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Urinary α_1 Microglobulin in Lead Workers

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Short-term heavy exposure to lead has been reported to cause nuclear inclusion bodies in renal proximal tubular cells, whereas prolonged exposure causes diffuse interstitial or peri-tubular fibrosis in the proximal tubules (Cramér et al. 1974). However, no functional impairment has been found by measurements of serum creatinine, or of inulin or p-aminohippurate (PAH) clearances. These renal functional parameters, which also include the concentration of blood urea nitrogen (BUN) and the total urinary protein concentration, are too insensitive to detect the early loss of renal function due to lead. Therefore, in order to clarify whether a relatively low level of occupational exposure to lead can cause renal damage, it is necessary to detect subclinical renal dysfunction or impairment using more sensitive measurements than the classical renal tests which can detect only symptomatic renal failure.

The excretion of low molecular weight proteins or renal specific enzymes in the urine may be a sensitive indicator of early renal injury. However, β_2-microglobulin (β_2-m) has been measured among lead workers, but elevated concentrations have not been found (Bernard et al. 1989; Endo et al. 1990; Gerhardsson et al. 1992). In the case of urinary N-acetyl-ß-D-glucosaminidase (NAG) excretion, elevated levels have been found in lead workers (Verschoor et al. 1987; Endo et al. 1990). In order to determine whether urinary NAG activity is the only marker responding at an early stage of lead nephropathy, we measured concentrations of another low molecular weight protein, α_1-microglobulin (α_1-m), in male lead workers. Many reports indicate that the increase of urinary α_1-m (U-α_1-m) can be used as an early indicator of renal tubular dysfunction (Itoh et al. 1983; Kusano et al. 1985) but no one has reported the relationship between α_1-m and lead exposure.

MATERIALS AND METHODS

This study was conducted on 99 male workers employed in two lead solder factories and in a secondary lead refinery. The subjects were battery dismantling workers, lead refiners, lead smelters, workers making lead solders, maintenance workers, office workers and managers. The airborne lead concentrations in the work-

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places handling lead in the two solder factories and in the secondary refinery were 6.6, 7.9 and 113 $\mu g/m^3$ as geometric means, respectively. The workers' age and duration of employment were 43.6 ± 13.3 and 11.0 ± 11.7 yr (mean ± S.D.), respectively. None of the subjects had a history of chelation therapy.

Blood and spot urine sampling were performed during medical examinations of the lead workers. The blood was carefully venipunctured to avoid lead contamination. Biological analyses were conducted as follows. Blood lead (Pb-B) was measured by anodic stripping voltammetry (Karai et al. 1980). Serum α_1-m (S-α_1-m) and U-α_1-m were measured by the double antibodies radioimmunoassay method (AMG RIA Shionogi, Shionogi, Osaka Japan) (Matsui et al. 1989). Serum and urinary creatinine concentrations were measured by Jeffés modified method (Fabiny et al. 1971). The value of U-α_1-m was adjusted for dilution by the concentration of creatinine in urine.

Non-parametric statistical methods, Mann-Whitney U test and Spearman rank order correlation coefficients between two indices, were used because group sizes and variances varied to some extent among groups. Probability values (p) less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Since the blood lead level is a good marker of lead exposure (ACGIH, 1991), the subjects were categorized into five groups according to blood lead levels as shown in Table 1. All workers in Group I were working in offices and had no occasion to receive occupational exposure to lead. This group was considered to be a control group. Workers in Group V were lead refiners, smelters and battery dismantling workers. The remaining groups consisted of workers with various occupations.

The average ages of Groups IV and V were significantly higher than those of Group I (p<0.01), but those of Group II and III were almost the same as those of Group I.

Table 1. Age and employment duration of 99 workers categorized by blood lead.

Group	I	II	III	IV	V
Number	16	27	26	14	16
Pb-B	7.9	14.4	27.6	49.6	76.2
(μg/dl)	(3.9-9.9)	(11.2-19.5)	(20.9-37.3)	(41.7-59.1)	(61.3-107.7)
Age	40	36	44	52 **	57 **
(yr)	(19-63)	(19-76)	(22-63)	(41-65)	(29-64)
Employment	1.5	4.5	9.4 *	11.4 *	8.4 *
(yr)	(0.5-35)	(0.5-44.3)	(0.2-43.6)	(1.2-42.3)	(2.0-42.3)

Subjects were categorized into five groups by blood lead levels (Pb-B) as follows: Group I, 0-9.9 μg/dl; Group II, 10.0-19.9 μg/dl; Group III, 20.0-39.9 μg/dl; Group IV, 40.0-59.9 μg/dl; Group V, 60.0- μg/dl. Values represent medians of the groups and the parentheses show the ranges. Values in Group II - V were compared with those in Group I -- level of significance: * p<0.05; ** p<0.01.

Table 2 presents U-α_1-m, S-α_1-m and serum creatinine (S-creatinine) data of the five groups. None of the workers had any symptoms of renal disease and none exceeded the normal range of serum creatinine (within 1.5 mg/dl). S-α_1-m was reported to be useful for estimating glomerular filtration rate (GFR), and the mean and the standard deviation were 2.13 ±0.25 mg/dl for 20 healthy Japanese male adults aged 21-54 yr (Matsui et al. 1989). Median concentrations of S-α_1-m in the five groups were similar, and the values were also similar to that of healthy Japanese males reported by Matsui et al. (1989). In addition, we measured the S-α_1-m of twelve healthy office workers employed in a company not included in this study. Concentrations of S-α_1-m ranged from 1.67-2.52 mg/dl and the median was 2.10 mg/dl, which was also similar to that of Groups I - V. Therefore the S-α_1-m in all of our subjects was considered to be within the normal range except for one worker in Group V who showed 6.79 mg/dl of S-α_1-m.

The upper normal limit of U-α_1-m concentration, calculated as 95% of the range, was reported as 14 mg/ g creatinine (Hofmann et al. 1989). The normal range of U-α_1-m has been reported to be 4.2 ±6.0 mg/g creatinine (mean ±2S.D.) (Yu et al. 1983). All workers in Groups I and II showed less U-α_1-m values than 14 mg/g creatinine. The percentages of workers who exceeded 14 mg/g creatinine in Groups III, IV and V were 11.5%, 28.6% and 12.5%, respectively. The U-α_1-m in Groups III, IV and V was significantly higher than in Group I ($p<0.01$, $p<0.01$, and $p<0.001$, respectively). Also, the increases were dose related. These results indicated that workers with blood lead levels higher than 20.0 μg/dl suffered to some extent from renal tubular dysfunction.

Table 3 shows the correlation among renal function parameters, blood lead and age. Urinary α_1-m correlated significantly with blood lead levels ($r = 0.482$, $p<0.001$) and also with age ($r = 0.526$, $p<0.001$). Next, in order to clarify whether the U-α_1-m elevation was due to lead exposure and/or aging, we separated the workers into ten groups according to age and Pb-B. U-α_1-m was normal for all workers of Groups I and II, but was significantly elevated in the workers of Groups III, IV and V (Table 2). Our results suggested the Pb-B level of 20.0 μg/dl as the separation level of high and low Pb-B groups (Table 4), and lead exposure in the low Pb-B group could be regarded as having no effect on U-α_1-m elevation. In low Pb-B

Table 2. Median and range values of renal functions in lead-exposed workers.

Group	I	II	III	IV	V
U-α_1 microglobulin [mg/g creatinine]	3.4 (1.2-13.9)	3.8 (1.1-11.0)	6.5 ** (1.3-21.4)	9.1 ** (2.6-34.8)	8.9 *** (2.5-19.9)
S-α_1 microglobulin [mg/dl]	2.16 (1.45-3.08)	2.02 (1.45-2.66)	2.05 (1.38-3.03)	2.30 (1.59-2.91)	2.00 (1.58-6.79)
S-creatinine [mg/dl]	1.0 (0.8-1.3)	1.0 (0.8-1.1)	1.0 (0.8-1.2)	1.0 (0.8-1.2)	0.9 (0.6-1.4)

The values are medians and the parentheses are ranges. ** $p<0.01$ and *** $p<0.001$ significant differences in the Mann-Whitney U test for Group I.

Table 3. Spearman rank correlation coefficients between renal function parameters, blood lead concentration, age, and duration of employment in male lead workers.

	Pb-B	U-α_1-m	S-α_1-m	S-Cre	Age
Urinary $\alpha_1$microglobulin	0.482 ***				
Serum $\alpha_1$microglobulin	0.080	0.219 *			
Serum creatinine	- 0.086	0.046	0.176		
Age	0.421 ***	0.526 ***	0.237 *	- 0.048	
Duration of employment	0.289 **	0.179	0.179	- 0.036	0.475 ***

Abbreviations are as follows: Pb-B, blood lead concentration; U-α_1-m, urinary $\alpha_1$microglobulin; S-α_1-m, serum $\alpha_1$microglobulin; S-Cre, serum creatinine. * $p<0.05$; ** $p<0.01$ and *** $p<0.001$ significant differences.

groups, the median of U-α_1-m increased with increasing age. This indicates that U-α_1-m is affected by age itself. The medians of U-α_1-m of the high Pb-B groups were higher than those of the low Pb-B groups for all age groups. A statistically significant difference between low and high Pb-B groups was observed in the 40's age group. This result indicated that lead itself led to an elevation of U-α_1-m.

No significant correlation was found between urinary α_1-m and duration of employment (Table 3). The workers in Groups I - V were also divided into subgroups by exposure duration of 5 years, and the difference of U-α_1-m levels was compared with the subgroups in each of the Groups I - V (Table 5). No significant differences were observed, suggesting that U-α_1-m is not affected by long-term exposure to lead.

The proteins α_1-m and $ß_2$-m flow freely through the glomerulus and are reabsorbed by proximal tubular cells. Both proteins in urine increase when the renal proximal tubule is impaired or the normal function is reduced. In our previous study, we found no increase of U-$ß_2$-m in lead workers (Endo et al. 1990). Other researchers also have found no increase (Bernard et al. 1989; Gerhardson et al. 1992). $ß_2$-M has been reported to be rapidly degraded in the bladder at pH lower than 6

Table 4. Urinary $\alpha_1$microglobulin categorized by age and blood lead concentration.

Age (yr)	19-29	30-39	40-49	50-59	60-
Low Pb-B group	2.4	3.7	3.7	4.2	6.0
	(1.1-8.2)	(1.8-9.5)	(1.1-16.9)	(4.0-7.6)	(4.6-9.6)
	n=13	n=10	n=13	n=3	n=4
High Pb-B group	3.3	6.0	7.9 *	9.6	8.0
	(1.3-6.5)	(2.3-11.2)	(2.0-3.8)	(3.6-21.4)	(2.7-17.9)
	n=6	n=8	n=12	n=19	n=11

Low Pb-B groups consist of workers with Pb-B levels lower than 20.0 µg/dl. High Pb-B groups consist of workers with Pb-B levels higher than 20.0 µg/dl. The values represent medians of urinary $\alpha_1$microglobulin (mg/g creatinine) and the parentheses represent the ranges. * ; $p<0.05$, significant difference between low and high Pb-B groups in the generations.

Table 5. Urinary $\alpha_1$microglobulin of the workers categorized by blood lead concentration and duration of employment.

Group	I	II	III	IV	V
Exposure duration of less than 5 years	4.5 (1.2-13.9) n=11	4.4 (1.1-11.0) n=14	6.0 (1.3-9.0) n=8	15.2 (4.6-34.8) n=4	7.1 (2.5-10.2) n=7
Exposure duration of more than 5 years	3.5 (2.0-4.8) n=5	4.7 (1.8-9.6) n=13	8.0 (2.0-21.8) n=18	9.0 (2.6-17.2) n=10	11.2 (5.2-19.9) n=9

Group I, 0-9.9 μg Pb-B/dl; Group II, 10.0-19.9 μg Pb-B/dl; Group III, 20.0-39.9 μg Pb-B/dl; Group IV, 40.0-59.9 μg Pb-B/dl; Group V, 60.0- μg Pb-B/dl. The urinary $\alpha_1$microglobulin values (mg/g creatinine) are medians and the parentheses are ranges. No significant differences were observed in blood lead levels between the groups with less and more than 5 years of duration of exposure.

(Schardijn et al. 1979). Generally, the cutoff value for determination of U-β_2-m is pH 5.5. However, the level is still correlated with the pH above 5.5 (Vershoor et al. 1987). This instability of β_2-m in the urinary tract is thought to hide the actual inhibition of β_2-m reabsorption in proximal tubules.

α_1-M is a glycoprotein with a molecular weight of 33,000 daltons (Takagi et al. 1979) and was first isolated from the urine of patients with chronic cadmium poisoning (Ekström et al. 1975). Elevated levels of U-α_1-m have been found in patients with cadmium poisoning (Itoh et al. 1983). Clinically, the level of U-α_1-m is elevated in patients with renal tubular disorders and the increase precedes a decrease of creatinine clearance (Kusano et al. 1985). U-α_1-m is stable in urine with a pH range of 4-8 for at least 11 days at 4°C (Yu et al. 1983). This stability of α_1-m in urine can reflect the actual reabsorption rate. The change of the U-α_1-m level is reported to precede that of U-β_2-m among people with various degrees of renal failure (Weber et al. 1985). Therefore, as this study clearly showed a significant elevation of U-α_1-m among lead workers with Pb-B levels higher than 20.0 μg/dl, a functional change in renal tubules can occur with a relatively low level of lead exposure. Also, in addition to NAG activity in urine, U-α_1-m can be a useful indicator of renal impairment due to lead exposure.

REFERENCES

American Conference of Governmental Industrial Hygienists (1991) Documentation of biological exposure indices, lead. In: Documentation of the threshold limit values and biological exposure indices, 6th edition vol. 3. ACGIH, Cincinnati, Ohio, p 99-104

Bernard A, Lauwerys R (1989) Epidemiological application of early markers of nephrotoxicity. Toxicol Lett 46:293-306

Cramér K, Goyer RA, Jagenburg R, Wilson MH (1974) Renal ultrastructure, renal function, and parameters of lead toxicity in workers with different periods of lead exposure. Br J Ind Med 31:113-127

Endo G, Horiguchi S, Kiyota I (1990) Urinary N-acetyl-ß-D-glucosaminidase activity in lead-exposed workers. J Appl Toxicol 10:235-238

Ekström B, Peterson PA, Berggård I (1975) A urinary and plasma α_1-glycoprotein of low molecular weight: isolation and some properties. Biochem Biophys Res Commun 65:1427-1433

Fabiny DL, Ertingshausen G (1971) Automated reaction-rate method for determination of serum creatinine with CentrifiChem. Clin Chem 17:696-700

Gerhardsson L, Chettle DR, Englyst G, Nordberg GF, Nyhlin H, Scott MC, Todd AC, Vesterberg O (1992) Kidney effects in long term exposed lead smelter workers. Br J Ind Med 49:186-192

Hofmann W, Guder WG (1989) A diagnostic programme for quantitative analysis of proteinuria. J Clin Chem Clin Biochem 27:589-600

Itoh Y, Enomoto H, Kawai T (1983) α_1-Microglobulin in cadmium poisoning. Nephron 35:211-212

Karai I, Fukumoto K, Horiguchi S (1980) Determination of blood lead by anodic stripping voltammetry equipped with mercury electrode. Osaka City Med J 26:39-46

Kusano E, Suzuki M, Asano Y, Itoh Y, Takagi K, Kawai T (1985) Human α_1-microglobulin and its relationship to renal function. Nephron 41:320-324

Matsui K, Kanao K, Fushimi H (1989) Determination of alpha-1 microglobulin by modified direct method (RIA) without pretreatment. Igaku to Yakugaku 21:91-96

Schardijn G, Statius van Eps LW, Swaak AJG, Kager JCGM, Persijn JP (1979) Urinary $ß_2$-microglobulin in upper and lower urinary tract infections. Lancet 1:805-807

Takagi K, Kin K, Itoh Y, Enomoto H, Kawai T (1979) Tissue distribution of human α_1-microglobulin. J Clin Invest 63:318-325

Verschoor M, Wibowo A, Herber R, Hemmen J, Zielhuis R (1987) Influence of occupational low-level lead exposure on renal parameters. Amer J Ind Med 12:341-351

Yu H, Yanagisawa Y, Forbes MA, Cooper EH, Crockson RA, MacLennan ICM (1983) Alpha-1-microglobulin: an indicator protein for renal tubular function. J Clin Pathol 36:253-259

Weber MH, Scholz P, Stibbe W, Scheler F (1985) Alpha-1-Mikroglobulin in Urin und Serum bei Proteinurie und Niereninsuffizienz. Klin Wochenschr 63:711–717

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