GFR and Blood Lead Levels in Gas Station Workers Based on δ-Alad Gene Polymorphisms

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GFR DAN KADAR TIMBAL DALAM DARAH PADA PEKERJA POM BENSIN BERDASARKAN δ-*ALAD GENE POLYMORPHISMS*

(GFR and Blood Lead Levels in Gas Station Workers Based on δ-Alad Gene Polymorphisms)

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ABSTRAK

Pendahuluan: Timbal merupakan zat beracun yang dikenal dapat menimbulkan kerusakan pada organ tubuh. Serum timbal sendiri dipengaruhi oleh δ-ALAD gene polymorphisms (Amino Levulinic Acid Dehydratase). Gen δ-ALAD mengkode enzim ALAD yang digunakan untuk sintesis darah. Karakteristik gen polymorphism mempengaruhi nilai GFR sebagai tanda kerusakan ginjal. Tujuan dari penelitian ini adalah untuk menemukan hubungan antara kadar timbal dalam darah dengan GFR, berdasarkan δ-ALAD gene polymorphisms. **Metode:** Desain cross sectional digunakan dalam penelitian ini. Sampel adalah pekerja 38 pom bensin yang ada di Banyumas. δ-ALAD gene polymorphisms dikarakteristikan dengan metode PCR-RFLP, di mana kadar serum timbal dikuantifikasi dengan AAS. Serum kreatinin diukur dengan AAS dan kadar GFR diformulasikan dengan metode Schwartz. **Hasil:** Hasil penelitian menunjukkan bahwa proporsi genotipe ALAD untuk ALAD 1-1, 1-2, dan 2-2 adalah 94,7%, 5,3%, dan 0%. Kadar serum dalam homozygous 1-1 adalah 15,94 ppb dan heterozygote 1-2 adalah 1,15 ppb. GFR responden berada pada rentang 71,11 mL/min sampai 185,20 mL/min dengan rata-rata 117,34 mL/min. Tidak ada hubungan antara serum Pb dan GFR (p = 0,19). Penelitian juga tidak bisa membuktikan hubungan antara GFR dan ALAD gene Polymorphism. **Diskusi:** Dari hasil penelitian dapat disimpulkan bahwa tidak ada hubungan antara kadar timbal dalam darah dengan GFR pada setiap δ-ALAD gene polymorphisms.

Kata kunci: Intoksikasi timbal, GFR, δ-ALAD, pekerja pom bensin

ABSTRACT

Introduction: *Lead is a well-known toxic agent that makes an organ's failure. Lead serum itself is influenced by δ-ALAD gene polymorphisms (Amino Levulinic Acid Dehydratase). δ-ALAD gene encodes an ALAD enzyme used for heme synthesis. The Characteristic of gene polymorphism may result in Glomerulo Filtration Rate (GFR) value as mark of renal failure. The goal of this study was to find correlations between blood lead levels with GFR in terms of δ ALAD gene polymorphisms.* ***Method:*** *A cross-sectional design was used to perform this research. Thirty-eight gas stations workers in Banyumas were recruited in this study. δ-ALAD gene polymorphisms were characterized using PCR-RFLP method, while lead serum levels were quantified by Atomic Absorption Spectrophotometer (AAS). In addition, Creatinin serum was done with a spectrophotometer and GFR value was formulated by means of the Schwartz method.* ***Result:*** *The study showed that the proportion of ALAD genotype for ALAD 1-1, 1-2 and 2-2 were 94.7%, 5.3%, and 0% respectively. The mean of serum levels in homozygous 1-1 was 15.94 ppb and heterozygote 1-2 was 1.15 ppb. GFR of participants ranged from 71.11 mL/min to 185.20 mL/min with a mean of 117.34mL/min. There was no correlation between serum Pb and GFR (p = 0.19). Study also could not determine the correlation between GFR and ALAD gene Polymorphism.* ***Discussion:*** *Study then concluded that there was no correlation between blood lead levels in the GFR on each δ-ALAD genotypes.*

Keywords: *Lead intoxication, GFR, δ-ALAD, gas station workers*

INTRODUCTION

Heavy metal intoxication was associated with industrialization and use of fossil fuels (Schwartz & Hu, 2007). One of the most potentially heavy metal intoxication is Plumbum (Pb) aka Lead. The data showed that Housing in the United States contributed about 17% of Lead contamination (Jacobs, et al., 2002), while in China there was 53.7% of population passing the Lead threshold (Ye & Wong, 2006). Other study estimates that no less than 17,000 liters of fuel are burned every day in Indonesia (Santi, 2007).

Elevated levels of Pb in the blood can cause such disorders including cardiovascular, gastrointestinal, hemolymphatic, urinary system, immune system, reproductive system, cancer and also cognitive impairment (Hirose, et al., 2004; ATSDR, 2007). Pb affects the enzyme of Aminolevulinic Acid Dehydratase

(ALAD) in the biosynthesis of heme (Dongre, et al., 2011). It can bind sulfhydryl groups of cysteine, amino groups of lysine, and the hydroxyl group of tyrosine in the enzyme ALAD (Kosnet, 2004).

δ-ALAD gene is a functional genes that produce ALAD enzyme which have δ-ALAD three gene polymorphisms encoded with ALAD 1-1, ALAD 1-2 and ALAD 2-2 (Schwartz & Hu, 2007; Kamel, et al., 2003). Previous study showed that there was a relationship between δ-ALAD gene polymorphisms and blood Pb intoxication rate. Homozygous subjects of ALAD-2 gene tend to have higher rates of Pb compare with ALAD-1 allele (Hopkins, et al., 2008). In addition, another study found that the increase of serum creatinin level in individuals of ALAD-2 gene was modified by the enhancement of Pb serum (Weaver, et al., 2008). Higher Pb in blood can cause a decrease of renal function and trigger aminoaciduria. Moreover, intranuclear inclusion bodies usually could be detected in the cells of human peritubuler (Futrakul, et al., 2011).

δ-ALAD genotypes were also reported could affect the kidneys performance. Previous data in a meta-analysis study stated that its clearly declared that ALAD-2 allele had significantly association with serum creatinin levels (Scinicariello, 2010). However, study using Indonesian subjects has not been performed. This study was therefore aimed to determine the relationship between δ-ALAD gene polymorphism and renal function using Glomerulo Filtration Rate (GFR) in Indonesian people.

MATERIALS AND METHODS

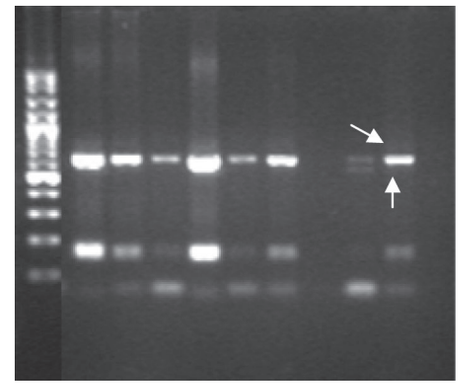
Study was performed using a cross-sectional design. Subjects had the following criteria; working as fuel filler, has been worked at least 1 year, and having at least 8 hours working a day, were recruited as participants. Those who suffered hypertension, diabetes, urinary tract obstruction, myopathy, athletes, alcohol consumer, and taking medications of anti-angiotensin II and vasopressin were excluded from the study. The sampling method used in this study was consecutive sampling.

Every participant underwent informed consent, questionnaires, and blood sampling. Interviews were conducted to obtain personal data and assess whether respondents meet the inclusion and exclusion criteria. GFR value was measured by Creatinin levels in serum (Csr) and calculated by the Schwartz formula (McPherson & Pincus, 2006).

Lead level in the blood was examined using atomic absorption spectrophotometer method (AAS). Constitution of δ-ALAD gene allele polymorphism (G to C) base substitution at position 177 (G177) was carried out by means of PCR-RFLP method. Primers used in this research were AGACAGACATTA GCTC AGTA and GGCAAAGAACAGGTCCATTC generated 916 bp PCR products. Enzyme MSP1 was used to characterize the ALAD polymorphism. ALAD 1 allele had 582 bp and ALAD 2 allele had 511 bp. All the data collected then analyzed by using Spearmen correlation test.

RESULTS

Subjects underwent genotyping of DNA and performed with various characteristics including age, smoking and, GFR, creatinin, Pb serum and also gender. Data in picture 1 and tables (1 & 2) showed that degree of Pb



Picture 1. ALAD Polymorphism pattern on PCR-RFLP gel electrophoresis. ALAD1 allele was depicted by upper arrow and ALAD 2 allele was lower arrow. ALAD 1 allele had 582 bp and ALAD 2 allele had 511 bp.

Table 1. The characteristic of respondents

Variable	N	Min	Max	Mean	f	p	SD
Sex							
Male	31				(81,6%)		
Female	7				(18.4%)		
Height	*38*	*153.90*	*192.00*	*169.08*			8.08
Cr serum	*38*	*0.51*	*1.27*	*0.84*			0.18
Pb serum	*38*	*0.14*	*55.51*	*15.16*		0.00	17.20
GFR	*38*	*71.11*	*185.20*	*117.34*		*0.56*	*23.35*
polymorphism	38						0.43
ALAD-1					36 (94.7%)		
ALAD-2					-		
ALAD-1-2					2 (3.3%)		
smoking	38						
Yes					21 (55.3%)		
No					17 (44.7%)		

Table 2. The characteristic of respondents by ALAD Polymorphism

Variable	N	Min	*Max*	*Mean*	*f*	*p*	*SD*
ALAD 1-1							
Age	*36*	*18*	*54*	*34.28*			10.91
Height	*36*	*153.90*	*192.00*	*169.02*			8.25
Cr serum	*36*	*.51*	*1.27*	*.84*			0.18
Pb serum	*36*	*.01*	*55.51*	*15.94*		0.00	17.34
GFR	*36*	*71.11*	*169.31*	*115.69*		0.64	23.80
Smoking	36						
Yes					52.8%		
No					47.2%		
ALAD 1-2							
Age	*2*	*30*	*40*	*35*			7.07
Height	*2*	*166.60*	*174.00*	*170.30*			5.23
Cr serum	2	*0.78*	*0.99*	*0.89*			0.15
Pb serum	*2*	*1.10*	*1.19*	*1.14*			0.06
GFR	*2*	*96.67*	*117.47*	*107.07*			14.71
Smoking	2						
Yes					100%		
No					0%		

and creatinin serum have wide value span from 0.14 to 55.51. Genotyping data demonstrated ALAD 1-1 as dominant allele and most of the subjects have smoking habit in their daily living. The average level of GFR stated in 1.5 mg/dl. Statistical analysis demonstrate the relationship between ALAD polymorphism and GFR showed a non-significant value ($p < 0.618$; $r = -0.084$).

DISCUSSION

The study showed that the frequency of ALAD 1-1 had a proportion of 94.7%, while only 5.3% was recorded for ALAD 1-2. Unfortunately, ALAD 2-2 allele was not found in this study. Research carried out by Weaver et al [15] reported that the proportion of ALAD 1-1 allele was more dominant than the ALAD 1-2, namely 90.1% versus 9.9%.

His study also stated that only 4% of the population had ALAD-2 allele. This leads to the possibility of decrease in ALAD 1-2 allele due to the presence of cross-breeding between ALAD-1 and ALAD-2 allele. The mean of Creatinin serum for all respondents amounted to 0.85 mg/dl. Creatinin Serum is affected by muscle mass and activity. The value among the respondents ranged from 0.51 to 1.27 mg/dl. It's comparable with the last study Wu et al (2003), which examined the relationship between Lead and renal function. They found the average serum creatinin of 1.2 mg/dl with a range of 0.6–2.5 mg/dl. The differences could be due to muscle mass and activity habit. American population is Caucasian while Indonesia is Mongoloid race. They have larger body than the Mongoloid so that muscle mass in Caucasians is relatively larger than the Mongoloid (Weaver, et al., 2005)

A mean serum creatinin in ALAD 1-1 is equal to 0.84 mg/dl, while in the ALAD 1-2 is 0.89 mg/dl. Population with ALAD 1-2 in this study was only 2 subjects so that we could not draw statistical analysis. These findings are consistent with previous research (Wu et al., 2003). They found that the mean of serum creatinin in ALAD 1-2 is greater than ALAD-1. However, there is contradiction result compared with another team (Weaver et al., 2003). Workers exposed to lead in Korea who had ALAD 1-1 allele had serum creatinin levels greater than ALAD 1-2. It could be explained that workers exposed to high lead exposure was in the battery factory and lead smelters in Korea, whereas in the Wu's study lead exposure was quite low (Wu et al., 2003).

GFR value was calculated using Scwahtrz formula. Serum creatinin is inversely proportional to GFR, while GFR is linear with the body height, so the variation of serum creatinin and body height will affect the value of GFR (Refaie, Moocchhala, & Kanagasundaram, 2007). The mean of GFR in ALAD 1-1 and ALAD-1-2 are 115.69ml/min and 107.07ml/min respectively. The relationship between ALAD polymorphism and GFR showed a non-significant value ($p < 0.618$; $r = -0.084$). This may be due to variations of the respondents, there were 36 people who had ALAD 1-1 allele and only 2 subjects who have ALAD 1-2 allele. Many studies support the fact that ALAD enzyme may modify different toxic effects in the organ. In addition, ALAD gene polymorphisms also related with several parameters of kidney function such as β_2 micro-globulin, α_1 micro-globulin and α_2 macroglobulin (Chia, et al., 2006).

The average level of Pb in the blood is 1.5 mg/dl. According to WHO statement, the threshold values of Pb in men is 40 mg/dl, while the female is 30 µg/dl. Our findings suggest that Pb levels in this study were low. Wu et al. (2003) found that the average level of Pb in blood is of 6.2 mg/dl. There were some issues concerning about this result. Subjects used in his study were elder people ranging from 43 to 93 years old. This wide difference in age may contribute the study result. Low levels of Pb in our study also can be caused by cessation of the use of lead in fuel oil process. Indonesian government through Pertamina has stopped production of fuel oil contain raising octane and anti-knocking agent since 2006 (Dellyani & RB, 2010). All respondents worked at least for 1 year and they make low exposure to Pb.

GFR relationship and blood Pb in this study was found not to be significant ($p < 0.195$; $r = 0.125$). This finding could be due to the low quantity of Pb so it did not cause damage to the nephrons. In line, previous study showed that there was no relationship between Pb with GFR. Low level of Pb caused no significant effect on GFR and intoxication (Zhao, et al., 2007)). On the other hand, a fieldwork conducted in the United States population from 1988 to 1994, found that at higher Pb levels > 4,21 mg/dl, subjects suffered hypertension while subjects with < 3,30 mg/dl did not affect hypertension. The study also depicted that GFR from those who suffered hypertension was higher than those who did not (Muntner, et al., 2003).

The effects of Pb on the kidneys failure in each ALAD genotype showed that there were differences between the renal effects of Pb on the ALAD 1-1 and ALAD 1-2 allele. There

was a reduction in GFR and increased levels of UNAG (urinary N-acetyl-β-Glucosaminidase) in the ALAD 1-2 [24]. In group of ALAD 1-2 there was an increase levels of UNAG urinary interpreted too many apoptosis in renal tubular cells. Exsposure of Pb led to damage the tubules and glomeruli in histopathological examination leading to a decline in kidney function (Weaver, et al., 2008).

Our study showed a replication data that the ALAD 1-1 had lower degree in Pb level compared with ALAD 1-2 allele. However, there was limitation study related to sample numbers, and study design.

CONCLUSION AND SUGGESTION

Conclusion

In conclusion, there was no correlation between GFR and blood Lead levels in the gas station employee concerning δ-ALAD gene alleles polymorphism.

Sugesstion

Further research could use more sample and prospective cohort design in order to assess the effects of Pb exposure on the kidneys as well as more control over confounding variables exist.

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