

## - aminolevulinic acid

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### - Abstract -

#### The Relationships between Plasma - aminolevulinic acid Concentration and Lead Exposure Indices in Lead Workers

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This study was carried out to investigate relationship between plasma - aminolevulinic acid (ALAP) and lead exposure indices in exposure to lead. The subjects were 218 male workers in 2 storage battery companies and 2 secondary smelting companies. Blood lead(PbB), blood zinc-protoporphyrin(ZPP), urinary - aminolevulinic acid (ALAU), hemoglobin(Hb), and hematocrit(Hct) were measured as lead exposure indices.

The results were as follows,

1. The means of blood lead and blood ZPP concentration of subjects were  $27.2 \pm 14.0 \mu\text{g/dl}$  and  $55.1 \pm 47.6 \mu\text{g/dl}$ , respectively. The means of plasma -ALA and urinary -ALA concentration were  $18.9 \pm 25.1 \mu\text{g/dl}$  and  $2.1 \pm 4.6 \text{mg/}$ , respectively.

2. The concentration of ALAP was  $11.2 \mu\text{g/}$  for below  $20 \mu\text{g/dl}$  PbB,  $12.8 \mu\text{g/}$  for from 21-40  $\mu\text{g/dl}$  PbB, and  $51.2 \mu\text{g/}$  for over  $40 \mu\text{g/dl}$  PbB, respectively.
3. ALAP was significantly correlated with ALAU( $r=0.829$ ,  $p<0.01$ ), ZPP( $r=0.724$ ,  $p<0.01$ ) and PbB( $r=0.552$ ,  $p<0.01$ ).

**Key Words** : Plasma - aminolevulinic acid, Lead exposure indices, High performance liquid chromatograph

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(NAG)가  
- ALA ( , 1999)  
, - ALA  
가 - ALA  
가 가 - ALA 가  $5.3 \pm 1.4$   
가  $\mu\text{g/}$  (Morita , 1993),  $8.6 \pm 1.3\mu\text{g/}$  (Morita ,  
1960 1994)  
- ALA  $8.2 \pm 4.7\mu\text{g/}$  (Tomokuni , 1992b)  
. 1987 - ALA  
가 - ALA  
, - ALA  
가 ( , 1992), - ALA  
- ALA가  
(PbB), Zinc- protoporphyrin(ZPP), (Hannan , 1989;  
- aminolevulinic acid( - ALA) Hosoda , 1989; Tomokuni , 1993a; Tomokuni  
1993b; Morita , 1993; Morita 1994), -  
aminolevulinic acid - aminobutylic  
acid(GABA)  
가 - ALA heme (Brennan , 1979)  
glycine active succinate - ALA  
synthetase( - ALA )가  
- aminolevulinic acid - ALA  
dehydratase( - ALAD)가  
- ALA 가 가 - ALA HPLC  
(Neuberger Scott, 1953). , , ZPP,  
- ALA 1956 Mauzerall - ALA  
Granick  
(Tomokuni Ogata, 1972)  
aminoacetone  
- ALA 가 가  
(原田 章 , 1991; , 1994),  
1.  
(HPLC) (Tomokuni ,  
1992a) - ALA 2  
- ALA  
(Endo , 1990) 218  
N- acetyl- -D- glucosaminidase 1 .

Table 1. General characteristics of study subjects.

	Mean ± SD	Min.	Max.
Age (year)	36.3 ± 8.7	22.0	74.0
Work duration (year)	8.0 ± 4.7	1.5	29.2
2.	High performance liquid chromatograph(HPLC, LC- 10AD, Shimadzu, Japan) (Tomokuni , 1992b). ALA 4.7 3μg/ . HPLC SAS(6.12 PC version) 2 .		
3.	(2) - aminolevulinic acid (ALA-U) 가 , (1) - aminolevlinic acid (ALA-P) . 3.5Mℓ acetylacetone (acetylacetone- ethanol- water 15:10:75 v/v/v) 10Mℓ (K3-EDTA)가 Vaccutainer 0.05Mℓ 10% formalin 0.45 3000 rpm 5 Mℓ 가 3 vortex mixer dry ice 100 10 pyrrole . pyrrole PVDF 50μℓ acetylacetone (acetylacetone- ethanol- water 15:10:75 v/v/v) 1.75Mℓ 10% for- maldehyde solution 225μℓ 10Mℓ 20μℓ HPLC(LC- 10AD, Shimadzu, Japan) (Tomokuni , 1992a). vortex mixer 3 100 1.024 . 10 pyrrole . pyrrole PVDF syringe filter(0.45μm, (3) (PbB) 13mm, Gelman) 120μℓ 6 , (K3-		

Table 2. Analytical condition of HPLC

Column	Sim- pack CLC- ODS, 150× 6.0mm
Pump	LC- 10AD
Detector	Rf- 10A spectrofluorometer (excitation 370nm , emission 460nm)
Autosampler	SIL- 10A Auto injector
Flow rate	1.0 Mℓ/min
Oven temperature	40
Mobile phase	methanol / acetic acid / dilution water (78 : 8 : 14 v/v/v, pH 3.4)

EDTA)가

Ice box

(AAS; Hitachi Z- 8100, Zeeman,

1% TX- 100

Japan)

(Fernandez, 1975).

가

가

3

Table 3. Analytical condition of AAS with graphite furnace

Lamp current	7.5 mA			
Wavelength	283.3 nm			
Curette	Tube type			
Sample volume	15 $\mu$ l			
Measurement mode	STD addition			
Instrument mode	Absorbance			
Calculation	Peak height			
Temperature program				
No.	Stage	Temp.( )	Time(se)	Carrier gas(Ml/min)
1	Dry	60 - 90	30	200
2	Dry	90 - 120	20	200
3	Ash	550 - 550	20	200
4	Atom	2000 - 2000	10	30
5	Clean	2400 - 2400	4	200

(4)

Zinc- protoporphyrin(ZPP)

- ALA(ALAU), (Hb),

20- 50 $\mu$ l

(Hct)

4

Hematofluorometer (AVIV 206D, USA)

PbB 27.1  $\pm$  14.0 $\mu$ g/

415nm, 596nm

d $\ell$ ( : 5.3- 72.9 $\mu$ g/d $\ell$ ) , ZPP 55.1  $\pm$

(Blumberg , 1977; Lamola , 1975).

47.6 $\mu$ g/ d $\ell$ ( : 21.0- 319.0 $\mu$ g/d $\ell$ )

, ALAP 18.9  $\pm$  25.1 $\mu$ g/ ( : 3.8- 180.7 $\mu$ g/ ),

(5)

(Hemoglobin), (Hematocrit)

ALA U 2.07  $\pm$  4.6mg/ ( : 0.1- 56.5mg/ )

(Coulter counter A.T series,

USA)

5 PbB ALAP, ALAU, ZPP

PbB 20 $\mu$ g/d $\ell$

ALAP 11.2  $\pm$  3.0 $\mu$ g/ , PbB 20- 40 $\mu$ g/d $\ell$

12.8  $\pm$  11.5 $\mu$ g/ , PbB 40 $\mu$ g/d $\ell$

126.3  $\pm$  73.0 $\mu$ g/ PbB 가 가

ALAP 가 가

4

218

(P<0.01). PbB 가 가 ALAU,

(PbB), ZPP(ZPP), - ALA(ALAP), ZPP 가 (P<0.01).

Table 4. Mean of lead exposure indices in 218 lead exposure indices

Variales	Mean ± SD	Minimum	Masimum
Hb(g/dℓ)	14.5 ± 1.0	11.4	16.5
Hct(%)	42.8 ± 2.9	34.0	50.0
PbB(μg/dℓ)	27.1 ± 14.0	5.3	72.9
ZPP(μg/dℓ)	55.1 ± 47.6	21.0	319.0
ALAP(μg/ )	18.9 ± 25.1	3.8	180.7
ALAU(mg/ )	2.1 ± 4.6	0.1	56.5

Hb : hemoglobin    Hct : Hematocrit    PbB : blood lead    ZPP : Zinc protoporphyrin  
ALAP : Plasma - aminolevulinic acid    ALAU : Urine - aminolevulinic acid

Table 5. The means of lead exposure indices by blood lead levels

Variable	- 20 (n=115)	blood lead level(μg/dℓ)		
		21- 40 (n=71)	41- (n- 32)	Total (n=218)
Hb(g/dℓ)	14.5 ± 0.9	14.7 ± 0.9	14.2 ± 1.1	14.5 ± 0.9
HCT(%)	42.6 ± 2.7	43.1 ± 2.6	42.7 ± 3.8	42.8 ± 2.9
ZPP(μg/dℓ)	33.0 ± 7.6	46.5 ± 22.9	126.3 ± 73.0	55.1 ± 47.6
ALAP(μg/ )	11.2 ± 3.0	12.8 ± 11.5	51.2 ± 45.4	18.9 ± 25.2
ALAU(mg/ )	1.4 ± 0.6	1.6 ± 1.2	4.1 ± 5.2	1.9 ± 2.5

Table 6. Correlation matrix between lead exposure indices in subjects

	Hb	Hct	ZPP	PbB	ALAP
Hb(g/dℓ)	0.889**				
HCT(%)	- 0.336**	- 0.248**			
ZPP(μg/dℓ)	- 0.056	0.058	0.676**		
ALAP(μg/ )	- 0.338**	- 0.202**	0.724**	0.552**	
ALAU(mg/ )	- 0.325**	- 0.263**	0.667**	0.404**	0.822**

\*\* : p < - 0.01

6 ALAP, ALAU, ALAP ALAU 0.822 가  
PbB, ZPP, Hb, Hct , ALAP ZPP 0.724.

ALAP와 PbB와의 상관성은 0.552인 것으로 나타나, ALAP와 다른 연노출지표들과의 상관성이 높은 것으로 분석되었다.

#### IV. 고 찰

연구 관련된 생물학적 노출지표로는 혈중 연량, 요중 연량과 같이 연이 체내에 흡수된 상태를 나타내는 연흡수(lead absorption)지표와 혈중 ZPP, 요중  $\delta$ -ALA 배설량과 같이 남에 의한 인체영향을 나타내는 연중독(lead intoxication)지표로 구분할 수 있다(Waldron, 1980). 혈중 연량과 혈중 ZPP 농도는 연작업자에 대한 건강진단시 중요검사항목으로 널리 사용되고 있으며, 특히 혈중 ZPP 농도의 측정은 많은 경험과 시설이 요구되는 혈중 연량의 분석과는 달리 portable hematofluoro-meter를 이용한 간편한 측정방법의 개발(Blumberg 등, 1977)로 연작업자에 대한 일차 선별검사항목으로 적극적으로 이용되고 있다.

분석방법 및 회수율의 차이는 있으나 정상인의 혈중  $\delta$ -ALA농도가  $5.3 \pm 1.4 \mu\text{g}/\ell$  (Morita 등, 1993),  $8.6 \pm 1.3 \mu\text{g}/\ell$  (Morita 등, 1994)로 보고된 바 있으며, 정상인의 혈청 및 혈장의  $\delta$ -ALA농도는  $8.2 \pm 4.7 \mu\text{g}/\ell$  (Tomokuni 등, 1992b)로 요중  $\delta$ -ALA농도의 100분의 1 수준의 낮은 농도로 분석에 어려움이 따르고 있다.

장 및 혈청  $\delta$ -ALA 분석은 비색법에 의한 분석방법(Chisolm, 1968; Suzuki 등, 1974)이 사용되었으나, 다량의 시료(4-20ml)가 필요하고, 검출한계가 매우 높아 저농도의  $\delta$ -ALA분석에 어려움이 있었다. Hosoda 등(1989)와 Takebayashi 등(1992)에 의해 전혈(3ml)을 탈단백질화하여 상층액을 형광 유도체화시켜 HPLC로 분석하는 방법이 소개되었다. 이 분석방법도 회수율이 낮고, 다른 peak에 의해 ALA peak가 방해되는 등의 단점이 지적되었다. Tomokuni 등(1992b)은 혈장을 탈단백질화 시키지 않고 직접 혈장

ALA를 형광 유도체화시킨 후 HPLC로 분석하는 개선된 방법을 제시하였다. 최근에는 Trichloroacetic acid 용액으로 혈장을 탈단백질화 시킨 후, 상층액을 acetate buffer solution(pH 3.8)을 사용하여 회수율을 개선한 분석방법(Morita, 1994)이 소개되기도 하였다. 이번 연구에서는 Trichloroacetic acid 용액으로 혈장을 탈단백질화시키지 않고 ethanol을 첨가하고 물증탕하여 혈장내 ALA를 형광유도체화시키는 과정에서 탈단백질화시켜 HPLC로 분석하는 Tomokuni법(Tomokuni 등, 1992b)을 사용하였으며, 혈장에  $\delta$ -ALA를 첨가하는 표준첨가법에 의해 검량선을 작성하여 혈장  $\delta$ -ALA 농도를 정량하였다.

전체 연구대상자의 혈장  $\delta$ -ALA는  $18.9 \pm 25.1 \mu\text{g}/\ell$ 로 분석되었으며, 연구대상자의 혈중 연농도는  $27.1 \pm 14.0 \mu\text{g}/\text{dL}$ (범위:  $5.3$ - $72.9 \mu\text{g}/\text{dL}$ ), 혈중 ZPP농도는  $55.1 \pm 47.6 \mu\text{g}/\text{dL}$ (범위:  $21$ - $319 \mu\text{g}/\text{dL}$ )로 분석되었다. 연구대상자간 혈중연 농도, 직력, 나이, 분석조건들이 달라 상대적인 비교는 어려우나, Morita 등(1994)은 혈중연 농도범위가  $2.5$ - $115.4 \mu\text{g}/\text{dL}$ 인 남성근로자를 대상으로 한 연구에서 혈장  $\delta$ -ALA가  $77.1 \mu\text{g}/\ell$ (범위:  $6.5$ - $441.5 \mu\text{g}/\ell$ )인 것으로 보고하였고, Pornchai 등(1999)은 평균 혈중연 농도가  $23.42 \pm 9.10 \mu\text{g}/\text{dL}$ 인 연폭로 어린이를 대상으로 한 연구에서는 혈장  $\delta$ -ALA가  $8.12 \pm 3.67 \mu\text{g}/\ell$ (범위:  $1.5$ - $27.08 \mu\text{g}/\ell$ )인 것으로 보고하였다.

이번 연구에서 혈장  $\delta$ -ALA와 혈중 연농도와와의 상관성은  $r=0.552$ , 혈중 ZPP와의 상관성은  $r=0.724$ , 요중  $\delta$ -ALA와의 상관성은  $r=0.822$ 로, 현재 연노출 근로자에 대한 검진시 활용되고 있는 연노출 지표중 요중  $\delta$ -ALA와의 상관성이 높은 것으로 나타났으며 통계적으로도 유의하였다. Pornchai 등(1999)의 연구(혈장  $\delta$ -ALA와 혈중 연 :  $r=0.38$ , 혈장  $\delta$ -ALA와 FEP :  $r=0.41$ )보다는 높은 상관성이 있는 것으로 분석되었고, 혈장  $\delta$ -ALA와 요중  $\delta$ -ALA의 경우 Morita 등(1994)의 결과( $r=0.921$ )보다는 상관성이 낮은 것으로 분석되었다.

이와 같이 혈장  $\delta$ -ALA농도는 혈중 연, 혈중

ZPP, - ALA 가 - ALA  
 . - ALA  
 . - ALA  
 , - ALA ZPP, - ALA  
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 - ALA

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