pyrimidine 5’-nucleotidase. Hereditary deficiency of this enzyme is characterized by basophilic stippling in erythrocytes, chronic hemolysis, and accumulation of pyrimidine nucleotides in intraerythrocytes (Novianti 2015). Biochemically erythropoietin hormone which functions for erythropoiesis is disrupted so that the formation of erythrocytes is inhibited. Lead can also inhibit the ALAD delta enzyme in the process of heme synthesis, so that heme is not formed (Santosa et al. 2020). Lead can increase membrane permeability and change erythrocyte morphology, giving rise to macrocytic and echinocytic forms and basophilic stippling (basophilic stippling). Lead toxicity causes coarse basophilic stippling due to suppressing pyrimidine 5'-nucleotidase, which prevents the breakdown of ribosomal RNA in circulating erythrocytes. Basophilic stippling is a pathognomonic feature in lead poisoning (Zamani et al. 2022).

Research by Putri Mayaserli, Rosita, and Oktafilinda, (2023) that analyzed the lead levels in the blood and their relationship with erythrocyte cell morphology in 20 samples of active smokers in Padang City showed that in 20 samples of blood, lead levels were still lower than the threshold. In the blood of respondents abnormalities of erythrocyte cells include anisocytosis in as many as one person (3.84%), color abnormalities in as many as two people (7.70%), Poikilocytosis as many as 10 people (38.46%), and the presence of objects inclusions in erythrocytes as much as 13 people (50%). Morphological analysis of cells in the respondents showed the presence of basophilic stippling in peripheral blood smears in 13 respondents. Basophilic stippling refers to the buildup of small granules seen in staining red

blood cells with basophilic staining. Silver men are among the professions potentially exposed to lead due to the many activities directly related to lead exposure sources, such as motor vehicle fumes and even the paint used to paint their bodies. The level of lead in silver men's blood can affect the effect of blood hematotoxicity shown in the presence of basophilic stippling. This study aimed to determine the relationship between lead levels and the percentage of basophilic stippling in the blood of silver men.

METHOD

The type of research is observational analytic with a cross-sectional design. Examination of lead levels in the blood was carried out at the Laboratory of Pharmaceutical Biology, University of Muhammadiyah Purwokerto. The percentage of basophilic stippling was examined at the Hematology Laboratory of the Medical Laboratory Technology Study Program D4 University of Muhammadiyah Purwokerto in July 2024. Samples of this study were the blood of silver men in Purbalingga regency and Purwokerto sub-districts as many as 14 respondents were taken by accidental sampling technique.Data collecting begins with blood sampling that takes as much as 3ml of silver men's blood into a purple cap vacutainer tube containing EDTA anticoagulants (Rahmatullah et al. 2023). Then it continues to the analysis of blood lead levels using the Atomic Absorption Spectrophotometer method. The raw solution concentration for blood lead level examination was as much as 10; 5; 4; 3; 2; 1; 0,5; 0,1; 0,05 and 0.01 ppm. For the blood samples, 1 ml of blood sample inserted into the erlenmeyer. Then concentrated HNO3 and HCL are added in a ratio of 1:5 and then homogenized and slowly heated to boiling (until as much smoke comes out as possible). Aquades were added to taste and then filtered into a measuring flask of 50 ml using Whatman filter paper. The solution was ana-lyzed with AAS at a wavelength of 283.3 nm. The data obtained in the form of blood lead levels expressed in ppm (Nasir 2018). Further, the calculation of basophilic stippling is carried out through the peripheral blood smears with giemsa staining that observed under a microscope, the calculation result is expressed in percent (%) (Putri Mayaserli, Rosita, and Oktafilinda 2023). Data collected in the form of blood lead levels and basophilic stippling percentage are presented in the form of tables and figures. To determine the correlation between blood lead levels with the percentage of basophilic stippling is using the Pearson Correlation test.

RESULT

Table 1.
Results of the descriptive test of blood lead levels (ppm) and the basophilic stippling percentage

Variable	Mean	Median	Min	Max	SD
Blood Lead Levels (ppm)	0,48	0,44	0,30	0,83	0,13
Basophilic Stippling Percentage (%)	1,24	1,28	0,00	2,79	1,01

The results of Table 1. show that the blood lead level is in the range 0,30-0,83 ppm, with an average of 0,48 ppm. These results showed that all respondents had exceeded reference values based on Kemenkes RI (2002) the decree of the Minister of Health of the Republic of Indonesia number 1406/MENKES/SK/IX/2002, with a reference value of 0,10-0,25 ppm. The result of basophilic stippling percentage show that in the range of 0,00-2,79% with an average of 1,24%. These results showed the presence of basophilic stippling found in 10 out of 14 respondents.

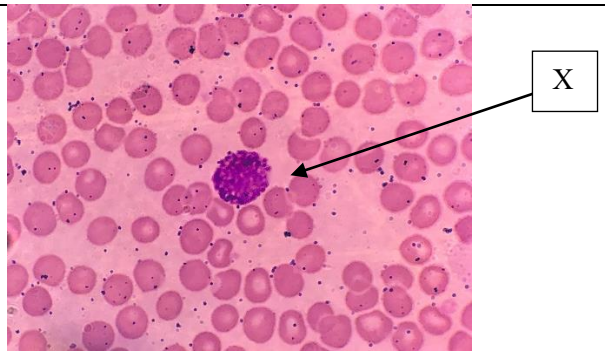

Figure 1. Basophilic Stippling with Giemsa staining (X = example of basophilic stippling)

Table 3.
The results of Pearson Correlation analysis to determine the correlation of blood lead levels with the Percentage of Basophilic Stippling in the blood of respondents

	Blood Lead Level (ppm)
Percentage of Basophilic Stippling (%)	r = 0.542 p = 0.045 N = 14

The results of Table 3. show that there is a correlation between blood lead levels and the percentage of basophilic stippling with a value of $p = 0.045$ ($p < 0.05$). Pearson Correlation value of 0.542 indicates a positive correlation with strong correlation strength.

DISCUSSION

Elevated levels of lead (Pb) in the "silver men" group are of grave concern, especially concerning the health impacts caused by exposure to this heavy metal. Based on research conducted in analyzing blood lead and malondialde-hyde (MDA) levels in silver men in Semarang City, it was found that 16 out of 20 silver men respondents had blood lead levels that averaged 37.3 mg/dl. This figure far exceeds the standard threshold set by WHO and CDC, which is between 10 to 25 mg/dl (Rosidah and Pranoto 2023). The study is in line with research conducted by showing that the analysis of lead (Pb) in the blood was detected from the results of laboratory tests with an average of 0.48 ppm, the highest of 0.83 ppm, and the lowest of 0.30 ppm. This study showed that all respondents had Pb levels in the blood above normal values.Several sources of lead pollution can be directly exposed to the human body. Sources of lead pollution can also come from motor vehicle fumes, industry, mining, coal burning, metal ore smelting, garbage burning, and wall paint that dissolves with rainwater (Nuradi 2018). Lead poisoning was reported in Kerala, India and there were several complications in the patient's body. Lead accumulates in the blood, bones, and soft tissues, including the brain, spleen, kidneys, liver, and lungs. Like many other heavy metals, excessive levels of lead cause the production of free radicals, which then cause oxidative damage to cellular components, including DNA and cell membranes. As an electropositive metal, lead has a high affinity for negatively charged sulfhydryl, resulting in the denaturation of enzymes such as delta-aminolevulinic acid dehydratase (ALA-D) and ferrochelatase. Both are important for heme synthesis, impaired heme synthesis leads to the accumulation of free erythrocyte protoporphyrins (Thomas et al. 2024). The activity of the silver men reinforces the possibility that the silver men had relatively high contact and exposure to lead. High exposure to lead will inhibit protoporphyrin synthesis and interfere with iron absorption, one of which is characterized by the appearance of basophilic stippling in red blood cells.

Lead can enter the body through several methods of inhalation, gastrointestinal tract and direct exposure through the skin. Lead is a toxic chemical and its presence in the body does not cause direct effects and without any specific symptoms. Lead exposure is chronic so it is less recognized so that the longer a person is exposed to lead, the cumulative dose will increase (Zahro and Purwati 2023). Systemic exposure to lead results in the binding of

these heavy metal ions to erythrocytes. The presence of toxic characteristics of lead makes the interaction of lead with erythrocyte cell membranes have the potential to result in premature hemolysis of erythrocytes before the normal regeneration cycle. The degree of erythrocyte destruction is dose-dependent and temporal, correlated with the intensity of exposure and the rate of erythropoiesis. Nevertheless, chronic exposure would lead to a progressive accumulation of lead in the circulation, followed by its deposition in various target organs, including the bone marrow, which has implications for impaired hematopoiesis (Prasetya 2021).

Basophilic stippling occurs mainly due to impaired ribosomal degradation during erythrocyte maturation. As a result, ribosomes and RNA remnants accumulate in the cytoplasm of developing red blood cells and produce distinctive spots called basophilic stippling (Kano et al. 2022). Basophilic stippling is a fine, medium-sized, coarse blue grain due to abnormal ribosomal aggregates. Fine basophilic stippling found in patients with thalassemia is different from basophilic stippling in lead poisoning which is usually coarse due to RNA deposition secondary to inhibition of pyrimidine 5'-nucleotidase. Basophilic stippling occurs in clinical conditions such as anemia, myelodysplasia, pyrimidine 5'-nucleotidase deficiency, and post-chemotherapy. Basophilic stippling cells are also often found in patients with se-vere lead poisoning (Kang et al. 2019)(Chan and Chan 2017). A case that went undiagnosed for 2 years was reported in Japan and when an examination found a lead concentra-tion of 1,315 ppm, basophilic stippling was found among the erythrocytes. Basophilic stippling can be found in people with lead poisoning, benzene poisoning, and pernicious anemia. Basophilic stippling found in a person with lead poisoning may be indicative of a diagnosis of lead poisoning (Kano et al. 2022). Basophilic stippling was found in 10 out of 14 re-spondents. The results of statistical tests in this study showed a p-value of 0.045 ($p < 0.05$) with Pearson Correlation value of 0.542, which means showing a positive correlation with strong correlation strength. Based on the strength of a strong correlation, it is interpreted that the presence of basophilic stippling will follow the increase in blood lead levels.

Pathologic basophilic stippling is more coarse and punctuate and is in dicative of disturbed rather than increased erythropoisisseen in many diseases like lead poisoning (Veena, Yadav, and Rameshbabu 2019) Basophilic stippling is a typical symptom that traces about the toxicity of heavy metals especially lead. Lead affects cell membranes, interfering with many energy systems and transport systems which may shorten the life of red blood cells, hemolysis and nephrotoxicity (Diab et al. 2020). Silver men allow for direct exposure to lead from a variety of sources. The increase in blood lead levels experienced is one of the evidence that high exposure occurs. The presence of basophilic stippling in peripheral blood smears is one of the side effects of exposure to lead obtained.Lead (Pb) emissions from mobile sources, such as motor vehicles, persistently contaminate the air at their various operational sites. Quantitatively, it is estimated that 10% of emissions will be concentrated within a radius of less than 100 meters from the source. In comparison, another 45% will be scattered polluting areas with a radius of up to 20 kilometers. A significant 35% of the pollution will be carried away by atmospheric movement over longer distances, expanding the geographical impact of lead pollution. This phenomenon is definitely due to the release of lead particulates from vehicle-based transport activities (Purwoko and Prastiwi 2019). Silver men in this study shows that their daily activities are carried out on the streets, so exposure to lead from vehicle fumes and the surrounding environment increases. This also reinforces the results of lead levels that are overall higher than the normal range of values in this study.

The risk of lead inhalation will be increased in individuals whose activities mostly intersect with motor vehicle fumes. The habit of smoking also causes high levels of Pb because in one cigarette there is 2.4 µg of lead and 5 % of it is found in cigarette smoke (Prabandari et al. 2024). Lead absorbed from the respiratory tract, digestion or skin will be transported by blood

to other organs. About 95% of lead in the blood is bound by red blood cells, 5% in blood plasma. Lead toxicity is affected by dose and duration of exposure, continuity of exposure, mode of contact, age, health status, nutritional status, immunity level, sex and tissue type exposed to lead (Shinta and Mayaserli 2020). The characteristics of the respondents obtained through interviews also stated that the overall respondents were smokers, that became a supporting factor for the existence of lead exposure in the blood of silver men

CONCLUSION

The results of laboratory tests showed that blood lead levels in silver men exceeded normal values, with an average lead level in the blood of respondents being 0.48 ppm. The results of laboratory tests showed that in 10 out of 14 respondents, basophilic stippling was found in the peripheral blood smears. The results of Pearson Correlation analysis in this study showed a p-value of 0.045 ($p < 0.05$) with a Pearson correlation value of 0.542, which means showing a positive correlation with a strong correlation strength. This study concludes that there is a strong correlation between blood lead levels and the percentage of basophilic stippling in silver men

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