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ORIGINAL ARTICLE

Lead Exposure Effect on Peripheral Blood Parameters among People around Bus Terminal in Yogyakarta

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Abstract

Background: Lead is a dangerous substance that can impact the blood components. Lead toxicity can cause imbalance in the homeostasis process of blood peripheral. The aim of this study is to search impact of lead exposure on peripheral blood parameter alterations among people around bus terminals in Yogyakarta.
Methods: This study is a cross sectional research design with convenience sampling method. According to calculation of sample size, 72 respondents had fulfilled the inclusion criteria. The independent variable was lead exposure and dependent variables were peripheral blood parameters including leucocytes, erythrocytes, hemoglobin, and platelets. Lead exposure was measured by atomic absorption spectrophotometer (AAS) and peripheral blood parameters were measured by automatic hematology analyzer. Those variables were analyzed by linear regression.
Results: Based on Independent T Test was found aged > 40 yo correlated with leukocyte (P =0.029), male workers correlated with leukocyte, erythrocyte, hemoglobin, and platelet (P =0.025,0.006, 0.000, and 0.031, respectively), smoking 1 packed per week associated with hemoglobin (P =0.006) and settlement 500 meter around terminal associated with leukocyte, erythrocyte, hemoglobin and platelet (P =0.025, 0.006, 0.000, and 0.031, respectively). Linear regression can predict level of leukocyte (β=0.32; CI 95= -0.207 to 0.643, P =0.006), erythrocyte (β=0.3; CI 95=-0.269 to 0.29; P =0.009), hemoglobin (β=0.33; CI 95=0.042 to 0.211; P =0.004), and platelet (β=0.25; CI 95=-0.548 to 0.73; P =0.029).
Conclusion: Age > 40 years old associated with leukocyte; male gender associated with leukocyte, erythrocyte, hemoglobin, and platelet level, respectively; smoking 1 packed per week correlated with hemoglobin level; settlement 500 m around terminal associated with level leukocyte, erythrocyte, hemoglobin, and platelet level, respectively. Finally, level of lead can predict positively leukocyte, erythrocyte, hemoglobin, and platelet level respectively.

Keyword: Bus Terminal; Lead Exposure; Peripheral Blood Parameters

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INTRODUCTION

Lead is a common element found in small amounts in the earth's crust (1). While it has some beneficial uses, it can be toxic to human and animals (2). Lead can be found in all parts of our environment, including the air, soil, water, and even inside our homes (2). Much of the exposure comes from human activities such as the use of fossil fuels including leaded gasoline, some types of industrial facilities, and lead-based paint in homes (1). Lead and lead compounds have been used in a wide variety of products found in and around our homes, including paint, ceramics, pipes and plumbing materials, solders, gasoline, batteries, ammunition, and cosmetics (1,2). People might be exposed to lead by eating and drinking food or water containing lead or from dishes or glass that contains lead (3). They may also breathe in lead dust by spending time in areas where lead-based paint is deteriorating and during renovation or repairing work that disturbs painted surfaces in older homes and buildings (2,3). Working in a job or engaging in hobbies where lead is used, such as making stained glass, can increase exposure as can certain folk remedies containing lead (1,2).

Long term lead exposure can affect almost every organ and system in the body. Children aged six years old and younger are most susceptible to the effects of lead (3). Even low levels of lead in infants (through breastfeeding) and children can result in behavior and learning problems, lower IQ and hyperactivity, slowed growth, hearing problems, and anemia. In rare cases, ingestion of lead can cause seizures, coma and even death (4). Lead is also harmful to adults. Exposure can lead to cardiovascular effects such as hypertension, decreased kidney function and reproductive problems in both men and women (3,5).

Lead is a hazardous substance that can impact blood

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components like hemoglobin, erythrocytes, leukocytes, and platelets. Lead toxicity can imbalance the homeostasis of blood circulation because of these effects. Lead inhibits the body's ability to make hemoglobin by interfering with several enzymatic steps in the heme synthesis pathway (6). Specifically, it decreases heme bio-synthesis by inhibiting d-aminolevulinic acid dehydratase (ALAD) and ferrochelatase (FECH) activity (6). Lead affects sblood coagulation actions through endothelial tissue injury, reduced nitric oxide, tissue plasminogen activator and an increased production of plasminogen activator inhibitor-1.20 (7). Lead affects leukocytes through initiating the inflammation process. A previous study indicated that inhaled lead acetate can increase total leukocyte percentage of eosinophil, neutrophil and basophil in broncho-alveolar lavage as well as IL-4 (8). The impact of biochemical and enzyme changes (and their possible sequelae) that may exist at lower blood lead levels remains unclear (8).

This study is very important to disclosing the mechanism of lead exposure on peripheral blood parameters. An animal model showed that long-term exposure can cause organ damage as well. Few studies state precisely the mechanism of lead exposure on the hematologic system, particularly leukocytes. The aim of this study is to search for correlations between lead exposure and health effects in people around bus terminals in Yogyakarta city. The results can help with providing information and recommendations to control lead exposure in the environment, particularly through public transportation.

METHODS

This study is a cross sectional research design with convenience sampling as a method to collect the samples. Prior to calculating sample size, it should be determined sample formula with continuous data such as $Z\alpha= 1,96$; $Z\beta= 0.84$; σ^2 and $\mu_1-\mu_2$ value. According to calculation of sample size, 72 respondents had fulfilled the inclusion criteria such as: agree to be respondents, age > 18 years old, and staying or working nearby bus terminal for more than one year. The exclusion criteria were being a bus passenger, mobile workers, and young workers < 18 years old.

The variables divided into two, independent variable is lead exposure and dependent variables are peripheral blood parameters including leucocyte, erythrocyte, hemoglobin, and platelet. Lead was measured by atomic absorption spectrophotometer (AAS) and peripheral blood parameters was measured by automatic hematology analyzer. Prior to analysis with regression model, Kolmogorov smirnov test was conducted to search normality data ($p>0.05$). Independent T Test was performed to search association of baseline characteristic with those variables. Linear regression provided significance $p<0.05$, coefficient (r), beta coefficient, standard error, and R^2. Due to several confounding can be identified, those variables were analyzed by linear regression using SPSS version 23 (IBM, Chicago, Illinois, USA).

Before collecting data, a preliminary study was piloted to observe research location, variables, and respondent characteristics. Those data were collected using questionnaires consisting of demographic data and possibility of comorbid diseases. Informed consent was obtained at the location. We began with introducing the research team, explaining the aim of the study and the study procedures, including how to take blood sample, risks related to the procedures and how to control these risks, and also compensation for the respondents after taking data. In the last step, we asked for the respondents' agreement to be study participants by obtaining their signed informed consent that was witnessed by one family member. Ethical approval was received from the Faculty of Medicine, Universitas Islam Indonesia (Approval number 32/Ka. Kom.Et/70/KE/IV/2018).

RESULTS

The demographic characteristics of the subjects was presented in Table 1. The most important of baseline characteristics of the respondents was aged more than 40 years old (68.1%), smoking subjects (66.6%), bus terminal workers (56.9%), length of work more than 8 years (66.6%), and all of the blood peripheral parameters within normal limits.

Table 1. Baseline characteristic of respondents

Variables		n	%
Age			
a.	≤ 40 y	23	31.9
b.	> 40 y	49	68.1
Gender			
a.	Male	51	70.8
b.	Female	21	29.2
Smoking			
a.	Yes	48	66.6
b.	No	24	33.4
Personal protective equipment (PPE)			
a.	Yes	10	13.9
b.	No	62	86.1
Education			
a.	Low	36	50
b.	High	36	50
Occupation			
a.	Terminal workers	41	56.9
b.	Nonterminal workers	31	43.1
Length of works			
a.	< 8 years	24	33.3
b.	≥ 8 years	48	66.6
Settlement			
a.	Around terminal	18	25
b.	Not around terminal	54	75
Comorbid disease			
a.	Yes	33	45.8
b.	No	39	54.2

Table 1. Continued.

Variables	Mean±SD
Leukocyte	9356.9±3808.5
Erythrocyte	5062777.7±526105
Hemoglobin	14.5±1.7
Platelet	257444.4±63257.5
Lead	0.78±0.68

PPE was wearing mask every day. Smoking had 1 packed/week. Education had graduated from senior high school was high. Terminal workers who stay in terminal. Settlement around terminal was 500 meters from terminal.

The association of peripheral blood parameters with baseline characteristics was showed in Table 2. It was elaborated age > 40 yo significant correlated with level of leukocytes (P =0.029). Male respondents associated with level of leukocytes (P =0.025), erythrocytes (P =0.006), hemoglobin (P =0.00), and platelet level (P =0.031). Smoking behavior 1 packed per week correlated with level of hemoglobin (P =0.006), while settlement around 500 meter from terminal associated with level of erythrocytes (P =0.006), leukocytes (P =0.025), hemoglobin (P =0.00), and platelet (P =0.006). Finally, to predict lead level with peripheral blood parameters was presented in Table 3. According to Table 3 was found lead level can predict level of leukocyte (P =0.006), erythrocyte (P =0.009), hemoglobin (P =0.004), platelet (P =0.029)

DISCUSSION

Age, gender, smoking behavior and settlement around the terminal were associated with several complete blood count (CBC) parameters. Age was associated with level of white blood cells (WBC), particularly in elderly (9). The study showed that elderly people > 65 yo were vulnerable to getting infection due to weaker immune systems. In our study, aged > 40 yo had mean level of WBC almost reaching upper limit of WBC level around 11.000 mg/dL that could be associated with the result study. Gender status was correlated with level of hemoglobin level, red blood cell (RBC), WBC and platelet level particularly in female workers (1). Females have a regular menstrual cycle that potentially decreases hemoglobin and RBCs, but in our study we did not ask about menstrual cycle in the questionnaire. Unfortunately, frequency of female respondents were 29.2 %, which cannot be generalized to the overall population. This finding seems interesting enough to conduct further study about it. Including level of WBC, several studies claimed prevalence of infection is more common among females than males, despite risk factors among them being multi factorial such as susceptibility, agent exposure and immune systems (10). In our study, we did not assess external factors in the subjects due to limit of resources.

Smoking was correlated with level of hemoglobin and leukocyte in previous studies (11, 12). Cigarettes contains thousands of hazardous substances that potentially damage molecule membranes of erythrocyte surfaces. Most respondents in our study were smokers (66.6%) with normal hemoglobin level. Length and frequency of smoking were questions in the questionnaire. Housing is an important aspect in lead level and inducing of peripheral blood parameters. In our study, we did not measure lead levels in the environment particularly 500 meter around the terminal that could be greater level than the outside of 500 meter of the terminal.

In our study, lead exposure was correlated with erythrocyte level among respondents, as shown in previous studies (3, 5, 6). The previous study showed lead toxicity on kidney function that was measured by glomerular filtration rate (GFR) and creatinine serum level (5). while in our study, we did not measure kidney function tests. Rather, we examined direct effect of lead on complete blood count (CBC) that found lead exposure affected level of red blood cell (RBC). In spite of the association being weak (0.3), mean of RBC and lead level in this study were normal range. We identified that there were confounding factors in this study, such as level of lead in air, food, and beverages, that were greater than in blood, however we did not measure these levels.

Lead has positively affected on hemoglobin level such previous study (13). The most common anemia reported is a microcytic hypochromic anemia. Our study claimed level of hemoglobin was in normal range, so we cannot identify the type of anemia. In addition, lead level was in normal range. We concluded there were other environmental factors that affected level of hemoglobin in our study.

The mechanism of lead induced erythrocyte changes is enzymatically process via heme production that inhibited by lead at basic dose 10 μg/dL (6). The most sensitive enzyme is delta-aminolevulinic acid dehydratase (13, 14). When the last enzyme in the pathway, ferrochelatase, is impaired, the precursor molecule protoporphyrin accumulates in the cells (15). Measurement of erythrocyte protoporphyrin levels in peripheral blood samples might be useful as clinical tool for following toxicity in patients with lead levels over 20 μg/dL (16). The hallmark of anemia in lead exposure is red blood cells with basophilic stippling, and ring sideroblasts on bone marrow (17). The mechanism of the ring sideroblasts is related to the accumulation of iron-laden mitochondria in the red blood cell precursors due to the inhibition of ferrochelatase by lead (13).

According to our study, lead has a positive correlation with coagulation effects via the destruction of platelets, which is similar to the findings of a previous study (18). That study was conducted among battery workers company that shown increasing level of plateletcrit (PCT), MPV, PDW, platelet large cell ratio (P-LCR), and mean platelet mass (MPM) were not determined , (18) while in our study we found that mean platelet level was normal. The level of platelets was in the normal range, because lead level in blood was normal. There could be other, unstudied sources of lead in the environment that could be confounding. Lead affects blood coagulation actions through endothelial tissue injury, reduced nitric oxide, tissue plasminogen activator and an increased production of plasminogen activator inhibitor-1 (18).

Table 2. Association of peripheral blood parameters with baseline characteristic

Variables	Mean ± SD Lead(µg/dL)	p	Mean ± SD Leukocyte (µl)	p	Mean ± SD Erythrocyte (mil/µl)	p	Mean ± SD Hemoglobin(g/d)	p	Mean ± SD Platelet (µl)	p
Age										
a. ≤ 40 yo	0.7±0.4	0.873	7934.7±3477.9	0.029*	5146521.7±497618	0.358	14.6±1.3	0.556	264478.2±50452.3	0.552
b. > 40 yo	0.7±0.7		10024.4±3806.2		5023469.3±539426.9		14.4±1.9		254142.8±68683.4	
Gender										
a. Male	0.7±0.5	0.126	8715.6±3666.4	0.025*	5169803.9±493191.6	0.006*	15.1±1.3	0.000*	247176.4±66182.9	0.031*
b. Female	0.9±0.9		10914.2±3780.2		4802857.1±523785.6		12.9±1.7		282380.9±48302.6	
Smoking										
a. Yes	0.7±0.7	0.831	8902±3363.9	0.198	5096666.6±543297.	0.443	14.9±1.6	0.006*	251479.1±65539.6	0.261
b. No	0.8±0.4		10266±4511.7		4995000±494025.1		13.7±1.9		269375±57897	
PPE										
a. Yes	0.5±0.3	0.267	9460±4446.7	0.927	5198000±378470.6	0.385	15±1	0.355	259100±41815.6	0.930
b. No	0.8±0.7		9340.3±3736.7		5040967.7±545478.3		14.4±1.8		257177.4±66324.9	
Education										
a. Low	0.9±0.8	0.099	9541.6±3401	0.684	5059166.6±569698.5	0.954	14.3±2.1	0.324	257777.7±57618	0.965
b. High	0.6±0.4		9171.2±4217.4		5066388.8±486721.7		14.7±1.3		257111.1±69262.5	
Occupation										
a. Nonterminal workers	0.8±0.8	0.715	9100±3854.3	0.622	5051935.4±398182.6	0.873	14.3±1.3	0.6	267838.7±53014.5	0.228
b. Terminal workers	0.7±0.5		9551.2±3809.7		5070975.6±610101.6		14.6±2		249585.3±69625.7	
Length of works										
a. < 8 years	0.9±0.8	0.205	9829.1±4398.5	0.461	5060833.3±516206.1	0.982	14.3±2.1	0.642	271416.6±77092.2	0.242
b. ≥ 8 years	0.7±0.5		9120.8±3503.3		5063750±536397		14.5±1.5		250458.3±54652.8	
Settlement										
a. Around terminal	0.7±0.5	0.126	10133.3±3737.2	0.025*	5006666.6±496292.1	0.006*	13.8±1.1	0.000*	257777.7±60236.3	0.031*
b. Not around terminal	0.7±0.7		9098.1±3831.2		5081481.4±538850.6		14.7±1.8		257333.3±64781.4	
Comorbid disease										
a. Yes	0.8±0.8	0.270	10157.5±3932	0.101	5022121.2±512596	0.550	14.3±2.1	0.358	262848.4±68047.8	0.509
b. No	0.7±0.5		8679.4±3613		5097179.4±541513.2		14.6±1.4		252871.7±59416.5	

*Independent T Test was significant (p<0.05). Variables were divided by status and median values. p is p value.

Table 3. Regression analysis to predict lead level with peripheral blood parameters

Variables	Beta coefficient (β)	p value*	CI 95%	
			Lower bound	Upper bound
Leucocyte	0.32	0.006	-0.207	0.643
Erythrocyte	0.3	0.009	-0.269	0.29
Hemoglobin	0.33	0.004	0.042	0.211
Platelet	0.25	0.029	-0.548	0.731

*Backward stepwise regression linear model was significant ($p<0.05$).

We also report that lead was positively correlated with leukocytes among subjects, similar to previous study findings (19, 20). Those studies discovered effect of lead to white blood cell in mice that altering structure, number of leukocyte, and nuclear arrangement was distorted (19, 20). In lead treated groups, the shape and structure of the monocyte was altered with reniform (kidney shaped) nucleus (20). These findings are also supported by another study with the evidence of reactive monocytes enclosing the cytoplasm became more intensely basophilic, and vacuolated (19). In our study, lead exposure associated with level of white blood cell (WBC), however, we did not measure specific WBC and another inflammation mediators. The WBC levels in our study were within normal range because the level of lead was normal. We did not measure sources of lead in the environment that could be within normal range. Lead has significant induced cytokines pro inflammation mediator substances via leukocyte destruction despite the study conducted on animal (21, 22, 23). These usually indicate chronic inflammatory process or might be seen with hemoplasmas. Toxicity in neutrophils is defined by the presence of *döhle bodies* (small, basophilic aggregates of RNA in the cytoplasm) and diffuse cytoplasmic basophilia (17).

LIMITATION

This study has limitations. The observational method does not allow for predicting between exposure and outcome. Also, further cohorts or experimental studies are needed to prove the association of lead exposure with peripheral blood parameters. We also identified that several factors could be counfounding, such as level of lead in the air, food and drinking water. These were not studied. If lead level was normal in the environment, it would be normal in blood of respondents. Further studies should be conducted comprehensively to investigate lead exposure on human and level in environment.

CONCLUSION

Age > 40 yo associated with leukocyte; male gender was associated with leukocyte, erythrocyte, hemoglobin, and platelet level, respectively; smoking 1 packed per week correlated with level hemoglobin; settlement 500 m around terminal associated with level leukocyte, erythrocyte, hemoglobin, and platelet level, respectively. Finally, level of lead can predict positively leukocyte, erythrocyte, hemoglobin, and platelet level respectively. Public health concern should be proposed to handle chronic impact on human such as controlling of air pollution, regulation of using lead in industry and personal protective equipment among workers or vulnerable population.

Conflict of interest: None to be declared.

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