



Original Article

Causality pattern of the blood lead, monoamine oxidase A, and serotonin levels in brass home industry workers chronically exposed to lead

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Abstract

The present study aims to analyse the effects of lead (Pb) chronic exposure on blood lead levels, Monoamine oxidase A enzyme (MAO A) and serotonin levels of brass craftsmen in Pati, Central Java, Indonesia, and to examine the connections among these three variables. The brass home industry area was polluted by lead. Thus, it chronically exposes the workers to lead pollution. Therefore, their blood lead level increased and later raised the level of MAO A and reduced the level of serotonin. Path analysis results show that the path coefficient (β) of lead effects in decreasing serotonin through MAO A pathway is -0.411. Furthermore, lead effects that directly affect serotonin level without passing through MAO A pathway is -0.391 with residual coefficient (ϵ) of 0.572. In conclusion, the increase of blood lead level causes an increase in level of MAO A and drop in the level of serotonin.

Keywords: chronic lead exposure, blood lead level, MAO A level, serotonin level, brass industry workers

1. Introduction

According to Kosnett (2012), chronic Pb exposure causes various health problems, for example, central nervous system disorder, peripheral nervous system neuropathy, anaemia, neuropathy, hypertension, and reproductive system disorder. There are several sources of Pb pollution such as means of transportation that use Pb-containing fuel, mining activity, and Pb smelting (Yang *et al.*, 2003; Li *et al.*, 2007). The usage of Pb-free fuel in recent years has reduced pollution sources by eliminating the means of transportation. However, the vehicle is not the only source of Pb pollution. Tong *et al.* (2000) in Zhang *et al.* (2012) stated that mining activity and metal smelting in China have emitted 357 to 857×10^6 kg/year of Pb to the environment.

Smelting activities are frequently conducted by local villagers in Growong Lor who mostly work as brass workers. Growong Lor village is located in Juwana District, Pati Regency, Central Java, Indonesia. Approximately 1% of its citizens (6668 people) work in the field of brass craft home industry. Each brass craft home industry employs 10 to 20 workers. Brass is an alloy made of several main elements such as 65.493 % of Copper (Cu), 34.506 % of Zinc (Zn) and other components including Pb, of which the highest level measured was 0.46% (Supriyanto, 2010).

Brass raw materials are obtained from scrap metal, which can be classified as toxic and hazardous waste. There is a high possibility that this poisonous waste will be released to the air. Brass craft workers are most vulnerable to Pb exposure in the smelting process, and they do not equip themselves with standard safety kit.

One of the most dangerous pollutants from smelting process is Pb. The lead pollutant contaminates the environment and can be absorbed by human through inhalation and ingestion. However, lead is absorbed more efficiently through

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inhalation than ingestion in the ratio of 10:1 (Neal and Guilarte, 2012). Tena and Clara (2012) stated that particles with mass median aerodynamic diameter (MMAD) more than 10 μm accumulated in the oropharynx, whereas particles sized 5 to 10 μm could pass through the respiratory tract; even worse, the small particle sized 0.5 to 5 μm could enter the bronchiole and alveoli. Pb is commonly inhaled by those who live and work in the metal smelting area (Hilts, 2003, Rodrigues *et al.*, 2010). The Occupational Safety and Health Association (OSHA) decided in 2005 that the threshold value of a work environment for lead in form of organic lead, dust and vapour is 0.05 mg/m^3 with not more than 8 hours exposure/day or 40 hours/week.

Pb is known as a potent neurotoxin. Pb can damage both structural and functional roles of neuron system. Wang *et al.* (2011) stated that Pb could penetrate into the blood brain barrier. Loss of the central nervous system caused by Pb was found in Antonio *et al.*, 2003; Erazi *et al.*, 2010; Sansar *et al.*, 2010. Pb is more often accumulated in the hippocampus (Marchetti, 2003). This is the location of neurons whose role is producing serotonin (Berger *et al.*, 2009).

Pb intoxication is also known for causing a decrease of monoamine neurotransmitter concentration, including serotonin (Sidhu and Nehru 2003). A low level of serotonin has been identified in blood lead by Kala and Jadhav (1995) and Naqvi *et al.* (2010). The drop in serotonin level is suspected to be stimulated by structural and functional damage of the neuron system. Damage to neuron tissue that produces serotonin has been detected by Prasanthi *et al.* (2010). In mice, the higher amount of blood lead has caused the greater activity of Monoamine oxidase A (MAO A) (Shin *et al.*, 2007). Meyer *et al.* (2006) stated that MAO A might play a vital role in activating several neurotransmitters including serotonin.

This study examines the increase of blood lead level in brass craft workers caused by Pb chronic exposure in the work environment. In addition, the effects of elevated blood lead level on the level of MAO A enzyme and serotonin neurotransmitters are also investigated. Finally, this study analyses the correlations which exists between blood lead level, MAO A level, and serotonin level in the body of brass craft workers.

2. Materials and Methods

2.1 Research design

This research was an observational analytic study and was conducted using the cross-sectional method. The sample comprised 55 brass craft workers who passed the expected inclusion criteria in Growong Lor village, Juwana District, Pati Regency, Central Java Indonesia. In detail, the inclusion criteria were (1) male or female brass craft worker; (2) aged between 17-70 years; (3) had been working continuously at brass smelting for the last 2 years; and (4) had passed elemen-

tary level of education. The independent variable was the blood lead level while the dependent ones were the levels of MAO A and serotonin.

2.2 The measurement of lead level in the air

The particular measurement of lead concentration in the air at the research site was made with a High Volume Air Sampler (HVAS) tool and filter medium for one hour. The bounded lead inside the suspended particles was digested by acid solution. Lead level analysis was made through wet destruction method using Atomic Absorption Spectrophotometry (AAS).

2.3 Blood sampling procedure

Before the blood sampling, the respondents were informed about the objectives of the research and some possible effects; they later filled in an informed consent form as evidence of their approval. Eight millilitres of blood were taken from respondents' vena by a medical crew.

2.4 Collection and storage of blood samples

During transport from the sampling site, blood samples were stored in a cooler box (4°C). The samples were brought to the laboratory of GAKI Medical Faculty of Diponegoro University Semarang, Central Java, Indonesia. Each blood sample (8 ml) was divided into two tubes; 5 ml in the first tube containing EDTA (Sigma-Aldrich), which used to measure blood lead level; and 3 ml in the second tube without EDTA. Both blood samples were centrifuged at 1000 g for 15 min. Produced blood serum was used to measure MAO A and serotonin level. Blood samples were stored at -20°C and were later analyzed for level of lead, MAO A, and serotonin.

2.5 The measurement of blood lead level

Blood lead level analysis was based on Atomic Absorption Spectrophotometry method and was started by separating Pb element from blood tissue. Prior to measurement, sample powderizing was performed for 8 h. The Pb ions contained in ashes were simultaneously dissolved by 0.1 M HCl (1 ml) (Sigma-Aldrich) and 0.1 M HNO_3 (1 ml) (Sigma-Aldrich). The next step was the atomization using graphite furnace. Pb ions reacted to Pb lamp rays. The interaction was in the form of absorbance of atomic radiation, and its amount was checked on AAS monitor. The amount of rays' absorbance was proportional to blood lead level.

2.6 The measurement of MAO A level

The amount of MAO A enzyme level and serotonin level were measured using enzyme-linked immunosorbent assay (ELISA) sandwich immunoassay technique. MAO A level assessment was based on ELISA Kit (CSBE-E10144h

CUSABIO). All reagents, working standards, and samples were prepared in accordance with the standard protocol. One hundred μL of standard and sample were added per well. After that, the wells were covered with an adhesive strip. The samples were incubated for 2 hours at 37°C . A plate layout was provided to record standards, and samples assayed. The liquid of each well was removed, without a wash. 100 μL of Biotin-antibody (1x) was added to each well, which was then covered by a new adhesive strip and it was incubated for 1 hour at 37°C (Biotin-antibody (1x)). Solution appeared cloudy; it was warmed up to room temperature and then mixed gently until a uniform solution appeared. Each well was aspirated and washed three times with Wash Buffer (200 μL) using a multi-channel pipette, and left for 2 minutes. Complete removal of liquid at each step was essential to good analytical performance. After the last washing, any remaining Wash Buffer was removed by aspirating or decanting. The plate was inverted and then blotted against clean paper towels. A 100 μL of HRP-avidin (1x) was added to each well, which was then covered with a microtiter plate with a new adhesive strip and incubated for 1 hour at 37°C . The aspiration/wash process was repeated five times. Subsequently, 90 μL of TMB Substrate was added to each well, which was then incubated for 15-30 minutes at 37°C protected from light. Finally, 50 μL of Stop Solution was added to each well and the plate gently tapped to ensure thorough mixing. The optical density of each well was measured within 5 minutes, using a microplate reader set to 450 nm. It was suggested to subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction would correct the optical imperfections in the plate. Readings were made directly at 450 nm without correction might be higher and less accurate (CSBE-E10144h CUSABIO).

2.7 The measurement of serotonin levels

The amount of serotonin level was measured using enzyme-linked immunosorbent assay (ELISA) sandwich immunoassay technique. The serotonin level was measured with Serotonin ELISA (RE59121 IBL International GMBH). All reagents, working standards, and samples were prepared as directed by the protocol. All reagents, standard, acylated control and acylated sample were prepared. 50 μL of each standard, acylated control and acylated sample were pipetted into the respective wells of a micro titer plate. Then, 50 μL of serotonin biotin was added into each well. Also, 50 μL of serotonin antiserum was pipetted into each well. The plate was covered with adhesive foil and was incubated for 90 min at RT ($18-25^\circ\text{C}$) on an orbital shaker (500 rpm). The adhesive foil was removed and then the incubation solution was discarded. The plate was washed $3\times$ with 250 μL of diluted wash buffer. Excess solution was removed by tapping the inverted plate on a paper towel. The amount of 150 μL of freshly prepared enzyme was conjugated into each well, and the plate covered with adhesive foil. The sample plate was incubated for 60 min at RT ($18-25^\circ\text{C}$) on an orbital shaker

(500 rpm). Subsequently, the adhesive foil was removed and incubation solution was discarded. The plate was washed $3\times$ with 250 μL of diluted wash buffer. Excess solution was removed by tapping the inverted plate on a paper towel. Pipetting was carried out at the same time intervals for substrate and stop solution. It was suggested to use positive displacement and to avoid formation of air bubbles. 200 μL of freshly prepared PNPP substrate solution was added into each well and then incubated for 60 min at RT ($18-25^\circ\text{C}$) on an orbital shaker (500 rpm). The substrate reaction was stopped by adding 50 μL of PNPP stop solution into each well. The contents were briefly mixed by gently shaking the plate. The optical density was determined with a photometer at 405 nm (Reference-wavelength: 600-650 nm) within 60 min after pipetting of the stop solution (RE59121 IBL International GMBH).

2.8 Statistical analysis

Statistical analysis was performed based on path analysis with the use of SPSS 17.0 for Windows. Path analysis was used to examine the effect of independent variables that is blood lead level on the dependent variable, which is serotonin level. The effect of blood lead level on serotonin was analyzed through direct and indirect pathways. Indirect pathways were through the intervening MAO A enzyme level.

3. Results and Discussion

Lead (Pb) level measurement in 4 research sites revealed Pb pollution levels of 0.008, 0.1014, 2.00, and 19.5 mg/m^3 , respectively. It is obvious that in all study sites, Pb air is still below the Permissible Exposure Limit (PEL) determined by OSHA's lead standard (2005) of 0.05 mg/m^3 of air, as average over an-8 hour period.

The average blood lead level of respondents was 59.71 ± 20.83 $\mu\text{g}/\text{dl}$ with the lowest and highest levels 24.21 $\mu\text{g}/\text{dl}$ and 98.60 $\mu\text{g}/\text{dl}$. From all respondents, 44 persons (80%) are proved surpassed the OSHA (2005) lead threshold for the worker, which is determined at 40 $\mu\text{g}/\text{dl}$.

Although Pb level in the workplace ambient air is not entirely above the permitted threshold, it is proven that the blood lead levels of 80% of workers are categorized as abnormal (higher than 40 $\mu\text{g}/\text{dl}$). This can be explained as follows. The workers were in the workplace for eight hours/day. They had already worked there for at least two years, so they have possibly chronically exposed by Pb. Consequently Pb accumulation happened. Pb accumulation is reinforced by the characteristic of Pb that difficult to be eliminated from the body. In twenty four hours, only 500 mg of Pb can be eliminated through urine (WHO 2011). This is because Pb is very readily binds with the tissue in the body. Pb can be bound to Haemoglobin for about 25 days, in adipose for about 40 days, even in bone tissue for 25 years (Nordberg, 1998). From the 50% of Pb that is absorbed, about 90% of it will be deposited in body's skeleton (Kosnett,

2012), and will be released to the blood if the blood lead levels decrease.

The average value of MAO A level was 6.72 ± 5.78 IU/ml and serotonin average level is 63.45 ± 36.66 ng/ml. According to the typical value of serotonin as approved by US National Institutes of Health which is 101-283 ng/ml, the serotonin level of 87% of the samples was below the standard value. The comparison between the normal and abnormal of blood lead, MAO A and serotonin levels is shown in Figure 1.

In order to examine the correlation between blood lead, MAO A, and serotonin, Pearson correlation matrix is used. The result showed that blood lead level significantly impacts to MAO A and serotonin level. Path analysis was chosen to analyze the relationship model among the variables, to find out the most influential pathway by which serotonin level is affected. Figure 2 shows the path of correlation.

Based on path analysis, the result suggests that the path coefficient value of blood lead level effecting MAO A level was 0.70. The coefficient of MAO A effects on serotonin level was -0.587. It is found that the strength of blood lead effects through MAO A pathway (indirect effects) in decreasing serotonin level was at -0.411 value, while path coefficient of blood lead effects on serotonin level without passing MAO A channel (direct effects) was only -0.381. This result indicates that the decrease in serotonin level is caused by the increase of blood lead level through the MAO A pathway (indirect effect), and it has more impact than the direct effect of blood lead on serotonin.

This study reveals that high blood lead level tends to be followed by an increase of MAO A enzyme level, whereas MAO A plays a significant role in neurotransmitter metabolism, and MAO A abnormality level will cause some psychological problems. MAO is an intracellular enzyme located in the mitochondrial membrane and is distributed throughout the human body. MAO oxidizes amine into aldehyde and peroxide. MAO oxidizes several substrates including monoamine neurotransmitters, which are amines obtained from food and xenobiotics (Kalgutkar *et al.*, 2001; Strolin Benedetti *et al.*, 2007). There are two forms of MAO;

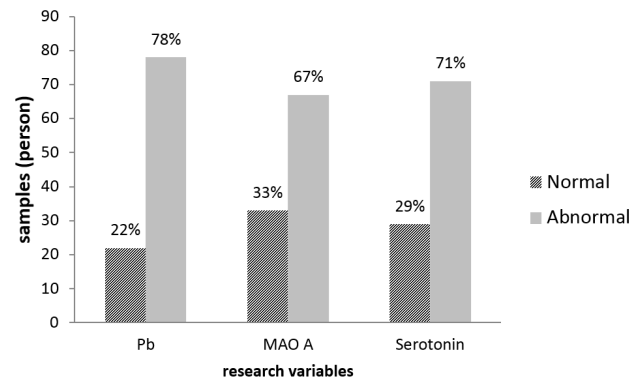


Figure 1. Comparison between normal level and abnormal level of Pb, MAO A and serotonin in blood of brass home industry workers.

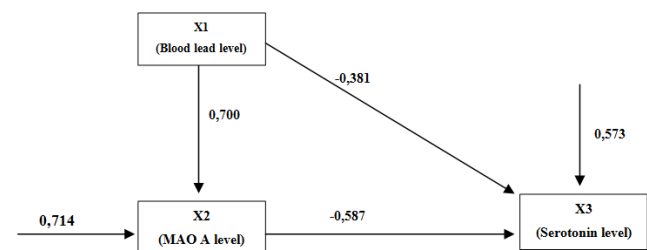


Figure 2. The model structure of simultaneous influences between Pb and MAO on serotonin level.

MAO A and MAO B; they have different substrates and specific inhibitors. MAO A is sensitive to clorgilin inhibitors and prefers to catalyze the reaction by oxidizing monoamine neurotransmitters includes serotonin. By contrast, MAO B works with Benzilamine and 2-feniletilamin as its substrates (Youdim *et al.*, 2006).

The increase of MAO A level is consistent with Shin *et al.* (2007) who stated that the high level of Pb triggered a greater increase of MAO A activity more than did a lower level of Pb. According to Chin *et al.* (1992, 1993 cited by Shin *et al.* (2007), the increase of MAO activity affected by Pb exposure is triggered by the increase of Tyrosine Hydroxylase

Table 1. The result of path analysis of Pb effects on serotonin level with MAO A as intervening variable.

Variable effects	Causal effects				Total effects
	Direct	Indirect (through a path)			
		X2	X3	X2 and X3	
X1 to X2	+0.700*	-	-	-	+0.700
X1 to X3	-0.381*	-0.411	-	-	-0.802
X2 to X3	-0.587	-	-	-	-0.587

*The (-) sign shows that when the value of first variable increases, then other variables value will decrease. The (+) sign indicates that the correlation between variables either increase or decrease

(TH) activity and Dopamine- β Hydroxylase (DBH) activity caused by chronic Pb exposure. Smith *et al.* (2005) stated that TH and DBH are enzymes that play roles in epinephrine production. TH enzyme functions as the oxidoreductase with tetrahydropteridin as its cofactor. This protein will change tyrosine into L-dihydroxyphenylalanine (L-dopa). L-dopa is later converted to 3,4-dihydroxyphenylethylamine (dopamine) using dopa decarboxylase enzyme and pyridoxal phosphate. Dopamine then passes through conversion stage and proceeds to become norepinephrine through dopamine β -hydroxylase (DBH).

The increasing activity of TH and DBH enzymes will cause an increase of epinephrine and norepinephrine levels. As the most responsible enzymes in regulating the balanced amount of monoamine neurotransmitter, TH and DBH are compensated by the rise of MAO A level. Together with Catechol-O-methyltransferase (COMT), MAO A will degrade catecholamine metabolites such as dopamine, epinephrine, and norepinephrine. However, Shin *et al.* (2007) also stated that the change of MAO A activity caused by Pb chronic exposure was more likely to happen rather than the change of TH and DBH activity. In the end, this causes an imbalance between the increase of TH and DBH activity and MAO A level, which later causes ambiguity in brain monoaminergic system.

The increase of MAO A level is actually followed by a decrease in serotonin level. The low level of serotonin will increase blood lead level as indicated by Kala and Jadhav (1995); Naqvi *et al.* (2010). A low level Pb exposure in mice could change the dopamine and serotonin specific metabolisms. High level of blood lead decreases the serotonin level in the nucleus acumbens, frontal cortex, and medulla. However, there are no chance of serotonin level in the striatum, hypothalamus, or hippocampus. The serotonin metabolite, 5-hydroxyindole acetic acid (5HIAA) is decrease in the frontal cortex, when the blood lead level is high. The drop in serotonin level is probably processed through serotonin metabolic pathway. High blood lead level has stimulated the increase of MAOA level (Shin, 2007). The increase of MAOA level will result in serotonin decrease because MAO A is the degrading enzyme for serotonin (Bortolato *et al.*, 2010).

An increase of blood lead level also causes structural damage effects in the central nervous system. The structural damage effect on the central nervous system is caused by Pb penetrating the brain. Other research that used radioactive scanning on mice demonstrated that Pb could break through brain tissue in the form of PbOH free ions compound. This complex passed the blood brain barrier through passive diffusion (Yoke, 2006). Pb ions could also be transported by cations transporters. Divalent metal transport 1 (DMT1) could rapidly transport Pb into the striatum, cortex, hippocampus, and cerebellum (Williams *et al.*, 2000). Pb ions are also capable of penetrating blood brain barrier (Wang *et al.*, 2011). In addition, research shows that Pb is often accumulated in the hippocampus (Marchetti, 2003). Prasanthi *et al.* (2010) proved that Pb exposure of adult and young mice increased

the level of oxidative stress in the cortex, hippocampus, cerebellum and medulla. In the central nervous system, the neurons that produce serotonin are located in the central brain (superior central nucleus and dorsal raphé nucleus), reticular pontine formation (pontine raphé nucleus), central inferior and medula (nucleus raphé obscurus, nucleus raphé magnus, and nucleus pallidus) (Berger *et al.*, 2009). Damage to neurons where serotonin produced causes the production of serotonin to decline. ‘

Pb can also affect many targets of the neural system (Devi *et al.*, 2005), one of them is catecholamine. Catecholamine plays crucial roles in emotional and motor coordination with other neurons. The intervention of the cholinergic system is the primary factor whereby Pb induction may cause brain dysfunction. Some researchers also reported that Pb toxicity reduced the concentration of monoamine neurotransmitter, including serotonin (Sidhu and Nehru, 2003).

This study has proven that there is a significant effect of Pb level on the increase of MAO A level and the decrease in serotonin level following a cause-effect pattern.

4. Conclusions

The study concludes that the increase of blood lead level in brass craft workers has significant implication on the elevated MAO A level and decreasing serotonin level. The reduction of serotonin level is strongly influenced by MAO A increase rather than by other factors. Therefore, the relationship pattern between blood lead, MAO A, and serotonin levels is of a cause-effect nature.

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