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RENAL FUNCTION IN MINERS INTERMITTENTLY EXPOSED TO ELEMENTAL MERCURY VAPOUR

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The authors investigated renal damage in 45 mercury miners under conditions of relatively short and low-level exposure to elemental (metallic) mercury vapour (Hg^0). The analysis included urinary mercury, immunoelectrophoresis of urinary proteins, immunofixation and high-resolution electrophoresis, quantitative analysis of urinary albumin, and urinary a_1-microglobulin before and after exposure. The activity of urinary *N*-acetyl-b-D-glucosaminidase (NAG) enzyme was determined after exposure. The average duration of exposure of miners was 37 (6–82) days. Urinary mercury significantly increased during exposure. Immunoelectrophoretic changes in the composition of urinary proteins occurred after exposure in 22 of 45 miners, of whom 15 showed high molecular weight (HMW) pattern of urinary proteins and seven showed low molecular weight (LMW) pattern. Only a slight increase in the urinary a_1-microglobulin concentration and NAG activity was found in miners with the LMW pattern of urinary proteins. The results point to a slight glomerular and tubular damage in a significant proportion of exposed miners with increased absorption of mercury vapour.

Key words:
electrophoretic analysis, increased absorption, occupational exposure, renal damage, urinary proteins

Mercury can cause organ damage if it accumulates in the body to a sufficient degree. The kidney has a remarkable capacity to concentrate mercury (1–3) and should be considered the target organ in the event of occupational exposure to mercury vapour. Manifestation of renal impairment varies from proteinuria at low levels of mercury exposure to the nephrotic syndrome (4, 5). Some authors suggest

that subclinical glomerular dysfunction may be present in some mercury-exposed workers (6, 7). Other authors detected only slight renal tubular effects manifesting through slightly increased urinary excretion of low molecular-weight proteins and enzymes (8–11).

The decision to study proteins in the urine of miners occupationally exposed to mercury vapour was made on the basis of results published by other investigators (6–11) and our own experience (12). The study was conducted on a sample of workers intermittently exposed to mercury vapour in the mercury mine in Idrija, Slovenia. The aim of the study was to evaluate the potential renal damage under conditions of relatively short and low-level exposure to mercury vapour using sensitive indicators of glomerular and renal tubular dysfunction.

SUBJECTS AND METHODS

Subjects and study design

Our investigation is a part of a programme of routine medical surveillance of miners intermittently exposed to mercury vapour and comprises target medical examinations and biological monitoring before and after workers' exposure to mercury vapour (13, 14). This study comprises 50 mercury miners periodically exposed to mercury vapour. The study has been conducted in accordance with the ethical standards laid down in Declaration of Helsinki. All participants gave their informed consent before their inclusion in the study. The data were obtained by specific questionnaires and from the workers' medical files. The targeted medical examinations were based on the findings of other authors (3, 15) and our own experience (16–18).

Medical examinations at the level of medical screening were performed before and after exposure to mercury vapour. The questionnaire was focused on medical history, in particular, the use of medicaments, smoking habits, alcohol consumption, and occupational history, typical non-specific neuropsychic symptoms, and oral and gastrointestinal symptoms of »micromercurialism«. The examination included the evaluation of neurological status: postural tremor of the upper extremities, coordination, reflexes, the presence or absence of pathological reflex, including snout reflex and pin-pain sensation, touch pressure, two-point discrimination and vibratory sensation (using a 128 Hz tuning fork).

The examinees were selected according to the following criteria: no symptoms or signs of "micromercurialism" or alcoholism, no history of occupational exposure to lead, cadmium, or other nephrotoxic substances, no history of renal disease, arterial hypertension (>140/90 mm Hg), diabetes mellitus or multisystemic diseases, no evidence of haematuria, pyuria, glycosuria, infections or neoplasia, and no consumption of analgesics or antibiotics for three weeks before the examination.

Pre-exposure medical examination revealed five miners with high and low molecular weight electrophoretic patterns of urinary proteins. They were excluded from the study to avoid any influence of previous mercury vapour exposure on the kidney function. Forty-five miners with normal electrophoretic patterns of urinary proteins

who remained in the study aged between 24 and 50 years. Their work experience in the mine ranged from 2 to 27 years. To avoid constant exposure, the miners were regularly switching between workplaces involving elevated mercury vapour concentrations and non-contaminated sites. Before our study, the miners had not been exposed to metallic mercury for 60–180 days. During exposure to mercury vapour, all miners used powered air-purifying helmets (Racal Airstream Inc.) with mercury-absorbing filters.

The miners selected for investigation were followed-up after the exposure to mercury vapour ceased. The first morning urine samples of all examined miners were taken before and after exposure period for analysis of total mercury to determine the internal mercury doses received during exposure. To compare the kidney function before and after exposure we used immunoelectrophoretical separation of urinary proteins and the Hoffman-Guder's screening programme (19) for quantitative single urinary protein determination in the second morning urine samples. The activity of urinary *N*-acetyl-β-D-glucosaminidase (NAG) was analysed only in post-exposure urine samples. All biological samples were sent for analysis to a clinical and toxicological laboratory immediately after collection.

Cumulative exposure to mercury vapour (cumulative urinary mercury index) was calculated by summing individual urinary mercury values in the last year (internal dose) and expressed in μg/L. Individual external exposure was assessed by the means of time-weighted averages (TWAs) of the daily profile of mercury vapour concentration in the air expressed in mg/m^3. Urinary mercury was used for biological monitoring of the exposed workers.

Methods

Indoor air mercury was measured in the working areas using the instant reading method (Mercury vapour indicator, MVI Shawcity, with the range 0–2 mg/m^3, sensitivity 1 μg/m^3, and repeatability ±5%). First morning urine samples were collected in mercury-free plastic containers. The total mercury concentration in urine was determined by reduction-amalgamation cold vapour atomic absorption spectrophotometry (CVAAS) after acid digestion in closed tubes at room temperature (20). The detection limit of mercury in a 0.5 ml urine sample was 0.05 ng, and the coefficient of variability (CV) ranged from 5% to 10%, depending on the concentration. Urinary mercury was expressed in micrograms per gram of creatinine. Urinary creatinine was determined according to Jaffé's picrate method (21). The results of the analysis of total mercury in urine were controlled by means of certified reference materials NBS (SRM 2672a, Freeze-Dried Urine Certified for Mercury, CV 105 μg/L±8) and by comparison with results from the laboratories of Jožef Stefan Institute (Ljubljana, Slovenia).

Immunoelectrophoresis (IE) was performed on agar gel using Dako antibodies, immunofixation (IF) on agarose gel using the same antibodies, and high-resolution electrophoresis (HR) on agarose gel or Cellogel-Mylar plates (22, 23). The following criteria were used to assess urinary protein patterns on the basis of results obtained by IE, IF, and HR using 300-fold concentration of urine samples:

- Normal pattern of urinary proteins: not more than four proteins (albumin, transferrin, IgA and IgG), visible in trace-faint thin bands (IF) or arcs (IE);
- High and middle molecular weight pattern of urinary proteins (HMW pattern of urinary proteins): at least five or more emphasised, broad, well-visible protein

bands (IF) or arcs (IE), usually albumin, α_1-acid glycoprotein, α_1-antitrypsin, transferrin, and IgG;

- Low molecular weight pattern of urinary proteins (LMW pattern of urinary proteins): broad thick bands (IF) or arcs (IE) for β_2-microglobulin or free kappa and lambda light chains in the absence of hypergamma-globulinaemia in the serum of the same patients.

Proteins were quantitatively analysed using a BNA 100 nephelometer (Behring, Germany). Antiserum, calibration, and control materials were also produced in Behring, Germany. The between-run precision of nephelometric procedures on the BNA 100 was as follows: albumin in urine CV 5.1%, sensitivity limit 11.0 mg/L; α_1-microglobulin in urine CV 9.4%, sensitivity limit 5.5 mg/L. The protein concentrations in urine were expressed in gram per mol creatinine. The upper normal limit was defined as the 95% ranges of values in »normal urine samples« (cut-off value). The upper normal limits for albumin and α_1-microglobulin are 2.26 g/mol creatinine (20 mg/g creatinine) and 1.58 g/mol creatinine (14 mg/g creatinine), respectively. All values that exceeded the upper normal limits were considered »abnormal values« and were used for screening.

The catalytic activity of *N*-acetyl-b-D-glucosaminidase (NAG) was estimated in diluted urine samples by means of the fluorimetric method using 4-methyl-umbelliferyl-2-acetamido-2-deoxy-beta-D-glucopyranoside as a substrate (24). The results were expressed in U/g creatinine, reference range 0.77–4.87 U/g creatinine, and CV varied from 5.4% to 6.7%. The upper reference range limit was defined as the 95% range of values in normal urine samples. The basic laboratory test also included a urine analysis with test strip procedures (Multistix 10 S G, Bayer diagnostics, albumin sensitivity limit 200 mg/L). Immunoelectrophoresis of serum proteins was performed before and after exposure to mercury vapour.

The data were statistically evaluated by the χ^2-test, *t*-test of paired samples, Pearson's correlation coefficient, and analysis of variance (ANOVA). In all cases PL0.05 was considered as the level of statistical significance .

RESULTS

Table 1 shows that in view of the basic characteristics of the examined miners (age, duration of work in the mercury mine, smoking habits, and alcohol consumption), cumulative urinary mercury index, and exposure of individuals in the group (days of exposure, mean of TWAs of metallic mercury in air), the subjects appear to be a relatively heterogeneous group. Mean urinary mercury levels increased during the exposure period in all workers three to four times (Figure 1).

A significant positive correlation was found between the cumulative urinary mercury index and urinary mercury concentrations before exposure. No correlations were detected either between the cumulative urinary mercury index and urinary mercury concentrations after exposure or between urinary mercury before and after exposure (Table 2). No correlation was found between external exposure indicators (days of exposure and the mean of TWAs) and urinary mercury concentrations after exposure (data not presented).

Table 1 *Characteristics of the studied group of mercury miners (N=45)*

Age (years)	37 (24–50)
Smoking status	
Cigarette smokers (N)	31
No. of cigarettes/day	20 (15–40)
[a]Alcohol consumption (N)	
<20 ml/day	27
20–140 ml/day	3
>140 ml/day	5
Work in the mercury mine (years)	8.4 (2–27)
[b]Cumulative mercury index (μg/L)	48 (22–1931)
Exposure (days)	35 (6–82)
[c]TWAs (mg/m^3)	0.36 (0.05–0.73)

The results are presented as absolute numbers (N) and medians with ranges in parentheses.
[a]Gerchow J, Schrappe O. Alkoholismus [Alcoholism, in German]. Köln: Deutscher Arzte-Verlag; 1989. p. 48.
[b]Sum of urinary mercury values from the last year of miners exposure.
[c]Time weighted averages of mercury in air

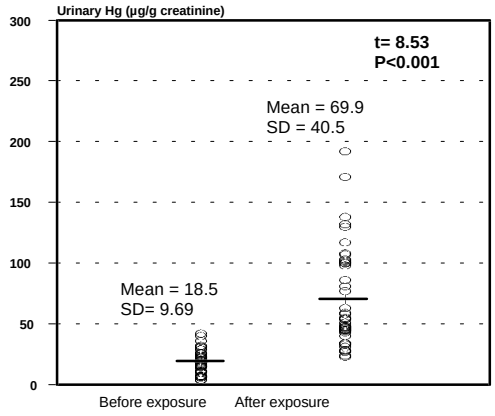

Figure 1 *Urinary mercury concentrations in miners (N=45)*

Table 2 *Correlation matrices between cumulative urinary mercury index and urinary mercury (U-Hg) concentrations before and after exposure (N=45)*

Variables correlated	**r**	**P**
[a]Cumulative U-Hg index: U-Hg before exposure	0.32	<0.05*
Cumulative U-Hg index: U-Hg after exposure	0.06	>0.05
U-Hg before exposure: U-Hg after exposure	0.16	>0.05

Variables were correlated by Pearson's correlation.
[a]Sum of urinary mercury values from the last year of miners exposure.
*Statistically significant correlation

Furthermore, the miners showed no symptoms or signs of »micromercurialism« or clinical renal impairment after exposure. The results of routine laboratory blood tests and urine analyses were within referent values. Urinary albumin analysed using a test strip was negative in all workers. Serum protein electrophoresis showed no hypergamma-globulinaemia or other significant abnormalities (data not presented).

After exposure, HMW patterns of urinary proteins were found in 15 of 45 workers and LMW patterns of urinary proteins in 7 of 45 workers, whereas in 23 workers the urinary protein composition did not differ from the protein composition determined before exposure (Table 3). Thus the immunoelectrophoretic changes in urinary protein composition were found in 22 of 45 miners with increased metallic mercury absorption. The prevalence rate of these changes was significantly higher ($c^2=15.7$,

Table 3 *Urinary concentrations of mercury, albumin, and α_1-microglobulin in subgroups of miners with normal, high molecular weight (HMW) and low molecular weight (LMW) elecrtrophoretic pattern of urinary proteins before and after exposure*

Subgroups of mercury miners (N=45)	N	Before exposure		After exposure	
		Mean±SD	Median (range)	Mean±SD	Median (range)
Miners with normal pattern of urinary proteins	**23**				
Urinary mercury (µg/g creatinine)		19.7 ± 9.9	20 (4 – 40)	62.4 ± 43.62**	49 (23.0 – 138)
Urinary albumin (g/mol creatinine)		1.40 ± 1.33	0.96 (0.55 – 6.83)	1.77 ± 1.30	1.26 (0.63 – 5.34)
Urinary α_1-microglobulin (g/mol creat)		0.43 ± 0.24	0.27 (0.27 – 1.08)	0.65 ± 0.51	0.27 (0.27 – 2.07)
Miners with HMW pattern of urinary proteins	**15**				
Urinary mercury (µg/g creatinine)		19.0 ± 10.0	19.0 (4 – 42)	70.9 ± 43.98**	54.0 (27 – 192)
Urinary albumin (g/mol creatinine)		1.39 ± 0.78	1.23 (0.57 – 3.26)	2.74 ± 4.72	1.34 0.48 – 18.9
Miners with LMW pattern of urinary proteins	**7**				
Urinary mercury (µg/g creatinine)		13.4 ± 7.76	11.0 (6.0 – 23.0)	92.3 ± 48.09**	101 (44.0 – 171)
Urinary α_1-microglobulin (g/mol creat)		0.52 ± 0.37	0.27 (0.27 – 1.26)	0.82 ± 0.30*	0.81 (0.23 – 1.15)

Statistical significance: *P<0.05; **P<0.01 (*t*-test of paired samples)

P<0.001) than the average prevalence rate in the entire group of miners observed during the period before exposure (in 5 of 50).

Table 3 shows that in all three subgroups of workers post-exposure urinary mercury concentrations significantly increased (P<0.01). No differences were found between the subgroups with either normal or HMW patterns of urinary proteins in urinary albumin and A_1-microglobulin concentrations before and after exposure. The prevalence rate of abnormal urinary albumin values was higher after exposure (26%) than before exposure (10%). Abnormal values of urinary albumin were found in both subgroups of miners, that is, those with normal and HMW patterns of urine proteins, with prevalence rates of 21% and 47%, respectively. Workers with the LMW pattern of urinary proteins showed a slightly increased urinary a_1-microglobulin mean after exposure (P<0.05, Table 3). No correlation was found between post-exposure urinary mercury concentrations and changes in urinary protein composition or urinary albumin and a_1-microglobulin concentrations (data not shown).

After exposure, workers with the LMW pattern of urinary proteins showed higher urinary NAG activity (Table 4) than in workers with either normal (P<0.01) or HMW pattern of urinary proteins (P<0.05). Positive correlations were established between the post-exposure urinary mercury concentration and urinary NAG activity (r=0.43, P=0.01), and between urinary α_1-microglobulin and NAG activity (r=0.47, P=0.001). No correlation was found between urinary NAG activity and the past-exposure indicator or pre-exposure urinary mercury (data not shown).

Table 4 *Urinary N-acetyl-β-D-glucosamidase (NAG) post exposure activity (U/g creatinine) in subgroups of miners with normal, high molecular weight (HMW) and low molecular weight (LMW) elecrtrophoretic pattern of urinary proteins*

Subgroups of mercury miners (N=45)	N	Mean±SD	95% confidence interval for mean
Miners with normal pattern of urinary proteins	23	2.9 ± 2.05[a]	2.06 – 3.74
Miners with HMW pattern of urinary proteins	15	4.17 ± 3.94[a]	2.18 – 6.17
Miners with LMW pattern of urinary proteins	7	8.29 ± 3.87[b]	5.41 – 11.16

[a,b]Differences among subgroups are indicated by different superscript letters (ANOVA at P<0.05)

DISCUSSION

It is known that occupational, mostly long-term uninterrupted exposure to inorganic mercury may affect glomerular and/or tubular renal function (4–11). Exposure to inorganic mercury may lead to the development of membranous glomerulonephritis or the nephrotic syndrome (5, 25), which can be immunologically mediated (26–29). The glomerulus is the main barrier hindering renal elimination of HMW proteins (>40,000 Daltons). Loss of selectivity of the glomerular filter can be detected at an early stage

by measuring HMW proteins in urine. The increased excretion of LMW proteins (<40,000 Daltons) in urine may be a sensitive indicator of tubular damage. They are freely filtered by the glomerulus and then taken up by the proximal tubular cells, where they are catabolised. High-molecular-weight lysosomal enzymes, which are mainly present in proximal tubular cells (30, 31), have been shown to increase in urine in the early stages of tubular damage due to cadmium, lead, and mercury exposure (11, 32, 33).

In this study, immunoelectrophoretic changes in the urinary protein composition and the quantitative analysis of urinary albumin and urinary a_1-microglobulin were selected as sensitive indicators of glomerular and tubular damage (functional indicators). Urinary NAG activity in post-exposure urine samples was chosen as a sensitive indicator of early tubular damage (cytotoxicity indicator) in order to verify the results obtained by immunoelectrophoresis of urinary proteins and quantitative analysis of urinary α_1-microglobulin. The elevated post-exposure urinary mercury, which was not in correlation with the pre-exposure urinary mercury and cumulative urinary mercury index, confirms that the increased absorption of metallic mercury was the consequence of exposure. After a relatively short and low-level exposure to mercury vapour, nearly half of the exposed miners showed an increased mercury absorption and a slight glomerular or tubular dysfunction determined by immunoelectrophoresis of urinary proteins. Abnormal values of urinary albumin were found in only 7 of 15 miners with the HMW pattern of urinary proteins. Those values kept within the range considered as minimal albuminuria (34). This finding is in agreement with the results of *Ellingsen and co-workers* (29). Increased values of urinary α_1-microglobulin and NAG activity in the subgroup of miners with the LMW pattern of urinary proteins could reflect damage to proximal tubular epithelial cells.

The results obtained by immunoelectrophoresis of urinary proteins are not directly comparable with the results of quantitative analysis of urinary albumin and urinary a_1-microglobulin. As the used methods are highly sensitive (300-fold concentrated urine samples for IE, IF, and HR electrophoresis), immunoelectrophoresis can give a very good picture of excreted proteins, but does not give any quantitative results and consequently no information about the degree of glomerular or tubular proteinuria. The question is whether the established changes in the composition and excretion of proteins in urine are the consequence of individual intra- and intervariability (15) or merely an indicator of reversible non-adverse biological effect of moderately increased metallic mercury absorption on individuals displaying increased susceptibility. The latter is partly supported by increased urinary NAG activity in the subgroup of miners with the LMW pattern of urinary proteins. A moderate positive correlation between urinary a_1-microglobulin and NAG activity and between urinary mercury and urinary NAG activity were indicative of tubular cytotoxicity, which is in agreement with published findings (35–37).

The results of our observations are partly in agreement with the results of some authors (4, 6, 7, 38) who found mainly a slight glomerular dysfunction in workers exposed to mercury vapour, and partly with the observations of other authors (8–11, 37) who found a slight tubular dysfunction. The type (intermittent) and intensity of exposure to mercury vapour, and partly the methods used for urinary protein analysis affect the results significantly. This is probably the reason for the partial disagreement of our results with those obtained in other studies. The post-exposure immunoelectrophoretical changes in the composition of proteins excreted in urine found in the subgroups of miners with HMW and LMW patterns of urinary proteins, as well as the

abnormal values of urinary albumin, differences in urinary a_1-microglobolin concentrations and NAG activity are probably acute responses to increased absorption of elemental mercury. However, in contrast to the findings of some authors (8, 9, 11, 37) these changes were not dose-dependent.

CONCLUSIONS

The results of our study point to a slight glomerular and tubular effect in a significant proportion of exposed miners with an increased absorption of mercury vapour. The specificity of immunoelectrophoretic changes in urinary protein composition obtained in our study is yet to be clarified. Our results suggest that the changes in the composition of proteins excreted in urine monitored by electrophoretic analyses (IE, IF, and HR electrophoresis) could very sensitively indicate early biological effects and help to assess the renal function of workers during increased absorption of elemental mercury under conditions of low-level and intermittent exposure. The magnitude of changes in urinary albumin and urinary α_1-microglobulin and the magnitude of urinary NAG activity were found to be small and clinically insignificant.

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Sažetak

FUNKCIJE BUBREGA U RUDARA INTERMITENTNO IZLOŽENIH PARAMA ELEMENTARNE ŽIVE

Pri profesionalnoj izloženosti parama elementarne žive (Hg^0) mogu se pojaviti oštećenja bubrega. U ovom radu procjenjivana je mogućnost učinaka relativno kraće izloženosti nižim koncentracijama živinih para na glomerularnu i tubularnu funkciju bubrega. U 45 rudara su prije i nakon izloženosti određena živa i proteini u urinu. Nakon izloženosti određena je i aktivnost *N*-acetil-β-D glukozaminidaze (NAG) u urinu. Proteini u urinu određeni su primjenom imunoelektroforeze, imunofiksacije i elektroforeze visoke rezolucije (velike sposobnosti razdvajanja), kao i pomoću kvantitativne analize albumina i α_1-mikroglobulina. Rudari, inače, već duže intermitentno izloženi živinim parama, bili su u vrijeme istraživanja izloženi u prosjeku 37 (6–82) dana pri prosječnim koncentracijama 0,37 (0,05–0,73) mg/m^3 zraka. Nakon izloženosti kod svih je rudara ustanovljena pojačana apsorpcija elementarne žive prosječnim koncentracijama žive u urinu 67,8±40,8 μg/g kreatinina. Imunoelektroforezom ustanovljene promjene u sastavu proteina u urinu nakon izloženosti su češće nego prije izloženosti (P<0,01). Kod tih rudara (u 22 od 45) s promjenama u sastavu proteina u urinu prevalencija uzoraka urina s proteinima velike molekularne mase veća je (15 od 45) nego prevalencija uzoraka urina s proteinima male molekularne mase (7 od 45). Nakon izloženosti koncentracije albumina u urinu nisu bile značajno povišene u odnosu na vrijednosti prije izloženosti. U podskupini rudara u čijim su urinima nađeni proteini male molekularne mase ustanovljen je blag porast α_1-mikroglobulina (P=0,05). Živa u urinu nakon izloženosti u bila je u blagoj pozitivnoj korelaciji sa NAG u urinu (r=0,43; P<0,01).

Prema ovim rezultatima moglo bi se zaključiti da je kod dijela promatranih osoba pojačana apsorpcija popraćena blagim – klinički neznačajnim – učincima na glomerularnu ili tubularnu funkciju bubrega. Promjene u sastavu proteina u urinu ustanovljene imunoelektroforetsim analizama, kvantitativne analize α_1-mikroglobulina i NAG u urinu mogu se upotrijebiti kao biološki pokazatelj u zdravstvenom nadzoru radnika koji su intermitentno izloženi parama elementarne žive u niskim koncentracijama.

Ključne riječi:
elektroforetske analize, oštećenje bubrega, pojačana apsorpcija, profesionalna izloženost, proteini u urinu

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