

USE OF HAEMATOLOGICAL AND IMMUNOLOGICAL BIOMARKER AS INDICATOR OF Pb INTOXICATION

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ABSTRACT

Transportation and industrial activities give high contribution to Pb exposure. The level of Pb in the air increases to 1.6-42 µg/m³ (WHO standard 0.001µg/m³) and in water reaches 120 µg/dl (WHO < 60 µg/dl). There are some researchers who examined the impact of Pb to health, but the results had insignificant impact. The objective of the study was to reveal the impact of Pb exposure to haematological and immunological system. The research was done by the metaanalysis method from similar researches. This article metaanalyses the research that used haematological and immunological system variables to Pb exposed objects. Variables in the haematological system which can be used as biomarker are the increase young reticulocyte, basophilic stippling, howel jolly bodies (normally not occurred), thrombocytosis, and blood zinc protoporphyrin > 1.3 µmol/l. Biomarker variable in the immunological system was the change of Th1/Th2 balance, in which Th1 showed an increase. In conclusion, haematological and immunological biomarker can be used as the indicator of Pb exposure. Further research is suggested in order to reveal the more specific and sensitive variables.

Keywords: *Plumbum (Pb) exposure, haematologic indicator, immunologic indicator*

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INTRODUCTION

Pb pollution has become an extensive problem in the community. The source of Pb pollution is the industrial activities as well as air pollution from the combustion gas of motor vehicles. Industrial pollution may have direct effect on the workers through emitted fumes as well as through underground water waste that in turn pollutes the source of drinking water (Beliles & Robert 1996). A study by Mukono (1988) found that Pb in well water located nearby an accu melting factory was 120 µg/dl (WHO value for Pb level in water surface was 60 µg/dl). For air Pb level in the same place was averagely 1.2 µg/m³ (according to WHO allowable air Pb level was 0.001 µg/m³). The average Pb level of population consuming the well water was 466.94 µg/dl (Mukono 1998). Pb pollution in main roads due to fuel combustion was ranging between 0.0016–0.042 mg/m³, and Pb level of police officer in duty in such roads is averagely 105 µg/dl (Darmasemaya 1998). These blood Pb levels are far higher than that allowed by WHO of 40 µg/dl blood.

The recent level of air pollution in Indonesia, particularly in urban area, has reached a level that needs a particular anticipation. The source of pollutant that comes from transportation activity provides a

contribution of 66.34%, industrial activity (accu, ceramic paint, metal melting, and coloring) 18.9%, human settlement activities 11.2%, and activities related to dumping 3.68% (Mukono 1998). These various health disorders occur because after being in the circulation, Pb moves forward to several target organs. The emergence of symptoms due to Pb intoxication depends on blood Pb level and duration of exposure.

Table 1. Blood Pb level and its impact on health (Tong et al. 2000)

Blood Pb level (µg/dl)	Effects
10	No effects
20	Increased Hb, reduced Vitamin D, reduced CNS development
40	Reduced urine ALA, increased urine coprophorphyrine, Reduced nerve conducting velocity
60	Severe anemia
80	Severe central nervous system disorder
> 100	Death

High blood Pb level was found in several studies among population and workers who were exposed to Pb in high

concentration. These studies were conducted by Mukono (1998, 1999), Rustam (1997), Chomsatun (2000), Darmasemaya (1999), and Rufiati (2002). These studies showed that blood Pb had no effect on the occurrence of anemia and health profiles complained by the respondents were not specific. If the results of those studies are used as reference, it can be perceived that Pb poses no harm for the community. The problem is that we need more specific indicator from other immunological and hematological biomarkers. The objective of this study was to determine the presence of Pb intoxication by using variables in hematological and immunological system. The changing in the systems was analyzed to find specific and sensitive biomarker in the emergence of Pb intoxication. This specific and sensitive biomarker can be used as indicator of Pb intoxication, so that it can be employed for early detection of Pb intoxication.

MATERIALS AND METHODS

This study used metaanalysis method. It is a technique combining several results of similar studies. The analyzed studies were those using variables in hematological and immunological system in objects exposed to Pb. It was expected that from those results the indicator of Pb intoxication could be determined by using hematological as well as immunological biomarkers.

RESULTS

Several reports of Pb-suspected cases treated at several medical centers showing similar symptom characteristics were as follows: First, the case from New Hampshire. A woman aged 37 years had abdominal pain, nausea and vomiting for 6 days. Blood examination revealed moderate basophilic stippling, blood lead level (BLL) above normal, Zinc protoporphyrine increased 7 times, and, after being given with chelating agent for 2 weeks, the BLL returned to normal (CDC 2006). The next case was from California, where a woman of 31 years-old complained nausea, vomiting, and lower abdominal pain. The result of laboratory examination revealed persistent microcytic anemia, prominent basophilic stippling, and blood lead level of 112 µg/dl. After being given with chelating agent for 2 months, BLL reduced to 22 µg/dl (CDC 2006). Another case was also in California. A man aged 34 years was treated due to the complaints of hip and abdominal pain. The BLL was 80 µg/dl, and after being given with chelating agent it reduced to 17 µg/dl (CDC 2006). In New Zealand, a woman of 51 years old had nausea, vomiting, abdominal pain, and myalgia.

Laboratory examination revealed normochromic anemia with prominent basophilic stippling, and the BLL was 69.3 µg/dl. After being given with chelating agent for 2 weeks, the BLL became 60 µg/dl. Chelating agent was continued until week 3, and the BLL became 55 µg/dl, and after 6 week therapy the BLL became 30 µg/dl (Anne et al. 2005). In another case, a woman aged 24 years with pregnancy age of 30 weeks had a symptom of abdominal pain. Blood examination revealed basophilic stippling, and BLL of 107 µg/dl. After receiving chelating agent, 36 hours later the patient had antepartum bleeding and induced delivery was carried out. The baby was born with bodyweight of 1.6 kg, female, Apgar score 4-6, Basophilic stippling was not found in neonatal blood examination. The BLL of the placenta was 157 µg/dl. The mother and the baby were given with chelating agent until BLL reached below normal value (Tait et al. 2002).

Anetor conducted an observational study to 86 workers exposed to Pb and 51 control groups not exposed to Pb. The result showed that the BLL of exposed group was higher than control group, while the levels of erythrocyte protoporphyrine (EPP) and probobilinogen (PBG) were the same in both groups. Hb, PCV and MCHC reduced significantly compared to that in control group. However, basophilic stippling was not found in both groups (Anetor et al. 2002).

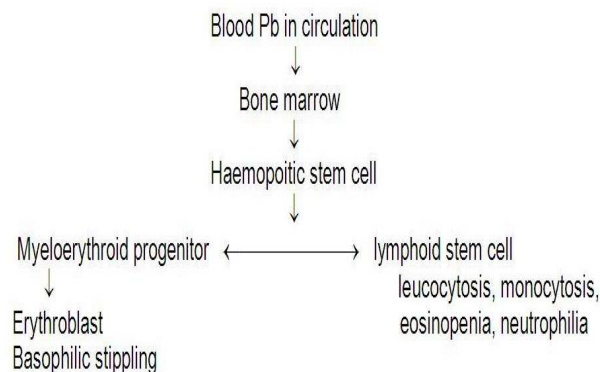


Figure 1. The effect of Pb exposure on haemopoietic stem cell (Mugahi et al. 2000).

The effect of Pb on immune system was revealed by McCabe (1999) who conducted a study on mice given with Pb acetate-containing drinks. The result of his study revealed the presence of delayed type hypersensitivity (DTH). The occurrence of such reaction depends on the way Pb is delivered, since intraperitoneal Pb administration did not induce DTH reaction (McCabe et al. 1999). Since the emergence of DTH the role of immune system are largely controlled by Th1 (McCabe et al. 1999). This finding was

confirmed by the result of study by Rodney (2000) who conducted immunotoxicological study based on immunological principles. This study was carried out in environment where Pb exposure was present. Rodney concluded that Pb exposure induce balance disorder between Th1 and Th2. This disorder indicated that Th1 increased more and the function of Th2 decreased. It also indicated the presence of cell mediated immunity or delayed type hypersensitivity reactions (Rodney 2002). Th1 had a role in cellular reaction, i.e., the delayed type hypersensitivity (Baratawidaja 1996; Rodney 2002; Stites 1998).

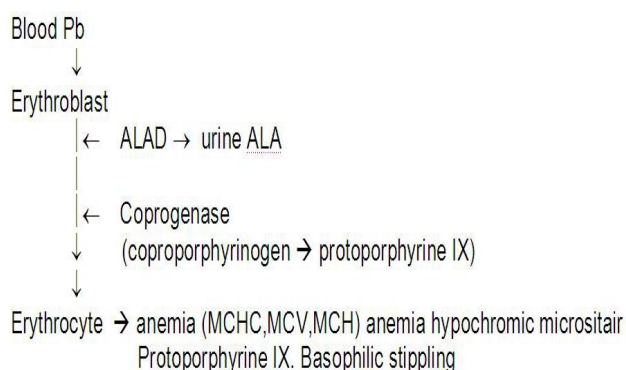


Figure 2. The effect of Pb on heme synthesis system (WHO 1999).

Bunn (2001) conducted an experimental study in rats given Pb-acetate containing drinking water at the beginning of pregnancy (day 3-9) and/or at the end of pregnancy (day 15-21). There was a significant occurrence of delayed type hypersensitivity (DTH) (Lane & Kemper 2001). Yan ZZ (2003) conducted an observational study to 217 pre-school children (96 male and 121 female) aged 3 to 6 years. Respondents lived permanently in rural area in Zhejiang Province. From his examination, it was found that 63 (23%) of the respondents positively had Pb intoxication (blood Pb level > 0.483 $\mu\text{mol/L}$). By conducting t test in case and control group, he found significant difference in immune function variables of lymphocytes (CD+4 and CD+8) and erythrocytes (Bunn et al. 2001). Delayed type hypersensitivity or cell-mediated immunity (CMI) are also called as delayed type hypersensitivity (DTH) or type IV reaction. This reaction occurs after previous antigen exposure. This reaction occurs because T cell response has been sensitized by certain antigen. In this case, there is no role of antibody. Due to such sensitization, T cell releases lymphokine, such as macrophage inhibition factor (MIF) and macrophage activation factor (MAF). These activated macrophages could induce tissue damages, and this increased Th1

would produce IFN-gamma, IL-2, and TNF (Baratawidaja 1996; Rodney 2002; Stites 1998).

CMI or DTH or type IV reaction requires sensitization period of 1-2 weeks to increase the number of cloned T cell specific for particular antigen. The antigen should be presented first by APC (antigen presenting cell). Antigen that can trigger such reaction may present as alien tissue (such as allograft), intracellular microorganisms (virus, mycobacterium), protein or skin-penetrable chemical substances that merge with protein that has a function as carrier (Baratawidaja 1996; Rodney 2002; Stites 1998).

DISCUSSION

Pb and its compounds come into the body through inhalation and digestion. Absorption through the skin is only significant in organic Pb (lead alkyl and lead naphthalene). Lead intake in general population is estimated between 100 to 350 $\mu\text{g/day}$. Although the primary sources are food and water, as much as 20 μg may be absorbed from inhalation of lead fumes and urban environmental particles that contain pollutant (Beliles 1996; Siswanto 1999; WHO 1999). Health hazard induced by Pb in the air depends on the size of the particle. Particle smaller than 10 μm can be halted in the lung, and larger particles precipitate in upper respiratory tract, and, through mucociliary movement, are brought upward, expelled or swallowed (Siswanto 1999; WHO 1999). The absorption level of respiratory tract mucosa depends on its solubility, shape, and the size of particle, smoking habit, and chronic respiratory tract disease. Averagely 40% of inhaled Pb-oxide absorbed through the lungs and approximately 5-10% of digested Pb will be absorbed in digestive tract. Tetraethyl lead fumes are well-absorbed in the lung. Pb absorption is increased in persons having Fe and calcium deficiency, and in those consuming much fats (Siswanto 1999). Pb absorption in children and infants is higher than that in adults (Siswanto 1999).

Pb is distributed to brain, kidney, liver, skin, muscles, and Pb is able to penetrate the placenta (Siswanto 1999; WHO 1999). Pb distribution mechanism in the body is through red blood cells, since 99% of Pb are bound by these cells, and 1% by plasma, and distributed to soft tissues. Pb half-life is 25 days. Pb in the body can be classified into, first, Pb in blood and soft-tissue (bone marrow, nerve system, kidney, and liver). There is balance between Pb level in blood and that in soft tissue (Siswanto 1999; WHO 1999), and it is estimated that Pb direct intoxication occurs only in soft tissue (WHO 1999). Second, Pb in bones and teeth. Pb in hard tissue remains bound tightly to the tissue and it becomes toxic

if the accumulation is distributed to soft tissue (WHO 1999). Pb effect in hematopoietic system can occur directly to bone marrow, and affect the erythropoietin system. The effects of hematopoietic system include hemoglobin synthesis inhibition, shortening of red blood cell age (both factors render the Pb-exposed workers to suffer from anemia), inducing disorders in iron metabolism and globin synthesis in red blood cells, and inhibiting the activities of various enzymes needed for haem synthesis, such as delta-aminolevulinate dehydratase (ALAD), haem synthetase (ferrochelatase), and coproporphyrinogen decarboxylase. The inhibition of these three enzymes may result in increasing excretion of delta-aminolevulinate acid (ALA) and coproporphyrine III in urine, and pro accumulation of zinc protoporphyrine IX (ZnPP) increase in erythrocyte (Beliles 1996; Siswanto 1999; WHO 1999).

Tong (2000) recommended variables of reduced ALA urine and increased coproporphyrine urine in men whose blood Pb level is 40 µg/dl. WHO (1999) recommended that reticulocytosis and basophilic stippling be present in peripheral blood examination. Basophilic stippling is ribosome basophilic-dotted erythrocyte. Such profile is found although there is no anemia (WHO 1999). Mugahi (2000) carried out a pre- and post control experimental study in adult male rats that were divided into treatment and control group, each comprising 15 rats, and receiving Pb-acetate containing drinks for 92 days. The results showed that there was significant increase of basophilic stippling, howell jolly bodies, anemia, leukocytosis, monocytosis, eosinopenia, neutrophilia and thrombocytosis. This suggested the presence of microcytic hypochromic anemia. This profile indicates the presence of interaction between Pb and Fe and Cu metabolism. The increase of leucocyte count is related to inflammatory effect in lymphatic organ. Valentine (1976) conducted an observational study in Pb-intoxicated patients, and found that 12% had hemolytic anemia, and 15% had basophilic stippling (Valentine et al. 2006).

The diagnosis of Pb intoxication should be established in several stages (WHO 1999). First, anamnesis. Anamnesis is directed toward the toxic effect on gestational system, peripheral and central nervous system disorder in kidney, such as intestinal colic (localized pain around or under the umbilicus), which is preceded by severe constipation. The symptom is usually occurs in chronic Pb intoxication. Symptoms that occur in nervous system in acute encephalopathy are convulsion, delirium or comatose, while those in chronic and subclinical encephalopathy include delayed action and psychomotoric disorder. Delirium, confusion, insomnia and sometimes schizophrenia are symptoms that occur in severe cases of alkyl Pb intoxication.

Anamnesis is also directed toward the possibility of air Pb exposure in workplace or housings, and the possibility of Pb intoxication in drinking water. Second, physical examination. Physical examination is performed completely from general condition to extremities. However, the predominant profiles in general condition are pallor or anemia, and the presence of gray pigmentation in the gums (lead lines). Third, exposure evaluation, in which air Pb exposure should be more than 0.001 µg/m³ or Pb exposure in drinking water is more than 60 µg/dl.

Fourth, laboratory examination, which include, first, to demonstrate the presence of Pb in blood. There are references indicating different standard values, i.e. by WHO (1997) of 40 µg/dl and by SHARP (2004) of 25 µg/dl. Second, hematological indicator examination, which is including the enzymatic changes occurring in erythrocyte maturity process, and the hematological change itself. To observe the enzymatic change, an examination is done to find whether there is an increase of the enzyme delta-aminolevulinate dehydratase (ALAD) in blood, an increase of delta-aminolevulinate (delta-ALA) acid in urine of more than 152.52 µmol/liter, and urine coproporphyrine level of more than 0.469 mmol/liter, and zinc protoporphyrine IX (ZnPP in red blood cells) of more than 1.779 µmol/liter (Siswanto 1999; WHO 1999). Then, an examination is carried out on the change of hematological system, which observes the reduction of hemoglobin content, indicating a profile of microcytic hypochromic anemia, increased young reticulocyte count, and basophilic stippling, howell jolly bodies, leucocytosis, monocytosis, eosinopenia, neutrophilia and thrombocytosis (Mugahi et al. 2000; Siswanto 1999; WHO 1999). The third step in laboratory examination was to measure toxic effect in immune system by using immunological indicators. Immunologically, the balance of Th1/Th2 function will change, in which the function of Th1 will be enhanced. Enhanced function of Th1 may affect the production of IFN-gamma, IL-2 and TNF (Baratawidjaja 1996; Rodney 2000; Stites 1998). Those examinations revealed that determining Pb intoxication is not sufficient by relying only on the examination of blood Pb, Hb, and environmental Pb. A number of other variables should also be scrutinized to reveal how much Pb exposure and intoxication had provided effects on health.

CONCLUSION

Since the effect of Pb intoxication on hematological system can be directly hit the bone marrow, it is necessary to evaluate the hematological and immunological indicators that may reveal the presence

of blood Pb intoxication. Hematological and immunological biomarkers that should be tested are microcytic hypochromic anemia (normal Hb 12- 14 g/dl), increased young reticulocyte count, presence of basophilic stippling, presence of howell jolly bodies (normally absence), thrombocytosis, blood zinc protoporphyrin (normal < 1.3 $\mu\text{mol/l}$), and increased activity of delta-aminolevulinate dehydratase (ALAD) in blood (normal < 2.8 $\mu\text{mol/mmol}$), and balanced Th1/Th2, in which Th1 is increased. A study should be undertaken to groups exposed to Pb using hematological and immunological variable. Validity test should also be carried out that include the sensitivity and specificity. Both tests use gold standard of Hb level of 25 $\mu\text{g/dl}$. From the sensitivity and specificity tests, we can find the most sensitive and specific biomarker that can be employed as an indicator of Pb intoxication. This indicator can be used for early detection of Pb intoxication.

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