African Journal of Nephrology (2003) 7:24-28

AJN

Original Article

Impact of cadmium, lead and mercury from smoking on renal integrity

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Abstract

Background: People are exposed to cadmium (Cd), lead (Pb) and mercury (Hg) from cigarette smoking. It is unknown whether nephrotoxicity is a possible complication or not.
Methods: A total of 68 adult males were included in this study. The studied population was grouped into those who are smokers (n= 35), and those who had never smoked (n= 33). Cd, Pb and Hg levels were estimated, were determined in blood, urine, hair and nails to assess the extent of exposure to these metals. Urinary excretion of B2-microglobulin (β_2M), N-acetyl-β-D-glucosaminidase (NAG), γ-glutamyltransferase (γ-GT) and alkaline phosphatase (ALP) were determined as markers of tubular damage. Albuminuria was determined as a marker of glomerular damage. Serum levels of creatinine, β_2M and blood urea nitrogen (BUN) were determined to assess glomerular filtration.
Results: Blood Cd level and Pb levels in blood and hair were significantly higher in smokers when compared to non-smokers. Blood levels of Cd and Pb correlated significantly with the smoking index (an indicator for the smoking degree) in the smokers group. The studied markers of kidney damage were neither elevated among the smokers, nor were correlated with exposure indices of these metals.
Conclusion: Smokers are exposed to Cd and Pb. This exposure is not high enough to produce nephrotoxicity. However, it may incite the nephrotoxicity signs in the presence of risk factors for kidney diseases.

Key words: Cadmium; cigarettes smoking, lead; mercury, nephrotoxicity

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Introduction

Cd, Pb and Hg have been recognized as nephrotoxic heavy metals, following occupational and enviromental exposure [1]. The salient feature of Cd nephrotoxicity is tubular dysfunction characterized by low molecular weight proteinuria and enzymuria [2]. With more severe exposure, irreversible glomerular damage and a decrease in the glomerular filtration rate occured [3].
Three stages of the renal effects of Pb were described: first, the phase of acute exposure with dysfunction of the proximal tubules, mitochondrial changes and the formation of characteristic inclusion bodies, a second stage of chronic Pb exposure, characterized by gradual tubular atrophy, interstitial fibrosis and reduction of glomerular filtration, and a third stage, characterized by renal tubular neoplasia, which was studied in experiments in rats [1].
The effects of Hg on the kidney is determined by its chemical form. Organic Hg compounds had no nephrotoxic effects [4]. The kidneys are the primary target for accumulation of inorganic Hg, and the proximal tubule is the initial site of toxicity [5]. Current evidence indicates that at least two mechanisms are involved in the proximal tubular uptake of Hg. One mechanism appears to involve the apical activity of γ-glutamyltranspeptidase, and the other mechanism appears to be the basolateral organic anion transport system [6]. Chronic low dose exposure to inorganic Hg salts or Hg vapor may also induce immunological glomerular nephritis [1].
Mercury vapor is rapidly oxidized in erythrocytes or tissues to inorganic Hg, and thus the tissue distribution and the toxic effects of Hg vapor and inorganic Hg are the same [1].
Human are beings exposed to these metals from smoking [7,8]. There are no studies relating this the exposure to renal integrity. Therefore our work aimed to study

exposure to Cd, Pb and Hg among smokers, and its impact on renal integrity.

Material and methods

The studied population

The studied population in the present work consisted of two groups of males. A group of 35 active smokers (mean age of 30 years, with a range of 25-35 years, current smoking habit is 16±7 cigarettes/day). The smoking index was used as a marker of the smoking degree. It is calculated as the average number of smoked cigarettes per day, multiplied by the average duration of smoking in years. The mean smoking index in our smokers was 229 and its range was 108-350. All smokers use a local trade mark of cigarettes called "Cleopatra". The control group included 33 healthy males (mean age of 31.8 years, with a range of 25-38 years) who had never smoked, and were without a known exposure to these heavy metals. Inclusion criteria for both groups, based on questionnaire, were: the absence of occupational exposure to these metals, dental amalgam fillings, drug intake, diabetes, hypertension, hepatic, renal or urological diseases. Renal and urological diseases were assessed by microscopic urine examination, ultrasound examination and clinical investigation. The two groups were matched for socioeconomic status, to eliminate the effect of nutrition on the body burden of these heavy metals.

Sampling

Blood for analysis of Cd, Pb and Hg was drawn into polyethylene tubes containing heparin as anticoagulant and stored at -20 °C. Serum samples obtained after centrifugation, (3000 x g, 10 min), were stored at -20°C until analysed for creatinine, β_2M and BUN. Early morning urine samples were collected in 50 ml polypropylene tubes, and were centrifuged at 1500 x g for 10 min to clear them of particulate matter. To prevent degredation of β_2M occuring in acidic urine (pH<5.5), each person took 4 grams of sodium bicarbonate dissolved in water, the night before the morning on which urine was collected. The pH of the urine was checked and adjusted to 5.5 - 7.5 using 1 N NaOH and stored at -20°C. For analysis of Cd, Pb and Hg, 10 ml of urine were stored in acid washed polypropylene tubes at -20°C. For urinary enzymes analysis, ethylene glycol was added to a final concentration of 30% (v/v) to the urine, the pH was adjusted to 7 by 1 N NaOH and samples were stored at - 20°C [9]. Urine samples for analysis of albumin and creatinine were mixed with sodium azide (0.1 % w/v) and stored at 4°C.

Hair and nail samples were obtained with the help of stainless steel scissors and nail clippers, respectively. The samples were cut into the smallest pieces possible, and washed three times with 1% w/v Triton X-100, then rinsed three times with de-ionized water. After washing, the hair and nail samples were dried in an oven at 80°C and protected in polyethylene bags until analysis.

Analytical methods

For analysis of Cd, Pb and Hg, samples were digested as previously described [10]. Measurements of Cd and Pb were performed on a model 210VGP Buck atomic absorption spectrometer, equipped with a M-220 graphite furnace system (Buck Scientific Ltd., England). Hg was determined on a Perkin Elmer atomic absorption spectrometer with a model of 2380 equipped with MHS-10 hydride generation system (Perkin Elmer Crop., USA). We used matrix-matched calibration curves (standard addition calibration technique) to overcome the matrix interferences. The accuracy of the method was determined by the analysis of Cd, Pb and Hg in three different standard reference materials, namely LIES No. 13 human hair (Dr. Yoshinaga J, National Institute for Enviromental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305, Japan), CONTOX heavy metal blood control level III (Kaulson Laboratories Inc., USA), and urine control level II (Sigma Chemical Co., USA). The results, reported in table 1, were in close agreement with the certified values. Calibration and quality controls were checked periodically during analysis.

Table 1: Accuracy of determination of Cd, Pb and Hg (n=7)

	Cd		*Pb*		*Hg*	
	Value found	Certified value	Value found	Certified value	Value found	Certified value
NIES No 13 for human hair (µg/g)	0.24 ± 0.016	0.23 ±0.03	4.49 ±0.42	4.6 ±0.4	4.29 ±0.28	4.42 ±0.20
CONTOX blood control, level III (µg/g)	15.6 ± 3.3	15.0 ±4.0	462.0 ±51.0	450.0 ±60.0	73.0 ±9.5	70.0 ±15.0
Sigma urine control, level II (µg/g) *	-	-	71.0 ±3.5	65 (39-91)	74.0 ±12.0	98 (59-137)

* Certified values for Cd is not available are sigma urine control

Creatinine was determined in serum and urine by a modified Jaffe method [11] on a Synchron CX7 system (Beckman Instruments, Inc. USA). BUN was measured by an enzymatic conductivity rate method [12] on a Synchron CX7 system. For enzyme activities in urine, samples were subjected to gel filtration on Sephadex-G 50 (fine) (Pharmacia, Sweden) as previously described [13]. ALP was measured using p-nitrophenyl phosphate substrate in 2-amino-2-methyl-1-propanol buffer, pH 10.3(14) on a Synchron CX? system. γ-GT was measured using γ-glutamyl-p-nitroaniline substrate [15] on a Synchron CX7 system. NAG was measured by colorimetry at 37 °C using 4-nitrophenyl-N-acetyl-β-D-glucisaminide, 10 mmolll in citrate buffer, pH 4.15 as substrate [16]. Albumin in urine was measured by an immunoturbidimetric method [17]. Albuminuria kits were purchased from Bayer (NY, USA). β2M in serum and urine was measured by the microparticle enzyme immunoassay method on the IMx system (Abbott laboratories, USA).

Calculations

Excretions of urinary analytes were related to urinary creatinine to compensate for the influence of urine flow rate on excretion rates. Data was expressed as mean±SD. For statistical calculations we used SPSS-PC software, version 8 (MAS Medical & Scientific Eq. C, IL, USA). Differences between groups were tested with the independent-samples t-test. Correlation between variables was assessed with Spearman's rank correlation coefficient (r). A probability value (p) less than 0.05 was considered significant.

Results

Comparison between smokers and non-smokers in relation to exposure indices of Cd, Pb and Hg: Table 2, shows the comparison between smokers and non-smokers in relation to concentrations of Cd, Pb and Hg in blood, urine, hair and nails. As shown, smokers had significantly higher levels of blood Cd, blood Pb and hair Pb than non-smokers. The other exposure indices were not statistically different between the two groups.

Relation between exposure indices of Cd, Pb and Hg with the smoking index among the smokers: The results of the correlation coefficient between the levels of Cd, Pb and Hg in blood, urine, hair and nails with the smoking index among smokers show that only blood levels of Cd and Pb significantly correlated with the smoking index (r= 0.581, p< 0.05; r= 0.506, p< 0.05, respectively).

Comparison between smokers and non-smokers in relation to the markers of kidney damage: Table 3, shows the comparison between smokers and non-smokers with respect to the studied markers of kidney damage. As shown, the differences between the markers of kidney damage between the two groups did not reach a significant level.

Relation between exposure indices of Cd, Pb and Hg with markers of kidney damage among smokers: The results of the correlation coefficient between exposure indices of Cd, Pb and Hg with the studied markers of kidney damage in the smokers group, show that none of the correlation coefficients were statistically significant (p> 0.05).

Table 2. Comparison between smokers and non smokers in relation to levels of Cd, Pb, and Hg in blood, urine, hair and nails

	Smokers (n=35)	*Non-smokers (n=33)*
Blood Cd (µg/l)	2.67 ± 1.21 *	1.37 ± 0.45
Urine Cd (µg/g Ucr)	3.15 ± 1.4	2.89 ± 1.09
Hair Cd (µg/g)	0.39 ± 0.14	0.31 ± 0.13
Nails Cd (µg/g)	1.44 ± 0.81	1.23 ± 0.83
Blood Pb (µg/l)	143.7 ± 33.8*	101.6 ± 30.9
Urine Pb (µg/g Ucr)	17.2 ± 6.05	17.16 ± 5.69
Hair Pb (µg/g)	8.09 ± 0.86*	5.37 ± 3.41
Nails Pb (µg/g)	4.75 ± 2.69	4.9 ± 2.73
Blood Hg (µg/l)	4.23 ± 2.21	3.57 ± 1.94
Urine Hg (µg/g Ucr)	0.44 ± 0.23	0.52 ± 0.24
Hair Hg (µg/g)	0.25 ± 0.07	0.21 ± 0.07
Nails Hg (µg/g)	1.06 ± 0.12	0.98 ± 0.17

* P < 0.05

Table3. Markers of kidney damage in smokers and non-smokers

	Non-smokers (n=35)	*Smokers (n=33)*
Serum creatinine (mg/dl)	0.79 ± 0.06	0.81 ± 0.06
Serum β2M (µg/l)	1.51 ± 0.36	1.53 ± 0.25
BUN (mg/dl)	11.63 ± 2.29	12.00 ± 2.33
Urine β2M (µg/gUcr)	36.84 ± 15.97	30.43 ± 15.64
NAG (U/g Ucr)	2.24 ± 0.60	2.15 ± 0.42
ALP (U/ g Ucr)	6.49 ± 2.08	5.31 ± 1.99
γ-GT (U/g Ucr)	25.13 ± 4.62	22.71 ± 8.34
Abuminuria (µg/g Ucr)	3.09 ± 1.76	3.40 ± 1.00

Discussion

The present study is the first in which the association between the exposure to Cd, Pb and Hg with markers of kidney damage among smokers was studied.

The determination of Cd, Pb and Hg in blood, urine, hair and nails is a valuable tool for diagnosis of metal over exposure. Generally, blood and urine concentrations reflect recent exposure [1]. An exception is urinary Cd which is not excreted in urine until all the available Cd-binding sites in the body (particularly thionein) are saturated [18]. Hair and nails content, indicate the accumulation of the metal during their growth phase. Thus, hair and nails provide a lasting record of metal content over some weeks or even months [19].

Whether the levels of these metals in the biological samples are influenced by smoking was investigated. The Cd levels in blood and urine are usually higher in smokers than non-smokers [20]. According to Shaham et al. [21], exposure to cigarette smoke via active and passive smoking increases blood Cd by an average of 0.01I-1g% over the background. Wolfsperger et al. [22] found that smokers had significantly higher levels of Cd and Pb in their hair than non-smokers. Higher blood levels of Pb were observed among smokers when compared with those in non-smokers [23]. Nevertheless,

the effect of smoking on the levels of these metals in nails has not been reported.

Our study illustrated that smokers had significantly higher levels of blood Cd, blood Pb and hair Pb than non-smokers, and the extent of the increase of blood Cd and blood Pb significantly correlated with the smoking index. These findings indicate that smokers are exposed to Cd and Pb via smoking, whereas they are not at risk to Hg exposure. This may be explained by Cd and Pb released into the mainstream smoke in significant amounts by comparison with Hg. Approximately 50% and 30% of the Cd and Pb, respectively, mobilized by the smoking process were found in the mainstream and then were inhaled by smokers [8]. However, only up to 3% of Hg was inhaled with mainstream smoke and the other part was found in the sidestream [7].

The question is, whether the exposure to Cd and Pb from smoking is enough to produce nephrotoxicity. To answer this, we used sensitive available markers of renal integrity. An effect on the glomerular filtration would have been detected by measuring serum levels of creatinine, β_2M and BUN. Tubular damage may be detected by increased urinary excretion of β_2M, NAG, ALP and γ-GT, and increased glomerular permeability may be assessed by albuminuria. Many investigators used these markers to study nephrotoxicity due to cigarette smoking. Cirillo et al [24] suggested that urinary excretion of albumin is significantly related to smoking. In another study [25], it was concluded that smoking cessation does not change urinary albumin excretion in normal subjects. Andron et al [26] found that smoking is not associated with the development of an increased urinary excretion of albumin, NAG and α_1-microglobulin. In all the above studies, exposure to Cd, Pb and Hg from smoking and its nephrotoxic effects was not investigated.

In the study of Koyama et al [27], the renal effects of low-level exposure to Cd due to smoking was examined. They measured Cd in blood and urine as the exposure marker of Cd. Renal effects were assessed in terms of urinary excretion of NAG and β_2M. Neither urinary NAG nor β_2M was increased in smokers compared with non-smokers. They explained the positive correlation between NAG and urinary Cd among the smokers, as an early Cd effect on the proximal tubules. Our results show that the differences between smokers and non-smokers in relation to the studied markers of kidney integrity did not reach significant levels. Moreover, there was no significant correlation between these markers of renal integrity and the exposure indices of Cd, Pb and Hg among the smokers. These observations imply that there is no association between the exposure to Cd and Pb from smoking and renal damage.

Recently, the critical concentrations of Cd, Pb and Hg that induce nephropathy have been reviewed [28]. Most of the structural or functional renal changes become significant when urinary Hg exceeds 50 µg/g Ucr. Several studies on Pb exposed workers with blood Pb levels usually below 700 µg/l have disclosed no renal effects. A threshold of 10 µg Cd/gUcr (corresponding to 200 µg Cd/g renal cortex: the critical concentration of Cd in the kidney) is confirmed by the occurrence of low molecular weight proteinuria, and subsequent loss of renal filtration reserve capacity. The mean levels of these biomarkers measured in our smokers were below these critical limits, and then this supports our finding that the exposure to these metals from cigarette smoking had no nephrotoxic effects.

Although the present study shows that exposure to these metals from smoking did not produce nephrotoxicity, it could still be a risk factor for some resons:

1. Long term exposure may cause accumulation of these metals in the kidney which may produce renal damage.
2. The exposure to these metals from smoking may be a co-factor for kidney diseases in the presence of other risk factors such as diabetics, astherosckrotics and hypertension.
3. Another factor that may affect the toxic effects of heavy metals via smoking is the background exposure from other environmental sources, such as everyday food, water and air pollution, etc. When the background exposure is just below the critical dose to induce kidney dysfunction, an additional burden via smoking may probably be sufficient to provoke the toxicity signs. When the background level is far below the threshold, however, additional burden via smoking will not cause any effects at all, as in the case of the present study.

In conclusion, cigarette smoking is a source of Cd and Pb exposure. However, this exposure is not high enough to produce nephrotoxicity. In the presence of other risk factors for kidney disease or other sources for heavy metal exposure, this exposure to Cd and Pb from smoking may motivate the nephrotoxicity signs.

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