ABSTRACT

BACKGROUND
Lead accumulation in the blood widely known affecting the formation of heme and oxygen transport processes in vital organs, leading to organ failure including the brain synapses. Lead affinity has been recognized influenced by constitutional genotype of aminolevulinate dehydrogenase (ALAD), which encodes for heme synthesis. This research aimed to determine the relationship between plumbum (Pb) and short term memory on each ALAD gene genotyping (ALAD 1-1, ALAD 1-2 or ALAD 2-2) in gas station workers.

METHODS
Seventy six probands from gas station workers were recruited to participate in this research. Each probands was carried out ALAD genotyping using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, lead serum level using atomic absorbent spectrophotometer (AAS), and short term memory was measurement by intelligence structure test (IST).

RESULTS
Proportion of $\delta$ ALAD 1-1, 1-2, and 2-2 were 91.8%, 8.2% and 0% respectively. Lead serum showed 15.84 ppb in homozygous 1-1, and 20.79 ppb in heterozygous. Short term memory in the probands varied from 85 until 117, with average in 99.71. There was significant negative relationship between lead serum and short term memory ($r$=-0.24; $p=0.038$). However, we could not find any significant correlation in each $\delta$ ALAD genotypes.

CONCLUSION
The $\delta$ ALAD genotypes did not modified the relationship between serum lead level and short term memory in gas station workers.

Keywords: Lead, short term memory, $\delta$ ALAD, gas station workers
Polimorfisme aminolevulinate dehidrogenase tidak memodifikasi hubungan antara kadar timbal dan memori

ABSTRAK

PENDAHULUAN
Akumulasi timbal (Pb) dalam darah mempengaruhi proses transportasi heme dan oksigen pada organ-organ vital, termasuk aktivitas sinaps di otak. Afinitas Pb dalam darah dipengaruhi oleh konstitusi genotip gen α aminolevulinate dehydrogenase (ALAD), yang mengkode protein untuk sintesis heme. Penelitian ini bertujuan untuk mencari hubungan antara menentukan adanya hubungan antara kadar Pb dan memori jangka pendek disetiap genotipe gen α ALAD (ALAD 1-1, 1-2 ALAD atau ALAD 2-2 pada pekerja pompa bensin.

METODE
Tujuh puluh enam responden dari pekerja pompa bensin direkrut untuk berpartisipasi dalam penelitian ini. Setiap subyek dilakukan genotyping α ALAD menggunakan metode polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Kadar serum timbal diukur menggunakan atomic absorbent spectrophotometer (AAS), sedangkan pengukuran memori jangka pendek menggunakan intelegence scoring Test (IST).

HASIL
Proporsi genotip αALAD 1-1, 1-2 dan 2-2 masing-masing adalah 91,8%, 8,2% dan 0%. Rerata Pb serum menunjukkan angka 15,84 ppb pada subyek homoizigot α ALAD 1-1, dan 20,79 ppb pada subyek heterozigot. Memori jangka pendek responden bervariasi mulai dari 85 sampai 117, dengan rerata 99,71. Terdapat hubungan negatif yang bermakna antara kadar serum Pb dengan memori jangka pendek (r=-0,24; p=0,038), tetapi tidak ada korelasi yang signifikan untuk setiap genotip α ALAD.

KESIMPULAN
Genotip α ALAD tidak dapat memodifikasi hubungan antara kadar Pb dalam serum dan memori jangka pendek pada pekerja pompa bensin.

Kata kunci: Timbal, memori jangka pendek, α ALAD, pekerja pompa bensin

Heavy metal poisoning becomes government worry especially in environmental issues. Latent effect and its potential to decrease human resources quality have attracted much concern. Nowadays, heavy metal contamination is increasing because of the development of industry, the increase of fuel use, the household modernization, and the change of environment support ability. In China, it was reported that about 53.7% citizens who were screened had blood lead level above the threshold, and mostly in children. Indonesia is also a risky country, moreover in the big cities with its pollution cases. World Bank record showed the impact of lead air contamination in Indonesia caused 350 cases of heart disease, 62,000 cases of hypertension, and 340 deaths per year. Augmentation of pollution in big city like Jakarta reached 36.6% above the
Lead contamination in Indonesia is caused by high consumption of fuel. From 1996, there are more than 9 million kiloliters fuels used with 7% annual growth. It’s about 36 million kiloliters of fuels in 2010 burns into gas in Indonesia.

The escalating of blood lead level can cause multi effect to many organs like cardiovascular system, hemolymphatic, urinary tract, immune system, reproductive system, and neural system. Neural system is the most sensitive system for lead intoxication. Lead over accumulation cause neuron and brain damage, and also autism. Other research showed that Lead poisoning more than 4 years could cause cancer and disturb cognitive functioning. Short term memory is one of the factors that affect cognitive function. Mechanism of short term memory involves transient modification in the function of pre-existing synapses. It is saved in the modification of specific Ca²⁺ channels.

Blood lead measurements were performed by a quantified using atomic absorbent spectrophotometer (AAS) on 17.0 nm wavelength.

ALAD genotyping

About 10 cc venous blood was drawn from each probands. ALAD genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method with the following primer forward and primer reverse used: 5'-AGACAGACATTAGCTCAGTA-3' and 5'-GGCAAAGAACAGGTCCATTC-3'. PCR profile was as follow: initial denaturation 95°C for 30'', amplicon multiplication with 30 cycles (denaturation 95°C for 30'', annealing 57°C for 30'', and synthesis 72°C for 30''), and polishing step in temperature 72°C for 5' and 26°C for 10''. Primer product then was cut with RFLP technique using MSP1 enzyme.

Assessment of memory function

Short term memory measurement was using intelligence structure test (IST) subtest that done by a psychologist. IST is an intelligence test constructed for subjects aged 14-60 years. The IST is an intelligence test battery that measures verbal, numerical and figural intelligence.

METHODS

Research design

The observational study using cross sectional design was used in this research and conducted from October to November 2011.

Research subjects

Seventy six gas station workers from seven gas stations in Banyumas were recruited to participate in this research. Inclusion criterias for the subjects were Javanese etnicity, age range from 20-60 years old, and have been a worker in gas station for minimum 1 year. As an exclution parameters, subject must be free from kidney disease history, and having no medication treatment for at least 1 month. Informed consent and interview were taken individually to collect the basic information about identity and medical history.
Statistics analysis

Descriptive statistics was used to calculate the frequencies and means of variables, including lead serum levels, short term memory score, ALAD gene polymorphism, sex, age, smoking habit, and alcohol consumption data. The Spearman correlation was used to test the relationship between lead serum levels and short term memory scores with p<0.05 was considered as statistically significant. Computer program STATA was used for the data analysis.

Ethical clearance

This study was performed after getting ethical clearance from ethic committee and conducted inline with Helsinki Declaration.

RESULTS

A total of 76 subjects were recruited into this study, with a mean age was 33.22 ± 10.25 years and most of gas station workers (81.6%) are male. There was also a tendency that most of gas station workers were having smoking habit (60%) but not consuming alcohol (93.4%). Mean blood lead level of all subjects was 14.16 ± 17.51 ppb and the mean score of short term memory was 99.97 ± 9.77. The results showed 45 probands (91.8%) had homozygous ALAD 1-1 and only 4 probands (8.2%) had heterozygous ALAD 1-2. There was no proband that recorded with homozygous ALAD 2-2 (Table1). However, 27 subjects could not be determined due to technical issues, then we excluded these subjects for further analysis.

The pattern of ALAD gene polymorphism was presented in Figure 1. The serum lead levels in workers with the ALAD 1-1 genotype were lower than those with the ALAD 1-2 genotype, and the difference was not statistical significance (p=0.595).

![Figure 1. Genotyping of ALAD polymorphism pattern in gel electrophoresis. Digestion of PCR product using MSP1 result two band available, 582 and 511 bp reflecting ALAD 1 and ALAD 2 alleles](image)

<table>
<thead>
<tr>
<th>Table 1. Characteristics of study participants (n=76)</th>
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<tr>
<td>Variables</td>
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<tr>
<td>Age (years) (\mu)</td>
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<tr>
<td>Sex</td>
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<td>Male</td>
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<td>Female</td>
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<td>Smoking habit</td>
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<td>ALAD 2-2</td>
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<td>铅血血铅水平 (ppb) (\mu)</td>
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<td>短期记忆评分 (\mu)</td>
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\(\mu\) Mean ± SD
Table 2 represents the relationship between lead serum level and short term memory score in all subjects and subgroups. The results indicated that there was a significant negative correlation between the serum lead level with short term memory \((r=-0.24; p=0.038)\). However, there was a different result when we tested the relationship between lead serum level and short term memory in each genotype of \(\alpha\) ALAD. The study also reported that there were no significant relationship on different sex, age group, smoking habit, and alcohol consumption (Table 2). There were no significant relationship between serum lead level and short term memory in subjects <40 years old and in ≥40 years old worker group, the coefficient correlation were \(r=-0.16\) (\(p=0.271\)) and \(r=-0.12\) (\(p=0.581\)) respectively. The relationship between serum lead level and short term memory in smoking and in non smoking worker were not statistically significant.

**DISCUSSION**

Lead is well known material that affects many organs, including the nervous system. In this research, the effect of lead towards short term memory was investigated in gas station workers. The \(\alpha\) ALAD gene polymorphism was conducted as a modifier effect. The results showed that most of gas station workers (81.6%) are male. There was also a tendency that most of gas station workers were having smoking habit (60%) but not consuming alcohol (93.4%). All female workers were not smoking and not consuming alcohol. The previous research on gas station worker in Purwokerto didn’t give the detail about the demographic characteristics such as sex, smoking habit, or alcohol consumption.(18)

The result showed that 45 probands (91.8%) had homozygous ALAD 1-1 and only 4 probands (8.2%) had heterozygous ALAD 1-2. There was no probands that recorded with homozygous ALAD 2-2. This result was consistent with previous research. Yang et al.[16] found that in Caucasian, allele ALAD-1 (88%) was greater than allele ALAD-2 (12%). Kamel et al.(11) also showed 78% of the respondent had ALAD 1-1, 20% of ALAD 1-2, and only 2% had ALAD 2-2.

This result showed that serum lead level serum was lower in worker with ALAD 1-2 than ALAD 1-1. It was also consistent with previous research that the person with ALAD 1-2 and ALAD 2-2 had higher susceptibility to have higher degree of serum lead level than ALAD 1-1.(19-22)
It is increasing the fact that ALAD plays an important role in the bioaccumulation of Lead; however it is still an open question for research. There are two established scenarios to explain these evidences. First, ALAD (particularly ALAD -2) act as the distributor of lead in target organs such as kidneys and brain structures. Second, it is possible that ALAD can serve as a sink, separate from the interaction of lead in blood. ALAD-2 could serve as a substrate with high affinity, lead levels are maintained in blood and protecting other organs. ALAD-2 could serve as a high-affinity substrate, retaining lead in the blood and therefore protecting other organs. In this case, people who have the ALAD-2 allele could experience less severe effects of lead on kidney and brain and lesser accumulation of Lead in bone while at the same time having higher blood lead levels than ALAD-1 homozygote exposed to the same doses of lead.\(^{(23)}\)

It may be also possible that ALAD-2 allele boost the retention of lead in blood and decreases the amount of chelatable Lead. Treatment with DMSA (chelator agent of lead), therefore, would not be as effective in ALAD-2 carriers. Other investigators found that ALAD genotype may influence kidney function, which may also affect excretion of Lead.\(^{(24)}\) The short term memory score mean was 99.97. Based on IST score categorization, the short term memory score mean for gas station worker was low. This low level of short term memory score could be affected by a lot of factor, such as genetic, intellectual stimulation, physical activities, nutrition, and also toxic exposure.

This study showed a significant negative relationship between serum lead level and short term memory in all subjects. It was a weak relationship. But this relationship could give a reason why even low level of lead in the blood could affect cognitive function. Kosnett\(^{(25)}\) already showed that accumulation dose of lead could be a predictor in poor performance of cognitive test in adult. It could be explained from Atkinson- Shiffrin model about short term memory. Short term memory is important in learning process because information should be stored in short term memory first before it could be stored in long term memory which bigger in capacity.\(^{(26)}\) Other significant relationship showed between serum lead level serum and short term memory in non alcohol consumer. It was assumed that the significant relationship could happen because almost all subjects are non alcohol consumer.

This study showed that there was no significant relationship found in the analysis between lead level serum and short term memory in ALAD 1-1 and ALAD 1-2. The results might happened because the small number of sample that analyzed in the group. It was statistically insignificant since proband with ALAD 1-1 only 45 and even in ALAD 1-2 only 4. It was consistent with Lidsky and Schneider statement.\(^{(15)}\) The insignificant relationship between lead level serum and short term memory in male in our study was inconsistent with Weisskopf\(^{(27)}\) and Payton et al\(^{(28)}\) studies. Weisskopf showed that low blood lead level could worse cognitive test result in elderly men. Payton et al also found that men with higher levels of blood and bone lead copied spatial figures less accurately. The relationship in female worker also showed insignificant relationship. It was consistent with Weuve study that relation only significant between bone lead and cognitive performance, not with blood lead level.\(^{(29)}\)

Smoking and age were two factors that could affect short term memory. It was said that smoking could affect cardiovascular and respiratory systems so it could hamper the oxygen and nutrition to the brain. The age itself affected the brain function since neural cell became less optimal in older people.\(^{(30)}\) However, there were no significant result when lead level serum and short term memory relationship in different group age and different smoking habit. Inabilities to record and control genetic factor that affect brain function, especially in short term memory may be important factor that could affect this study.
One limitation of this study was the lack of statistical power and the consequent inability to reproduce associations in the present study to a larger population. Another limitation was the observational nature of the study, making confounding a concern.

CONCLUSIONS

There was significant relationship between lead serum level and short term memory. This study also showed that lead serum level in gas station worker with ALAD 1-2 was higher than ALAD 1-1. However there was no modification effect of ALAD genotypes on the relationship between serum lead level and short term memory among gas workers. The present findings as well as lead toxicity complications will be examined in further longitudinal studies.

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REFERENCES