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Urinary excretion of proteins in chromeplaters, exchromeplaters and referents

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LINDBERG E, VESTERBERG O. Urinary excretion of proteins in chromeplaters, exchromeplaters and referents. *Scand j work environ health* 9 (1983) 505–510. β_2-microglobulin was measured in the urine of 24 presently exposed chromeplaters, 27 previously exposed chromeplaters, and 37 referents. The concentration of β_2-microglobulin and the number of "elevated" values (> 0.30 mg/l) was higher in the presently exposed group than in the referents. Within the presently exposed group there was a dose-effect relation between the concentration of hexavalent chromium in air and the number of elevated values of urinary β_2-microglobulin. However, no difference between the previously exposed chromeplaters and the referents could be demonstrated regarding urinary β_2-microglobulin. There were no indications that the exposure could raise the excretion of albumin in urine. The results seem to indicate an acute effect on the kidney tubules, which is reversible even in workers who have had a relatively high exposure to chromic acid.

Key terms: albumin, β_2-microglobulin, chromic acid, occupational exposure, urine.

In man the kidneys can be affected by acute peroral intake of hexavalent (VI) chromium compounds. The main pathological alterations are renal failure, acute tubular necrosis, and proteinuria (6, 7, 15, 17).

In animals an acute administration of chromates will cause tubular damage of the kidneys (5) and induce massive but reversible tubular proteinuria and a very increased excretion of β_2-microglobulin (2, 12). However, small doses of chromates given intraperitoneally to rats 5 d a week have resulted in alterations in the proximal renal tubules – the longer the period of administration, the severer the alterations (1). When the injections were continued for more than seven months, cell necroses resulted. Chromates in general selectively damage proximal convoluted tubules (18).

Franchini et al (5) have demonstrated that the renal clearance of diffusible chromium increased in heavily exposed chromeplaters with total exposure time. The findings were interpreted as a decreased reabsorption of chromium due to the effect on the proximal renal tubules.

Elevated protein excretion in urine is a sensitive indicator of kidney malfunction (10). We have therefore studied whether industrial exposure to chromates or chromic acid can result in effects on the kidney, demonstrable as an increased excretion of β_2-microglobulin or albumin in urine. No information on this topic could be found in the literature. The urinary concentration of these two proteins are written as U-β_2 and U-alb, respectively, in the following presentation.

Subjects

The study was made on (i) male chromeplaters with present exposure, (ii) previously exposed chromeplaters, referred to as exchromeplaters, and (iii) referents.

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Presently exposed chromeplaters

The presently exposed group included 24 male hard chromeplaters working at four plants. Their exposure was measured with personal air samplers worn by every individual, as described by Lindberg & Hedenstierna (9). The 8-h mean value ranged between 2 and 20 μg of chromium (VI)/m³ and averaged 6 μg/m³. Individual interviews had been performed for the assessment of total exposure time, medical history, and symptoms of the nose, throat and chest. The nose had been inspected with a speculum prior to the interview (9). The total exposure times ranged between 0.1 and 26 years and averaged 5.3 years, the median being 4 years. Most of the 24 workers had irritation symptoms of the airways. Two of them had an ulcerated nasal septum; another two had a complete perforation.

Exchromeplaters

The exchromeplaters consisted of 27 men who had worked in an old-fashioned chromeplating plant which existed between 1940 and 1968. The group included all male workers who could be found in the personnel register, who had worked at least one year in the plant, and who were alive and still living in Sweden, except one 80-year-old worker with a diabetic nephropathia, who was excluded. They were investigated in the same way as the presently exposed group. Five other workers were dead, and one had emigrated. The exposure time of the group ranged from 1 to 22 years with an average and median of 4 years. The ventilation system in the old plant had been very poor. Almost all the workers had had marked irritation symptoms in the airways. Seven of the 27 workers had a permanent perforation of their nasal septum at the time of the investigation in 1983. Thus it can be concluded that their exposure to chromic acid had been at least as high – probably higher – than the exposure determined for the presently exposed group by Lindberg & Hedenstierna (9).

Table 1. Age of the chromeplaters, the exchromeplaters, and their corresponding referents.

Group	N	Age (years) Mean	SD	Range
Chromeplaters	24	36	15	20–70[a]
Referents	27	38	15	18–60
Exchromeplaters	27	52	13	34–82
Referents	27	56	14	34–80

[a] Only one person over 60 years of age.

Referents

Two reference groups were used, one younger for the presently exposed group and one older for the exchromeplaters. Both were selected from a pool of 37 factory workers or exworkers living in the same area and working in the same company as the exchromeplaters. The age range was 18–80 years. When 10 individuals older than 60 years were excluded, the remaining 27, ranging between 18 and 60 years of age, became comparable to the presently exposed group. Analogously, when the 10 youngest referents were excluded, the remaining 27, ranging in age from 34 to 80 years, became comparable to the exchromeplaters. Thus the groups are overlapping, and 17 individuals aged 34–60 years are included in both groups (table 1).

In all the groups 50–60 % were smokers, but, as no differences concerning proteins in urine between smokers and nonsmokers could be demonstrated, smoking habits were ignored. Both exposed groups were, on the average, a few years younger than their referents (see table 1).

Methods

The examinations were performed on Wednesday or Thursday of a work week. Late in the evening of the previous day the exposed persons took a glass of water with Samarin [This contained 4 g of a mixture of sodium bicarbonate (52 %), tartaric acid (44 %), sodium carbonate (3 %), and potassium-sodium-tartrate (1 %).] to elevate the pH of the urine and thus minimize the risk of the protein's decomposing. Morning urine samples, taken immediately after the subjects arose, were submitted by the exposed persons. For those persons who had had to urinate during the

night, the urine sample with the highest pH value was selected for the analysis.

Urinary β2-microglobulin and albumin analyses

The analyses were performed for β_2-microglobulin with a radioimmunoassay (Phadebas Pharmacia, Sweden) (4) and for albumin with zone immunoelectrophoresis assay (20). The detection limit was 0.002 mg/l for U-β_2 and $<< 2$ mg/l for U-alb. The coefficient of variation for assaying a sample on different days was < 10 % for both analyses, except for a few very low values, for which the relative uncertainity is a little higher.

The concentrations of U-β_2 and U-alb were adjusted to the density 1.024 according tc the formula

$$C_{corr} = (C \times 0.024)/(d-1),$$

where C = concentration of chromium in the urine sample and d = density of the urine sample.

Urine samples were transported at 0°C and then stored at −20°C for one to three weeks before being assayed.

For U-β_2 a "normal" range of < 0.30 mg/l was used, which is a rounded off value determined by Kjellström et al (8) as the 95 % tolerance interval when using the same method of analysis. U-β_2 values exceeding 0.30 mg/l are referred to as "elevated" in the present communication.

An analogous discussion about U-alb in healthy persons based on studies by Vesterberg led us to define the U-alb level of > 20 mg/l as elevated (20).

Even moderate exposure to cadmium can cause a considerable elevation of U-β_2. Therefore all urine samples with elevated levels of U-β_2 were analyzed for cadmium by atomic absorption spectrometry.

Statistics

The distributions of the concentrations of proteins in the urine of the groups were skewed. Furthermore the variances differed for the groups. Therefore, when the differences between the groups were tested, an approximate test using the normal distribution was used; this procedure was justified by the size of the groups according to the central limit theorem (3). The hypothesis was that the exposed groups had elevated values. This hypothesis was supported by the fact that acute exposure in human and animal studies have never shown a reduced excretion of protein and β_2-microglobulin. Thus the p-values refer to one-tailed tests.

The dose-effect relation within groups was tested with the Mantel extension test (one-tailed) (11).

Results

β2-microglobulin

In the presently exposed group the U-β_2 values were higher than in the reference group ($p = 0.045$). Moreover elevated values occurred for more and younger presently exposed individuals than for the referents (table 2). On the other hand there was no difference between the exchromeplaters and their referents with regard to U-β_2 levels (table 2). In all the groups all the urine samples had a pH value of > 6.0.

The presently exposed group included 24 chromeplaters, five of whom worked in the most heavily exposed factory. Three of these five intensively exposed workers had an elevated U-β_2. Two of the three also had a perforated nasal septum. In the plant with the lowest exposure there were no elevated U-β_2 values. The dose-effect relation was significant ($p = 0.01$). The scoring of the exposure is in accordance with table 3.

There was no correlation between total exposure time and U-β_2.

Cadmium

All urine samples with elevated U-β_2 had a urinary cadmium level of < 10 nmol/l, and therefore cadmium exposure can be excluded as the cause of the elevated U-β_2 (13).

Albumin

In the presently exposed group there were no elevated U-alb values.

Among the exchromeplaters there were three with elevated U-alb values. One of the three was 82 years old, one had

Table 2. Concentration of β_2-microglobulin in the urine (U-β_2) of the chromeplaters, the exchromeplaters, and the referents. The number and the age of the individuals with elevated concentrations (> 0.30 mg/l) are given separately.

Group	N	Mean age (years)	U-β_2 (mg/l)			Persons with elevated U-β_2 concentration	
			Mean	SD	Range	N	Ages
Chromeplaters	24	36	0.23 } *	0.27	0.04–1.24	5	20, 21, 21, 37, 58
Referents	27	38	0.15 } *	0.08	0.06–0.19	1	57
Exchromeplaters	27	52	0.25 } **	0.26	0.06–1.20	4	55, 57, 61, 82
Referents	27	56	0.29 } **	0.41	0.06–2.10	4	57, 67, 73, 80

* The difference between groups significant (p = 0.045).
** The difference between groups not significant (p = 0.35).

Table 3. Distribution of the urinary β_2-microglobulin (U-β_2) concentrations of workers presently exposed in plants with different mean values of exposure.

Plant	Exposure range ($\mu g/m^3$)	Number of workers	Mean age (years)	U-β_2 range (mg/l)	Persons with an elevated (U-β_2 > 0.30 mg/l) concentration	
					N	Ages (years)
1	11–20	5	39	0.23–1.30	3	21, 37, 58
2–3	4–8	13	37	0.04–0.44	2	20, 21
4	2–3	6	29	0.06–0.18	–	–

hypertension and bronchitis, and one had had nephrolithiasis twice.

Of the total pool of referents seven had elevated U-alb levels. One was 80 years old. Two others had earlier had albuminuria after infections. Another two had infections at the time of investigation, one of whom had a normal U-alb when reexamined two months later.

No significant differences between the exposed groups and the referents were found for the mean and median values although the values of the exposed groups were somewhat lower than those of the referents.

There were no indications that the exposure increased the excretion of U-alb.

Discussion

A moderate influence of exposure may be difficult to prove because of some nonoccupational causes for elevated U-β_2 and U-alb. Some reports describe elevated U-β_2 values without a corresponding influence on U-alb due to upper urinary tract infections (16) and common analgesics (14). Various immunologic diseases and certain tumors can raise the β_2-microglobulin concentration in blood (19), and perhaps even in urine, if the capacity of tubular reabsorption is not sufficient. Many nonoccupational causes of elevated U-alb have also been described [cited in the report of Vesterberg (20)].

About half of the elevated values of the exchromeplaters and referents in the present study could possibly be related to well-known nonoccupational causes, but this was not the case for any of the individuals under 40 years of age in the presently exposed group.

The difference between the mean values of U-β_2 in the presently exposed group and the reference group is further strengthened by the fact that the exposed workers with elevated U-β_2 levels were young. The dose-effect relation within the presently exposed group is another indication.

The absence of any indications of differences between the exchromeplaters and the referents makes it probable that the influence on U-β_2 is reversible at the exposure levels studied.

A possible remaining effect on U-β_2 in the exchromeplaters should be indicated by a dose-effect relation in that group. Seven exchromeplaters had a perforated nasal septum. Only one of them also had an elevated U-β_2 level. This result is an indication against a dose-effect relation for the exchromeplaters and is thus against irreversibility.

The results seem to contradict those of Franchini et al (5), who found a positive correlation between total exposure time and urinary chromium clearance. This finding was interpreted as an impaired reabsorption of chromium because of tubular damage. However an influence on cells reabsorbing chromium may not necessarily mean that there is tubular damage resulting in impaired reabsorption of proteins. Moreover the platers investigated by Franchini et al (5) may have had a higher level of exposure.

To sum up, there are good reasons to conclude that the exposure we studied, which was high enough to cause severe subjective and objective symptoms of the airways, can also give rise to a temporarily increased excretion of β_2-microglobulin in urine. It may also be mentioned that, among the five deceased chromeplaters, no one had a diagnosed renal disease as a primary or contributing cause of death.

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