

ORIGINAL ARTICLE

Reassessment of the threshold of the blood lead level to increase urinary δ -aminolevulinic acid based on their relationship in recent lead workers in Japan

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Abstract

Objectives: The present study investigated the quantitative relationship between blood lead (Pb-B) and urinary δ -aminolevulinic acid (ALA-U) in lead workers, and examined the Pb-B level that induces increases in ALA-U and the corresponding ALA-U.

Methods: We collected 10 562 data sets on Pb-B, ALA-U, age, and smoking habits from 808 workers (771 males and 37 females) who underwent multiple lead poisoning medical examinations at a lead-acid battery and lead smelting plant in Japan between 1995 and 2018. Females were excluded, and data collected in 169 subjects prior to engaging in lead work were used as the control. Pb-B and ALA-U levels were measured by graphite furnace atomic absorption spectrophotometry and high-performance liquid chromatography respectively.

Results: A significant dose-response relationship was observed between Pb-B and ALA-U based on Pb-B-classified observations of increases in ALA-U values and the prevalence of over-reference ALA-U as well as regression analyses independent of smoking habits. The results obtained revealed that the threshold of Pb-B to increase ALA-U was 25.1–35.0 $\mu\text{g/dL}$ based on the significant elevation point of the prevalence of over-reference ALA-U and 16.2–22.3 $\mu\text{g/dL}$ from a 3rd degree regression equation.

Conclusions: We proposed a threshold of Pb-B to increase ALA-U of 20 $\mu\text{g/dL}$ and a biologically acceptable value of ALA-U of 1 mg/L , corresponding to the threshold.

KEYWORDS

biological exposure indices, blood lead, dose-response relationship, lead workers, threshold, δ -aminolevulinic acid

1 | INTRODUCTION

Lead is a major metal that has been used in various industrial fields since ancient times. Factory workers who

engaged in industries such as lead refining, printing, and storage battery manufacturing developed lead poisoning, characterized by anemia, damage to the central and peripheral nervous systems, abdominal pain, and the gingival

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lead seam line, through the inhalation of lead-containing dust. Regarding anemia, lead affects several enzymatic processes involved in heme synthesis. δ -aminolevulinic acid (ALA) is synthesized from glycine and succinyl-CoA by δ -aminolevulinic acid synthetase (ALAS). Porphobilinogen is biosynthesized from ALA by δ -aminolevulinic acid dehydratase (ALAD). Lead exposure enhances ALAS activity and inhibits ALAD activity, which elevates blood ALA levels, followed by increases in urinary δ -aminolevulinic acid (ALA-U) levels.¹

Blood lead (Pb-B) levels are a representative of the Biological Exposure Indices (BEI) for occupational lead exposure.^{1,2} ALA-U is also accepted in occupational health as an indicator of the most sensitive health effect of lead exposure.² The relationship between Pb-B and ALA-U in lead workers has been examined since the 1950s, and the Pb-B level that induces an increase in ALA-U has also been investigated worldwide since the mid-1970s. Although a linear regression, the simplest assumption, has been applied to elucidate the relationship between Pb-B and ALA-U,³⁻⁶ a curve regression has been reported as a better fit for this relationship.^{7,8} Curve regression analyses revealed that ALA-U significantly increased at Pb-B higher than 40 $\mu\text{g}/\text{dL}$.^{7,9} Furthermore, ALA-U did not change at Pb-B lower than 30–45 $\mu\text{g}/\text{dL}$ ¹⁰ or decreased at levels lower than 20 $\mu\text{g}/\text{dL}$.¹¹ These findings suggest that Pb-B has a specific threshold at which ALA-U increases.

Based on this threshold, the biological tolerance level of Pb-B was proposed to be 40 $\mu\text{g}/\text{dL}$ in 1994,¹² and was subsequently amended to 15 $\mu\text{g}/\text{dL}$ in 2013^{12,13} in Japan, while the BEI of Pb-B set by ACGIH is 20 $\mu\text{g}/\text{dL}$.¹⁴ Although the biological tolerance level of Pb-B has been reduced, that of ALA-U has remained unchanged since it was set at 5 mg/L in 1994¹⁵ in Japan. In addition, the biological tolerance level of ALA-U was based on findings obtained using colorimetric measurements, the accuracy of which is limited not only for ALA-U, but also other urinary substances.¹⁶ The background for the current biological tolerance level of ALA-U indicates the necessity for reassessments, particularly based on the relationship between Pb-B and ALA-U in recent lead workers measured using high-performance liquid chromatography (HPLC), which is more reliable than colorimetric methods.^{17,18} Furthermore, since the subjects of previous studies that set the biological tolerance levels of Pb-B and ALA-U consisted of workers with high levels of lead exposure, the values described above may be not suitable for current lead workers whose lead exposure levels are markedly lower. Therefore, the threshold of Pb-B to increase ALA-U and the corresponding value of ALA-U need to be reassessed based on the relationship between Pb-B and ALA-U in recent lead workers with lower lead exposure.

In the present study, we investigated the quantitative relationship between Pb-B and ALA-U in lead workers in

Japan using their recent records of lead poisoning medical examinations to establish the threshold value of Pb-B that increases ALA-U and the reference value of ALA-U measured by HPLC.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Target factories in the present study consisted of a lead-acid battery factory and lead smelting factory. The lead-acid battery factory was manufacturing lead-acid batteries for automobiles, emitting lead oxide dust and fume. At the lead smelting plant, old lead-acid batteries were dismantled and lead was regenerated, and lead oxide dust and fume were generated under high temperatures. In these factories, 808 workers (771 males and 37 females) underwent lead poisoning medical examinations between 1995 and 2018 under the Ordinance on the Prevention of Lead Poisoning. Background information on workers, such as age and smoking habits, as well as data on Pb-B and ALA-U were collected from their records. Data included the results of multiple lead poisoning medical examinations of each worker. The records of lead poisoning medical examinations of workers before they engaged in lead work were used as control data. Some records lacked information or data because blood or urine were not collected on the day of the examinations, for example, and, thus, were handled as missing values.

2.2 | Measurement of Pb-B and ALA-U levels

Pb-B levels were measured using a graphite furnace atomic absorption spectrophotometer (ZA3700, ZA3000, Hitachi High-Tech Science Corporation) with a wavelength of 283.3 nm. The analysis was performed using the methods of Subramanian et al with modifications.¹⁹ A peripheral blood sample was diluted with ultrapure water containing Triton X-100 as a surfactant and diammonium hydrogen phosphate as an interference inhibitor for the analysis. A linear calibration curve was prepared by the simple standard addition method and quantified.

ALA-U was measured by HPLC (LaChrom Elite L-2000 Series, Hitachi High-Tech Corporation). The analysis was performed by modifying the methods of Okayama et al²⁰ and Endo et al¹⁷ Reaction solution A (acetylacetone/ethanol/sodium chloride/ultrapure water) and reaction solution B (formaldehyde/ultrapure water) were added to urine and heated in boiling water to make ALA a fluorescent derivative for the analysis. In the liquid chromatograph, the mobile phase (methanol/acetic acid/ultrapure water) and the

ODS column (TSK-gel ODS-80Ts, 4.6×150 mm, 5- μ m particles, Tosoh) were separated, and the fluorescence detector (excitation wavelength 363 nm, measurement wavelength 463 nm) was used for detection. A linear calibration curve was prepared by the absolute calibration curve method and quantified. ALA-U was corrected with a urine specific gravity of 1.020.

Pb-B and ALA-U levels were measured at Keio University until the spring of 2005, and after the fall of 2005, measurements were conducted at the Special Reference Laboratory Inc (Tokyo, Japan, which is a nationwide clinical laboratory). The limits of quantification were 1.0 μ g/dL for Pb-B and 0.1 mg/L for ALA-U.

2.3 | Statistical analysis

Lead workers and controls were divided by their smoking habits because of the high content of lead in tobacco smoke, and Pb-B and ALA-U levels in all subjects, non-smokers, and smokers were presented as geometric means (GMs) based on the assumption of a lognormal distribution. Spearman's rank correlation coefficients between age, Pb-B, and ALA-U were calculated for controls and lead workers. In all subjects, comparisons of Pb-B or ALA-U between controls and lead workers were performed using the Student's *t*-test. In subjects divided by smoking habits, comparisons of Pb-B or ALA-U between non-smokers and smokers were performed using the Games-Howell test. Dunnett's multiple comparison test and Fisher's exact test with Bonferroni corrections were used to compare the GMs of ALA-U and the proportions of ALA-U between controls and subjects divided by Pb-B levels, respectively. A regression analysis was performed to investigate the relationship between Pb-B and ALA-U.

Statistical analyses were performed using IBM SPSS Statistics V25 (SPSS Japan) along with Excel 2016 for simple calculations.

3 | RESULTS

3.1 | Subjects

An outline of data collected and the data used in each statistical analysis is shown in Figure 1. The total number of data sets collected was 10 562 from 808 workers. We excluded 37 female workers because of their small number. As a result, the numbers of control and lead worker data sets were 169 from 169 subjects and 10 067 from 704 subjects, respectively.

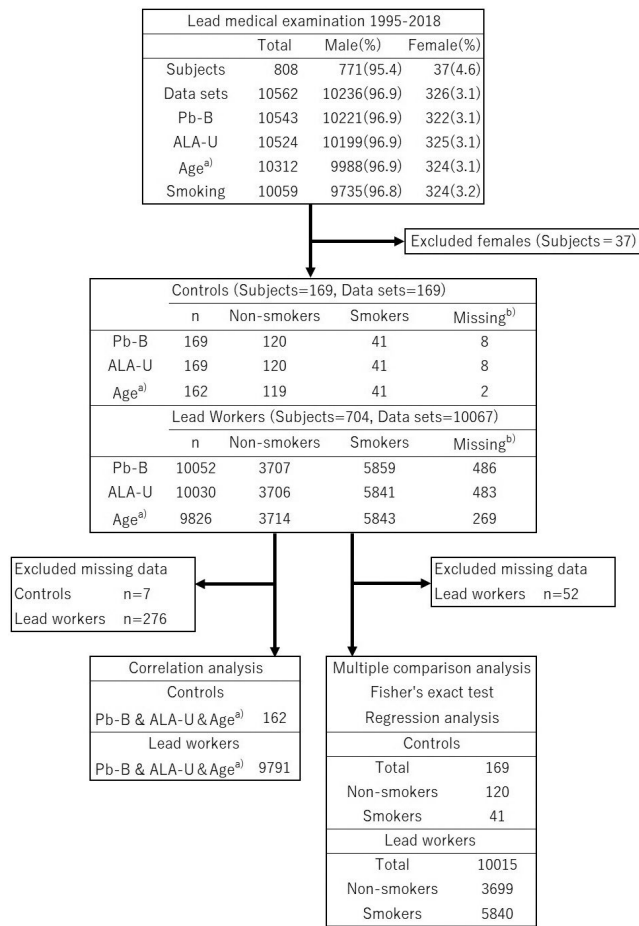


FIGURE 1 Schematic diagram showing the study outline.

ALA-U, δ -aminolevulinic acid in urine; Pb-B, Lead in blood. ^aAge at the implementation of the lead poisoning medical examination. ^bThe number of subjects without information on smoking habits

3.2 | Relationships between age, Pb-B, and ALA-U

Table 1 shows Spearman's rank correlation coefficients between age, Pb-B, and ALA-U in controls and lead workers. Analyses were performed after the exclusion of data sets with missing values for any of the three items. In the controls, correlation coefficients between age and Pb-B and between age and ALA-U were 0.195 ($P < .05$) and 0.188 ($P < .05$), respectively, while in lead workers, correlation coefficients between age and Pb-B and between age and ALA-U were 0.052 (n.s.) and -0.122 ($P < .05$) respectively. These results indicated that age did not correlate with Pb-B or ALA-U in the controls or lead workers. Therefore, even if a data set had no information on age, other data items were used in subsequent statistical analyses.

3.3 | Pb-B levels

Table 2 shows Pb-B levels in total data sets and subgroups divided by smoking habits. The total data set of lead workers

	Spearman's correlation coefficient (ρ)					
	Controls (n = 162)			Lead workers (n = 9791)		
	Age	Pb-B	ALA-U	Age	Pb-B	ALA-U
Age (years)	1			1		
Pb-B ($\mu\text{g/dL}$)	0.195*	1		0.052	1	
ALA-U (mg/L)	0.188*	0.203*	1	-0.122*	0.298*	1

Abbreviations: ALA-U, δ -aminolevulinic acid in urine; Pb-B, lead in blood.

* $P < .05$ (two-tailed).

included those who did not have information on smoking habits. Lead workers had significantly higher Pb-B levels than the controls in the total and both subgroups, and smokers had significantly higher Pb-B levels than non-smokers in both controls and lead workers. Similar results were obtained after the exclusion of subjects with no information on smoking habits.

3.4 | ALA-U levels

Table 3 shows ALA-U levels in the total data sets and subgroups divided by smoking habits. The data sets of lead workers were further classified by Pb-B levels up to 70.0 $\mu\text{g/dL}$ in increments of 5-10 $\mu\text{g/dL}$. The total data set of lead workers included those who did not have information on smoking habits. Lead workers had significantly higher ALA-U levels than the controls in the total data set and non-smoking and smoking subgroups. In addition, the ALA-U levels of lead workers in all Pb-B-classified groups, except for the group of ALA-U ≤ 5.0 in non-smokers and the group of ALA-U 5.1-10.0 in smokers, were significantly higher than those of the controls. The group of non-smokers with Pb-B >70.0 had a very small number of subjects ($n = 3$) and, thus, was not suitable for statistical analyses. A comparison of controls by smoking habits showed no significant differences in ALA-U levels between non-smokers and smokers.

TABLE 1 Correlation matrix for age, Pb-B, and ALA-U

3.5 | Prevalence of elevated ALA-U levels

We initially calculated the reference upper limit value of ALA-U: after converting the values of ALA-U of the controls ($n = 169$) into logarithmic values, the mean value and standard deviation (SD) were calculated to obtain the mean + 2SD, which was, in turn, converted to an exponent to obtain the value 1.842 mg/L as the reference upper limit value of ALA-U.

Figure 2 shows the prevalence of ALA-U over the normal reference value in the total data sets and subgroups divided by smoking habits, and charted the data in Table 3. The prevalence of over-reference ALA-U in controls of the total was 3.0%, and was significantly higher in all lead workers with Pb-B 25.1-30.0 $\mu\text{g/dL}$ (13.6%) or higher. The prevalence of over-reference ALA-U in control non-smokers and smokers were 2.5 and 2.4%, respectively, and were significantly higher in non-smoking and smoking lead workers with Pb-B 30.1-35.0 $\mu\text{g/dL}$ (24.6% and 24.2%, respectively) or higher.

3.6 | Relationship between Pb-B and ALA-U

We investigated the relationship between Pb-B and ALA-U in lead workers to obtain parameters for 1st to 3rd degree regression equations (Table 4). The coefficient of determination (R^2) slightly increased from the 1st to 3rd degree as the order of the

TABLE 2 Geometric mean of blood lead levels

Group	Total			Non-smokers			Smokers		
	n	GM	Range	n	GM	Range	n	GM	Range
Controls	169	2.2	0.5-9.0	120	2.0	0.5-9.0	41	2.9 [†]	1.0-6.0
Lead workers	10 015	16.7*	1.0-108.0	3699	14.4**	1.0-88.0	5840	18.0** [‡]	1.9-90.0

Note: Comparisons between controls and lead workers in the total data sets were performed using the Student's t -test.

Comparisons among the four subgroups of controls and lead workers divided by smoking habits were performed by the Games-Howell test.

Abbreviations: GM, geometric mean; n, number of data sets.

* $P < .05$ (two-tailed) vs controls in total, the unpaired Student's t -test.

** $P < .05$ (two-tailed) vs controls in non-smokers, the Games-Howell test.

[†] $P < .05$ (two-tailed) vs non-smokers in controls, the Games-Howell test.

[‡] $P < .05$ (two-tailed) vs non-smokers in lead workers, the Games-Howell test.

TABLE 3 Urinary δ -aminolevulinic acid levels and the prevalence of over the normal reference value in total data sets and subgroups divided by smoking habits in controls and lead workers. Data sets of lead workers were divided by blood lead levels

Group	Total				Non-smokers				Smokers			
	n	GM	Range	Prev (%)	n	GM	Range	Prev (%)	n	GM	Range	Prev (%)
Pb-B ($\mu\text{g/dL}$)												
Controls	169	0.90	0.30-3.06	3.0	120	0.88	0.35-3.06	2.5	41	0.95	0.30-2.72	2.4
Total ^a	10 015	1.21 ^{***}	0.13-74.6	12.4	3699	1.1 [†]	0.24-60.08	8.4	5840	1.26 ^{‡,§}	0.13-74.60	14.0
≤ 5	418	1.03 [*]	0.30-4.35	3.8	264	0.98	0.30-3.74	2.3	137	1.16 [*]	0.43-4.35	6.6
5.1-10.0	1653	1.05 [*]	0.24-6.96	5.8	742	1.00 [*]	0.24-4.28	3.9	853	1.10	0.34-6.96	7.0
10.1-15.0	1968	1.09 [*]	0.13-14.18	6.3	832	1.03 [*]	0.26-3.93	4.2	1042	1.12 [*]	0.13-14.18	6.9
15.1-20.0	1793	1.10 [*]	0.18-4.23	6.5	684	1.05 [*]	0.31-4.22	5.3	1055	1.13 [*]	0.18-4.23	7.4
20.1-25.0	1647	1.17 [*]	0.28-6.47	9.3	497	1.08 [*]	0.28-6.47	5.6	1096	1.21 [*]	0.33-5.70	10.9
25.1-30.0	1154	1.25 [*]	0.37-5.22	13.6 ^{**}	314	1.18 [*]	0.43-4.49	10.8	782	1.30 [*]	0.42-5.22	15.1
30.1-35.0	628	1.43 [*]	0.36-9.39	24.2 ^{**}	187	1.41 [*]	0.37-6.90	24.6 ^{**}	401	1.45 [*]	0.36-9.39	24.2 ^{**}
35.1-40.0	306	1.61 [*]	0.51-36.10	32.7 ^{**}	86	1.55 [*]	0.57-6.90	34.9 ^{**}	202	1.61 [*]	0.51-6.50	31.7 ^{**}
40.1-45.0	121	1.91 [*]	0.67-13.17	45.5 ^{**}	24	1.83 [*]	0.85-4.17	45.8 ^{**}	82	1.96 [*]	0.67-13.17	43.9 ^{**}
45.1-50.0	85	2.19 [*]	0.36-19.19	62.4 ^{**}	22	1.99 [*]	0.36-8.54	59.1 ^{**}	44	2.02 [*]	0.73-5.30	54.6 ^{**}
50.1-55.0	48	3.80 [*]	1.02-30.37	83.3 ^{**}	12	5.01 [*]	1.49-21.80	91.7 ^{**}	31	3.60 [*]	1.25-30.37	87.1 ^{**}
55.1-60.0	76	5.20 [*]	1.07-54.70	89.5 ^{**}	21	5.68 [*]	1.25-46.80	81.0 ^{**}	35	5.04 [*]	1.07-54.70	88.6 ^{**}
$\geq 60.1-70.0$	81	7.67 [*]	0.64-65.40	98.8 ^{**}	11	4.20 [*]	0.64-28.46	90.9 ^{**}	58	8.05 [*]	2.35-65.40	100.0 ^{**}
> 70.0	37	13.42 [*]	1.15-74.60	97.3 ^{**}	3	19.17	9.00-60.08	100.0 ^{**}	22	14.8 [*]	6.08-74.60	100.0 ^{**}

Abbreviations: GM, geometric mean; n, number of data sets; Pb-B, lead in blood; Prev, prevalence of δ -aminolevulinic acid in urine over the normal reference value.

^aTotal number of lead workers.

^{*} $P < .05$ (two-tailed) vs controls, Dunnett's multiple comparison test.

^{**} $P < .00357$ (Bonferroni $\alpha = 0.05/14$) vs controls, Fisher's exact test.

^{***} $P < .05$ (two-tailed) vs controls in all subjects, the unpaired Student's t -test.

[†] $P < .05$ (two-tailed) vs controls in non-smokers, the Games-Howell test.

[‡] $P < .05$ (two-tailed) vs controls in smokers, the Games-Howell test.

[§] $P < .05$ (two-tailed) vs non-smokers in all lead workers, the Games-Howell test.

1st to 3rd degree equations changed. We adopted the 3rd degree regression equation, which was the best fit for the regression equation. A scatter diagram of Pb-B and ALA-U and the 3rd degree regression curve of lead workers are shown in Figure 3. As Pb-B levels increased, ALA-U levels did not change at the lower range of Pb-B levels, but began to increase at a specific Pb-B level, and then continuously increased.

The local minimum value X (Pb-B) was calculated using the 3rd degree regression equation with the largest R^2 . The 3rd degree regression equation was obtained as follows: total number of lead workers $Y = 0.00005x^3 - 0.00133x^2 + 0.00309x + 1.2045$, non-smokers $Y = 0.00011x^3 - 0.00594x^2 + 0.10596x + 0.55713$, smokers $Y = 0.00008x^3 - 0.00373x^2 + 0.05937x + 0.92392$. The local minimum value X is the value when the differential equation is equal to 0. The calculation method is $dy/dx = b + 2cx + 3dx^2 = 0$. As a result of the calculation, the local minimum value Xs of lead workers were 16.2, 22.3, and 18.6 in all subjects, non-smokers, and smokers respectively.

4 | DISCUSSION

In the present study, we analyzed data obtained from recent records of lead poisoning medical examinations of lead workers with or without smoking habits in Japan, and found a significant dose-response relationship between Pb-B and ALA-U by Pb-B-classified observations of increases in ALA-U values and the prevalence of over-reference ALA-U as well as regression analyses. Based on these results, we derived a threshold of Pb-B to increase ALA-U of 25.1-35.0 $\mu\text{g/dL}$ from the significant elevation point of the prevalence of over-reference ALA-U and 16.2-22.3 $\mu\text{g/dL}$ from the 3rd degree regression equation.

Prior to analyses, we set 1.842 mg/L of ALA-U as the reference upper limit value, calculated as the mean + 2SD of ALA-U values in control subjects. The calculation was performed by the method of Fukutake.²¹ According to the concept of the reference interval proposed by The National Committee for Clinical Laboratory Standards (NCCLS),²²

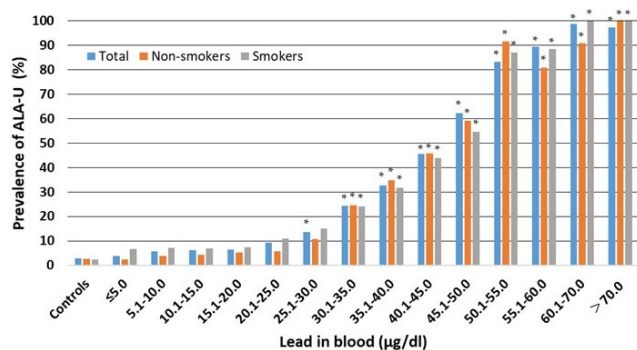


FIGURE 2 Prevalence of ALA-U over the normal reference value in total data sets and subgroups was divided by smoking habits from controls and lead workers. The data sets of lead workers were divided by blood lead levels. Data in Table 3 were charted here. ALA-U, δ -aminolevulinic acid in urine. * $P < .00357$ (Bonferroni $\alpha = 0.05/14$) vs respective controls, Fisher's exact test

the upper limit of the reference interval of ALA-U was calculated from the interval that includes approximately 95% of all measured values of ALA-U. Some individuals with high levels of ALA-U without lead exposure due to factors other than lead, such as acute intermittent porphyria (AIP) that increases ALA-U, have been included in analyses.¹⁵ Therefore, it is reasonable to set the reference upper limit value of ALA-U as the mean + 2SD.

Using the normal reference value, we demonstrated that the rates of subjects with abnormally elevated ALA-U were significantly higher at Pb-B levels of 25.1–30.0 and 30.1–35.0 $\mu\text{g/dL}$ in all subjects (13.6%) and in non-smokers (24.6%) and smokers (24.2%), respectively, among lead workers than among controls. As shown in Table 3 and Figure 3, the rates of elevated ALA-U remained unchanged or were slightly elevated as Pb-B levels increased to 20.1–25.0 $\mu\text{g/dL}$, and then an abrupt elevation occurred from the points described above. A similar pattern was noted in the GMs of ALA-U; however, a significant turning point for Pb-B to

increase ALA-U was not identified (Table 3). These results indicate that NOAEL of Pb-B is approximately 20.1–25.0 $\mu\text{g/dL}$. Makino et al¹¹ previously reported that ALA-U decreased when Pb-B was less than 20 $\mu\text{g/dL}$, which is consistent with the present results.

We then made regression models for the relationship between Pb-B and ALA-U based on scatter diagrams, including straight lines and polynomial curves. Using these models, we found that the 3rd degree polynomial regression curve was the best fit for the relationship, yielding local minimum values for Pb-B of 16.2, 22.3, and 18.6 $\mu\text{g/dL}$ in all, non-smoking, and smoking subjects, respectively, from which ALA-U started to increase. These results were consistent with previous findings reported by Higashikawa showing that the coefficient of determination slightly increased as the order of the 1st to 3rd degree equations changed.²³

Pb-B levels were significantly higher in smokers than in non-smokers among lead workers (Table 2), and as a result of its adverse effects, ALA-U levels were also significantly higher in smokers than in non-smokers among lead workers (Table 3). However, no significant differences were noted in ALA-U levels in Pb-B-classified groups, in other words, at the same Pb-B levels, although some groups showed a higher prevalence of elevated ALA-U levels in smokers (Table 3). In addition, although Pb-B levels were significantly higher in smokers than in non-smokers among controls (Table 2), these values were low and ALA-U levels did not significantly differ between these groups (Table 3). These results indicated that although smoking elevates Pb-B levels, the resultant increases in Pb-B, not smoking itself, affect ALA-U; therefore, it is not necessary to consider a smoking habit when establishing the threshold of Pb-B that increases ALA-U.

In comparisons of the threshold of ALA-U in the present study with those previously reported, differences in the methods used to measure ALA-U need to be considered. While ALA-U in the present study was measured using fluorescent

TABLE 4 Coefficients to X of various powers in regression equations, and coefficients of determination in lead workers

Group	Degree ^a	Coefficients (a to d)				R ²	P-value
		X ⁰	X ¹	X ²	X ³		
Total (n = 10 015)	1st	−0.31420	0.09111			0.196	<.05
	2nd	2.26175	−0.15040	0.00419		0.417	<.05
	3rd	1.20450	0.00309	−0.00133	5E-05	0.437	<.05
Non-smokers (n = 3699)	1st	0.17157	0.06431			0.133	<.05
	2nd	2.02233	−0.14023	0.00417		0.346	<.05
	3rd	0.55713	0.10596	−0.00594	1E-04	0.411	<.05
Smokers (n = 5840)	1st	−0.38360	0.09226			0.187	<.05
	2nd	2.53925	−0.16854	0.00445		0.408	<.05
	3rd	0.92392	0.05937	−0.00373	8E-05	0.437	<.05

Note: Coefficients are in the regression equation $Y = a + bX + cX^2 + dX^3$, where X is lead in blood ($\mu\text{g/dL}$) and Y is δ -aminolevulinic acid in urine (mg/L).

^aDegrees of the regression equation.

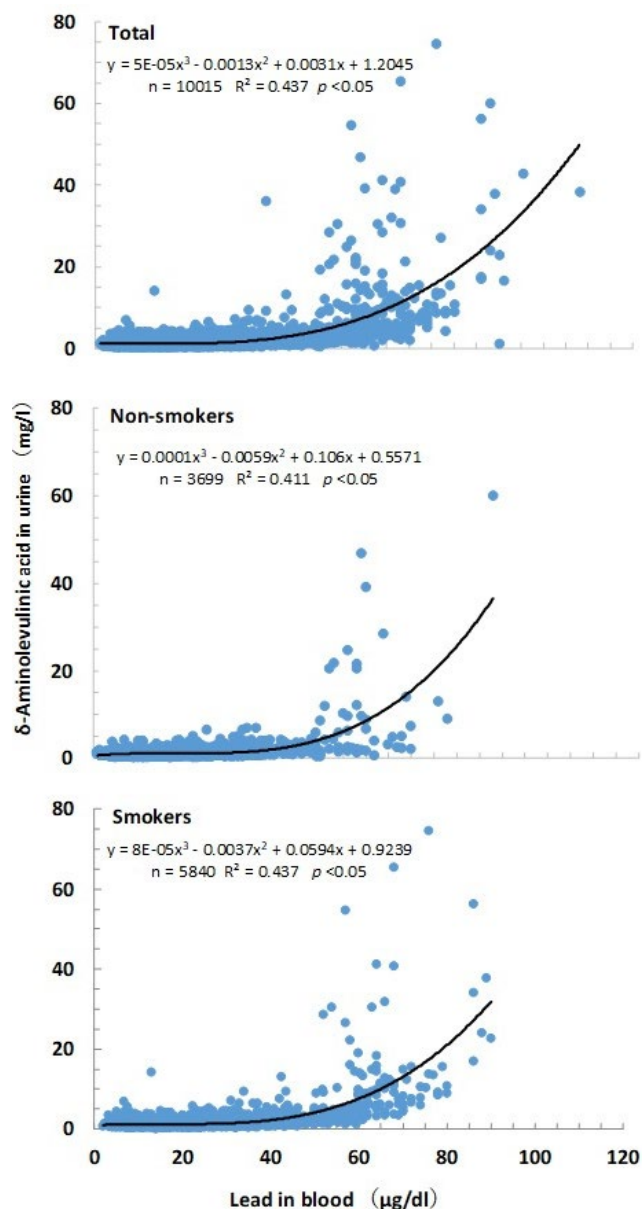


FIGURE 3 Scatter diagrams and regression curves between Pb-B and ALA-U in lead workers. Each dot shows one case

HPLC, many previously reported ALA-U values in lead poisoning medical examinations to calculate the threshold were assessed by various colorimetric methods. When ALA-U was >5 mg/L, the values of ALA-U measured by the two methods were consistent with each other; however, when ALA-U was lower than 1 mg/L, values obtained by colorimetry may have been twofold higher than those obtained using HPLC.²⁴ Furthermore, a previous study reported that the values of ALA-U measured by HPLC were 1/3 those by colorimetry at low Pb-B.¹⁸ The colorimetric method has the drawback of producing pyrroles, such as aminoacetone, which interfere with the measurement of urinary substances.¹⁶ Okayama et al²⁰ developed a method for fluorescently deriving ALA with acetylacetone and formaldehyde and measuring it using

HPLC, and successfully eliminated the effects of these urinary substances. Therefore, ALA-U at low Pb-B in previous studies analyzed by colorimetry may be higher than the values obtained in the present study.

Based on the present results, we propose a threshold of Pb-B to increase ALA-U of 20 µg/dL. Consequently, the values of ALA-U (measured by HPLC) that correspond to the threshold of Pb-B among lead workers were 1.17, 1.08, and 1.21 mg/L in all subjects, non-smokers, and smokers respectively. However, the current biologically acceptable value of ALA-U is 5 mg/L and is based on the measurement of ALA-U by various conventional colorimetric methods. For example, in Japan, the safety criteria for the distribution of ALA-U in the lead poisoning medical examination is set as distribution-1, -2, and -3 for 5 mg/L or less, 5 mg/L or more and 10 mg/L or less, and 10 mg/L or more respectively. Therefore, a biologically acceptable value of ALA-U of 1 mg/L is recommended on the safe side.

Our proposed threshold of Pb-B of 20 µg/dL is higher than those reported previously. A WHO study group found that lead exerted adverse effects on the hematopoietic and peripheral nervous systems in the Pb-B range of 40–49 µg/dL, and proposed 40 µg/dL of Pb-B as the standard.²⁵ In addition, IPCS²⁶ and ATSDR²⁷ estimated that the threshold level for decreased peripheral nerve conduction velocity and sensorimotor dysfunction in lead workers was 30–40 µg/dL of Pb-B. ACGIH sets the BEI of Pb-B as 20.0 µg/dL.¹⁴ In a review by Araki et al, decreased peripheral nerve conduction velocity was observed at 30–40 µg/dL of Pb-B on average.²⁸ Since the Pb-B level that affects heme synthesis is lower than that affecting the peripheral nervous system, these differences in the threshold of Pb-B may be derived from the targeted organs.

A strength of the present study is that it included subjects with a wider range of lead exposure levels, especially a large proportion of those with relatively lower lead exposure, enabling more precise analyses at a lower range of Pb-B than previous studies. Another strength is that ALA-U was measured by HPLC, which is more specific and sensitive than various colorimetric methods.

This study has some limitations. We did not obtain information on lead levels in the work environment and exposure periods to lead by workers. However, the resultant Pb-B level was itself a reliable indicator of lead exposure. Since ALA-U was not measured by a colorimetric method in workers in the present study, it is not possible to statistically compare the values measured by the two methods. Furthermore, subjects in the present study were solely male; therefore, the results obtained may not be applicable to females. Pb-B and ALA-U have different metabolisms; Pb-B has a half-life of 28–36 days,²⁹ and ALA-U returns to a normal level soon after discontinuation of exposure.¹² This difference may have an influence on the relationship between them, but it would be negligibly small

because most subjects in this study worked in the constant condition. Drugs such as barbiturates can affect ALA-U, but we did not obtain information on it from the subjects.^{30,31}

5 | CONCLUSIONS

Based on the relationship between Pb-B and ALA-U in male lead workers in Japan using the recent records of lead poisoning medical examinations, we propose that the threshold of Pb-B to increase ALA-U is approximately 20 µg/dL, and the corresponding ALA-U as a biologically acceptable value is 1 mg/L, independent of smoking habits.

ACKNOWLEDGMENTS

The authors are grateful to Honorary Professor Kazuyuki Omae of Keio University for discussing the statistical analysis of this study, Yoko Eitaki of Keio University who provided the measurement information of Pb-B and ALA-U, and Mayumi Saito of Furukawa Battery Co., Ltd. for collecting data.

DISCLOSURE

Approval of the research protocol: This study was approved by the Ethics Committee of the School of Medicine of Kitasato University (approval no. B18-121). *Informed consent:* N/A. *Registry and the registration no. of the study/trial:* N/A. *Animal studies:* N/A. *Conflicts of interest:* The authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

AO collected and analyzed the data and wrote the manuscript. HH interpreted the data and supported the writing of the manuscript.

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How to cite this article: Ono A, Horiguchi H. Reassessment of the threshold of the blood lead level to increase urinary δ -aminolevulinic acid based on their relationship in recent lead workers in Japan. *J Occup Health*. 2021;63:e12202. <https://doi.org/10.1002/1348-9585.12202>