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Association between urinary indicators of renal dysfunction and metal concentrations in workers chronically co-exposed to cadmium, zinc and lead

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The study was carried out in 31 workers co-exposed to cadmium, lead and zinc fumes and dusts in a zinc ore refinery. Urinary cadmium, lead, zinc, β_2-M levels and NAG activities were determined to evaluate the possible dose–effect relationship between these parameters. A correlation was found between urinary cadmium, lead and zinc concentrations, and urinary β_2-M levels and NAG activities of the exposed group. A statistically significant increase was also observed for urinary NAG activity in exposed workers who had urinary cadmium concentrations $> 2\,\mu g\,g^{-1}$ creatinine. However, in the same exposed group, the increment of β_2-M was not statistically significant. In conclusion, the present study thus confirms the earlier observations and may suggest the notion that the urinary NAG seems to be a more sensitive indicator than urinary β_2-M level in early stages of renal injury of moderately cadmium co-exposure with lead and zinc even at urinary cadmium concentration as low as $2\,\mu g\,g^{-1}$ creatinine. When the earlier studies on the irreversibility of cadmium-induced tubular dysfunction and the present results were taken into consideration, the present health-based biological limit proposed by the WHO ($5\,\mu g\,g^{-1}$ creatinine) seems to be high for the occupational exposure to cadmium.

Keywords: cadmium exposure, nephrotoxicity, renal markers, NAG, β_2-M

Introduction

A number of industrial and environmental chemicals may adversely affect the kidney. One or more of its anatomical structures, such as the glomerulus, the proximal or distal tubule, or the interstitium, may be targets of nephrotoxic substances. Long-term exposure to cadmium, which is one of these types of chemicals, results in its accumulation mainly in the kidney and this causes renal tubular damage by affecting reabsorption functions of the proximal tubule. Recently, it was indicated that cadmium-induced tubular dysfunction was progressive in spite of reduction or cessation of cadmium exposure (Roels *et al.* 1997). Occupational exposure to cadmium occurs primarily through inhalation of dust and fumes. The highest occupational exposures occur in the metallurgical industries (particularly cadmium refining) in the production of nickel-cadmium batteries, cadmium pigment and plastic stabilizers (Friberg *et al.* 1986, Thun *et al.* 1991). On the other hand, chronic occupational exposure to lead also causes renal

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dysfunction, which is characterized by glomerular and tubulo-interstitial changes (Loghman-Adham 1997).

Many population groups exposed to cadmium have been studied to find early indicators for the detection of the nephrotoxic effects of cadmium. The most sensitive renal tests included the urinary enzymes *N*-acetyl-β-D-glucosaminidase (NAG) and urinary low molecular weight proteins β2-microglobulin (β_2M) (Kawada *et al.* 1990, Jung *et al.* 1993, Taylor *et al.* 1997, Yamanaka *et al.* 1998). The present study was carried out to assess these two biomarkers as early indicators in the detection of nephrotoxicity of cadmium co-exposure with zinc and lead at low concentrations by investigating the dose–effect relationship between the urinary cadmium, lead, zinc concentrations, and urinary NAG activity and β_2M levels in a group of workers at a zinc ore refinery.

Materials and methods

Subjects studied

The study was conducted on 31 male workers employed in a zinc ore refinery in Turkey and exposed to cadmium, zinc and lead together. Ages ranged from 37 to 52 years (mean 43.29) and 22 of the subjects were smokers. The average duration of exposure to cadmium was 19.9 years (range 15–24 years). Thirty controls belonged to the same socio-economic class as did the workers exposed, with ages ranging from 28 to 53 years (mean 42.23); 17 of them were smokers.

Biological monitoring

Urine samples were collected in metal-free polyethylene containers at the end of the shift. The samples were kept frozen (-20°C) until analyses were performed. For β_2M determination, urinary pH was adjusted to 6.0 if necessary to avoid degradation of β_2M. For β_2M determination, urinary pH was adjusted to 6.0, if necessary, to avoid degradation of β_2M. The urinary β_2M level was determined with a Coat-A-Count® β2 Microglobulin IRMA. Urinary NAG activity was measured according to the MCP-NAG method of Noto *et al.* (1983).

The urine samples (2.5 ml) were digested in 5 ml HNO_3 by using MILSTONE mls 1200 mega, High Performance Microwave Digestion Unit (HPMDU) in order to determine the urinary Zn, Pb and Cd by means of VARIAN, Model 30/40, Atomic absorption spectrophotometer (AAS). Because of the higher urinary excretion rate, the flame AAS technique was preferred to determine the urinary concentrations of Zn. However, urinary Pb and Cd were determined by using the flameless AAS technique because of the relatively lower urinary concentrations when compared with Zn.

For the flame AAS technique, a 10-cm slit burner was attached to the spectrometer to produce an acetylene–air flame. For the flameless AAS technique, a VARIAN graphite furnace atomizer was attached to the spectrometer and the equipment was set to background correction (deuterium lamp) mode. The determination of Zn, Pb and Cd was performed at wavelengths of 213.9, 283.3 and 228.8 nm respectively. Stock solutions of Zn (1000 μg ml^{-1}, Sigma Z-2750), Pb (1000 μg ml^{-1}, Sigma L-4885) and Cd (1000 μg ml^{-1}, Sigma C-5527) were used as calibration solutions by diluting in appropriate de-ionized water in order to provide an adequate quantification range. After digesting the standard dilutions of Zn, Pb and Cd in HPMDU, the metals were determined by AAS, as was mentioned above for the urine samples. De-ionized water was produced by using a Millipore Reverse Osmosis water system, MA (combined with distillation), and was used for the final washing and diluting procedures.

The urinary measurements were corrected with the urinary creatinine concentration and expressed as g^{-1} creatinine.

Statistical analysis

The measured urinary values were not normally distributed. Therefore, log transformation was applied to the variables before comparing the control and exposed groups by using the Student's *t*-test. Logs were used also by calculating the correlation (Pearson Correlation Analysis) between the groups.

Results

The general characteristics of the study population and urinary cadmium, lead, zinc, β_2M, creatinine levels and NAG activities of the control and exposed workers are summarized in table 1. As expected, urine cadmium and lead concentrations were statistically higher in the exposed group compared with that of the control group (3.60 versus 1.62 $\mu g\,g^{-1}$ creatinine, and 14.03 versus 6.02 $\mu g\,g^{-1}$ creatinine respectively). No significant differences between urinary zinc concentrations, β_2M levels, NAG activities of exposed and non-exposed group were observed.

In the exposed group, a good, positive correlation was evident between urinary cadmium concentration and NAG activity ($r = 0.57$, $p < 0.001$), and β_2M concentration ($r = 0.45$, $p < 0.01$), urinary zinc concentration and NAG activity ($r = 0.67$, $p < 0.001$). Again there was a weak correlation between urinary zinc concentration and β_2M concentration ($r = 0.43$, $p < 0.05$), urinary lead concentration and NAG activity ($r = 0.39$, $p < 0.05$), and β_2M level ($r = 0.39$, $p < 0.05$). We also observed a significant correlation between NAG activity and β_2M concentration of the same group ($r = 0.60$, $p < 0.001$) (table 2).

By using as landmark the concentration of 2 $\mu g\,g^{-1}$ creatinine for the urinary cadmium excretion as done by Roels *et al.* (1993), while we found a significant increase in the mean of the urinary NAG activity for the higher cadmium excretion than 2 $\mu g\,g^{-1}$ creatinine, we did not find a significant increase in the urinary β_2M level (table 3).

Urinary cadmium concentrations, NAG activities and β_2M levels in smokers and non-smokers were also compared and no statistically significant difference was evident between the groups.

Discussion

We tested the diagnostic sensitivity of two proximal tubular indicators (NAG and β_2M) for the detection of cadmium-induced nephropathy at an early stage in workers co-exposed to cadmium, zinc and lead. The most sensitive renal tests

Table 1. Characteristics and the urinary Cd, Pb, Zn β2-M and NAG levels of the control and exposed workers.

Parameters	Control group	Exposed group
n	30	31
Age (years)	42.23 (28–53)[a]	43.19 (37–52)
Years of employment	–	20 (15–24)
Smokers	17	22
Duration of smoking (years)	22.29 (10–35)	23.73 (5–40)
Smoking habit (cigarettes day^{-1})	20.70 (3–60)	19.82 (6–40)
Urinary Cd ($\mu g\,g^{-1}$ creatinine)	1.67 ± 1.75[b]	3.60 ± 5.34*
Urinary Pb ($\mu g\,g^{-1}$ creatinine)	6.68 ± 6.40	14.03 ± 12.02*
Urinary Zn ($\mu g\,g^{-1}$ creatinine)	330.89 ± 307.79	558.36 ± 731.19**
Urinary β2-M level ($\mu g\,g^{-1}$ creatinine)	83.55 ± 79.57	88.48 ± 58.15**
Urinary NAG activity ($U\,g^{-1}$ creatinine)	4.01 ± 1.91	4.90 ± 4.47**
Creatine (gL^{-1})	1.24 ± 0.80	1.35 ± 0.82**

[a] Mean (range).
[b] Mean ± SD.
* Significantly high from the control group ($p < 0.01$).
** Not significantly different from the control group ($p > 0.05$).

Table 2. Statistical evaluations between the urinary metal (Cd, Pb, Zn) excretion and the early indicators of renal dysfunction.

	Urinary NAG activity ($U g^{-1}$ creatinine)				Urinary β2-M level ($\mu g\, g^{-1}$ creatinine)			
	Control group		Exposed group		Control group		Exposed group	
Parameters	r^a	p^b	r	p	r	p	r	p
Urinary Cd ($\mu g\, g^{-1}$ creatinine)	0.12	>0.05	0.57	<0.001	0.06	>0.05	0.45	<0.01
Urinary Pb ($\mu g\, g^{-1}$ creatinine)	0.24	>0.05	0.39	<0.05	0.24	>0.05	0.39	<0.05
Urinary Zn ($\mu g\, g^{-1}$ creatinine)	0.08	>0.05	0.67	<0.001	0.32	>0.05	0.43	<0.05
Urinary NAG activity ($U g^{-1}$ creatinine)	–	–	–	–	0.02	>0.05	0.60	<0.001

[a] Correlation coefficient.
[b] Statistical significance (Students t-test).

Table 3. Average levels of urinary β2-M and NAG versus urinary Cd exretion of higher and lower than $2\,\mu g\, g^{-1}$ creatinine.

	Urinary β2-M ($\mu g\, g^{-1}$ creatinine)	Urinary NAG ($U g^{-1}$ creatinine)
Control group		
Cd $< 2\,\mu g\, g^{-1}$ creatinine ($n = 23$)	86.10 ± 60.76[a]	3.86 ± 1.86
Cd $> 2\,\mu g\, g^{-1}$ creatinine ($n = 7$)	95.33 ± 53.72*	4.76 ± 2.05*
Exposed group		
Cd $< 2\,\mu g\, g^{-1}$ creatinine ($n = 15$)	69.06 ± 45.15	3.38 ± 1.87
Cd $> 2\,\mu g\, g^{-1}$ creatinine ($n = 16$)	97.15 ± 92.28*	6.34 ± 5.67**

[a] Mean $\pm$ SD.
* Not significantly different ($p > 0.05$).
** Significantly high ($p < 0.05$).

included the tubular lysosomal enzymes NAG and the low-molecular-weight proteins β_2M (Jung *et al.* 1993, Taylor *et al.* 1997, Yamanaka *et al.* 1998). It is clear that these markers do not respond to exposure to these metals with the same sensitivity.

In the factory where this study was carried out, workers were chronically co-exposed to cadmium, lead and zinc dust and fumes. The workers were only moderately exposed to cadmium (mean $3.60\,\mu g\, g^{-1}$ creatinine) as only four subjects had urinary cadmium exceeding the previously proposed critical level ($5\,\mu g$ cadmium g^{-1} creatinine). Fels *et al.* (1994) estimated on the basis of urinary cadmium excretion. An excretion of 1.5–5.0 μg cadmium g^{-1} creatinine led to the classification of moderate, and an excretion $> 5.0\,\mu g$ cadmium g^{-1} creatinine led to the classification of a high cadmium body burden. Although urinary lead concentration is not a useful indicator of lead body burden, mean urinary lead excretion of $14.03\,\mu g\, g^{-1}$ creatinine in workers indicates a low level of lead exposure. On the other hand, zinc in urine was relatively elevated in workers.

But the results were not statistically significant. While occupational exposure to cadmium and lead occurs primarily through inhalation of dust and fumes, no data were available about zinc absorption through inhalation. It is suggested that zinc decreases cadmium uptake and cumulates in the kidney with competition (Endo *et al.* 1997). Many studies of occupational cadmium poisoning have not taken into account the co-exposure to other toxins such as lead and zinc.

Although the tubular effects of cadmium are well recognized, there is some controversy about the threshold value of urinary cadmium concentration at which nephropathy appears. The World Health Organization's (WHO 1991) recommended Health-Based Biological Limit (HBBL) for cadmium in urine of occupationally exposed workers has been set at $5\,\mu g\,g^{-1}$ creatinine. In the present study, when urinary cadmium concentration was $> 2\,\mu g\,g^{-1}$ creatinine, although urinary NAG activity was significantly increased, the urinary β_2-M level did not. It is well known that NAG is a sensitive parameter for renal effects and it is considered to be specific to the proximal tubule. NAG is classified as a renal marker which excretion may increase when cadmium in urine is $>4\,\mu g\,g^{-1}$ creatinine by Lauwerys *et al.* (1994) and $5\,\mu g\,g^{-1}$ creatinine by Fels *et al.* (1994). β_2-M, a major component of the low molecular weight proteins in the urine of cadmium workers, increases with renal tubular damage. An increase in urinary excretion of low molecular weight proteins is known as microproteinuria. Microproteinuria can be defined as β_2-M in urine $>300\,\mu g\,g^{-1}$ creatinine. In the present study, it was observed that only one worker had β_2-M in the urine of $>300\,\mu g\,g^{-1}$ creatinine (the urinary cadmium and NAG were $27.71\,\mu g\,g^{-1}$ creatinine and $25.85\,U\,g^{-1}$ creatinine respectively) as the workers were only moderately exposed to cadmium. (Only three workers had cadmium in urine $>5\,\mu g\,g^{-1}$ creatinine.) Some researchers have observed that increased microproteinuria was often diagnosed in cases with a urinary cadmium $>10\,\mu g\,g^{-1}$ creatinine (Roels *et al.* 1997). In a previous study (Karakaya *et al.* 1993), it was found that an increased prevalence (55%) of microproteinuria occurred in workers with a urinary cadmium of $>5\,\mu g\,g^{-1}$ creatinine. However, at that time, mean urinary cadmium concentration was $5.77\,\mu g\,g^{-1}$ creatinine (high cadmium body burden).

The present study found a significant correlation between urinary cadmium and NAG activity and the β_2-M level. However, the correlation between urinary cadmium and NAG activity was more significant than that between urinary cadmium and the β_2-M level. It was also observed that a weak correlation existed between urinary lead concentration and NAG activity. This was the expected result. Although the most sensitive test appears to be urinary excretion of NAG for lead, renal failure is generally observed when the blood lead level remains below a critical threshold of $60\,\mu g\,dl^{-1}$.

In conclusion, the present study thus confirms the earlier observations and may suggest the notion that the urinary NAG is a sensitive indicator in the detection of minimum renal effects of moderately cadmium co-exposure with lead and zinc even at urinary cadmium concentration as low as $2\,\mu g\,g^{-1}$ creatinine. Additionally, the stabilities in pH change, simplicity, low cost and the reliability of measurements were also satisfactory. When the earlier studies on the irreversibility of cadmium-induced tubular dysfunction and the present results are taken into consideration, the present health-based biological limit proposed by the WHO seems to be high for the occupational exposure to cadmium.

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