Urinary Metal Levels with Relation to Age, Occupation, and Smoking Habits of Male Inhabitants of Eastern Iran

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Abstract

In low-income and middle-income countries such as Iran, smoking is becoming increasingly popular, especially among young people. This has led to additional exposure to a variety of substances, including metals which may exert a toxic influence and lead to severe diseases. In order to evaluate the influence of smoking on metal concentrations, a case-control study of levels of metal in urine was carried out in smokers ($n = 64$) and non-smokers ($n = 35$) from the city of Birjand (Iran). They were divided according to their age and socioeconomic status. Concentrations of cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb), and zinc (Zn) were measured using ET-AAS. We found higher concentrations of Cd (0.03 vs. 0.12 μg/L), Co (0.6 vs. 1.22 μg/L), and Cr (14.00 vs. 18.17 μg/L) in the urine of smokers. Age and occupation are factors that also influence the levels of metals. Young smokers demonstrate higher Cd and Pb levels than other age groups. It would also appear that public sector workers and self-employed are the sectors most susceptible to high levels of metals.

Keywords Smoking · Cadmium · Lead · Exposure · Urine

Introduction

According to the World Health Organization, 40% of men and 9% of women admit to smoking, which gives in a total of 2.7 billion people worldwide [1]. By the year 2030, another billion will take up smoking by the age of 18 [2]. Smoking was responsible for 6 million deaths worldwide in 2015, and that number is set to rise to 8.3 million in 2030 [3]. A general trend that tobacco-attributable deaths tend to decline in high-income countries and to double in low-income and middle-income countries has been observed [4, 5].

Tobacco contains metals that were absorbed from soil by the plants during their growth. This transfer is influenced by a number of factors such as soil type, climate, and plant species [6–8]. Some metals that enter the body via smoking may, in conjunction with occupational exposure, increased air, food, and water pollution, lead to an increase in the development of a disease, such as cardiovascular disease, lung cancer, and COPD in humans [9–12]. The risk increases with age and poor occupational status [13].

Several studies have been conducted on the levels of essential and non-essential metals in cigarettes from Iran, but the levels in the blood, hair, and urine samples from smokers have barely been considered [14, 15]. This is a significant gap in the knowledge, since urine samples have already been identified as the best matrix (and the easiest to collect) for daily monitoring of the excretion of metals. They may thus reflect exposure [16–19]. There is a serious need to present data for human biomonitoring of that region and so to estimate the probability of the development of severe diseases according to the concentrations of metals, occupational exposure, and age.

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The purpose of this paper is therefore to determine the concentrations of metals (Cd, Co, Cr, Cu, Fe, Ni, Pb, and Zn) in the urine of people from Iran. The comparison of the levels of metals was made with respect to smoking status, age group, and the occupation of subjects. The relationships between the levels of metals in urine were also studied.

Materials and Methods

Sample Collection

In this case-control study, 99 first morning urine samples were collected (between 6 a.m. and 7 a.m.) from male smokers and male non-smokers between March and July 2018 at Birjand Health Center (Birjand University of Medical Sciences). All subjects were selected via the snowball sampling method and gave informed consent to take part in that study. The smokers consisted of subjects who smoked > 10 cigarettes per day and had done so for at least 5 years. The control (non-smoking) group included people who had no history of smoking and had not been exposed to second-hand smoke. All subjects filled in a questionnaire in order to collect demographic information, e.g., age (all subjects were divided into three age groups: young (under 35), middle-aged (between 35 and 50), and old (over 50)) and occupational status (public sector worker, laborer, self-employed, home-maker, and retirees) (Table 1).

Sample Preparation and Measurements

After collection, all the urine samples (10 mL each) were stored at -20 °C until analysis. For the mineralization, the urine samples were mixed with 2 mL of nitric acid (HNO$_3$, 65%, Merck, Germany) and 1 mL of perchloric acid (HClO$_4$, 70%, Merck, Germany) and mineralized on a water bath (TW12, Julabo GmbH, Germany) at 100 °C. Mineralized solutions were diluted with ultrapure water (18.2 MΩ-cm at 25 °C, Fistreem, WSC044, UK) up to 25 mL. The levels of Cd, Co, Cr, Cu, Fe, Ni, Pb, and Zn were quantified with an electrothermal atomic absorption spectrometer (Analytik Jena AG, ContrAA 700). The limits of detection were expressed for raw urine samples. The validity of the whole procedure was checked against the certified reference material (Table 2). Spikes and control samples were run every 10 analyses and were adjusted to fit the mid-point of the working range of the methods.

Statistical Analysis

The general characteristics of participants in that study were expressed as a median with lower (Q1) and upper (Q3) quartiles and an interquartile range (IQR). Since the data was not always normally distributed, the Mann-Whitney and Kruskal-Wallis tests followed by Duncan's post hoc were used to compare average metal concentrations in respect to smoking status, age, and occupation. Spearman's correlation coefficient analysis and Ward's method-based (and Euclidean distances) cluster analysis (CA) were also carried out to evaluate the relationships between metals in the urine. From all the correlations tested, only the strong (higher than 0.6) and significant are presented in the paper. All the analyses were performed with the SPSS 20 software (Chicago, IL, USA) with the significance level applied at 0.05.

Results

Metal Concentrations

The general scheme of the increase of metal concentrations in smokers was Cd < Co < Pb < Ni < Zn < Cr < Cu < Fe. In non-

Table 1 Levels of factors used in the research, followed by the number of corresponding individuals (data for smokers followed by data for non-smokers)

Factor	N
Smoking status	
All groups	64 vs. 35
Age	
Young (under 35 years old)	29 vs. 19
Middle-aged (35–50 years old)	24 vs. 9
Old (over 50 years old)	11 vs. 7
Occupational status	
Home-maker	3 vs. 1
Laborer	10 vs. 3
Public sector worker	26 vs. 22
Retirees	4 vs. 5
Self-employed	21 vs. 4

Table 2 Characteristics of the analytical method used along with quality assurance parameters

Metal	Wave λ (nm)	LoD (µg/L)	CRM*	Recovery (%)
Cd	228.8	0.01	–	98
Co	240.7	0.1	–	100
Cr	357.9	0.1	–	99
Cu	324.8	2.0	CRM No. 18	102
Fe	248.3	1.0	–	98
Ni	232.0	0.1	–	99
Pb	217.0	1.0	CRM No. 18	104
Zn	213.9	2.0	CRM No. 18	99

*CRM No. 18—human urine, National Institute for Environmental Studies, Japan

For Cd, Co, Cr, Fe, and Ni, no appropriate CRM was found on the market

smokers, it was the same except for Cr and Zn, which switched their positions (Table 3). Smokers had significantly higher concentrations of Cd (0.12 vs. 0.03 µg/L), Co (1.22 vs. 0.6 µg/L), and Cr (18.17 vs. 14.00 µg/L) in the urine, while at the same time, they showed significantly lower levels of Cu (20.98 vs. 25.51 µg/L), Fe (32.56 vs. 61.48 µg/L), and Zn (17.96 vs. 24.25 µg/L) than non-smokers.

The age group affected only Zn concentrations among the smokers ($p = 0.016$; lowest among the middle-aged (15.9 µg/L) and highest among the old (21.8 µg/L)) and Cu concentrations among the non-smokers ($p = 0.023$; lowest among the young (19.6 µg/L) and highest among the old (29.9 µg/L); Table 4).

Among smokers, significant differences were found in the case of Cd ($p = 0.003$), Ni ($p = 0.001$), and Pb ($p < 0.001$) levels according to their occupation. In this respect, public sector workers had the highest Cd and Pb levels in their urine, while the self-employed presented the highest Ni levels (Table 5). Among the non-smokers, significant differences were found according to their occupation only in the case of Ni level ($p < 0.001$), which again was highest in the self-employed (22.5 µg/L).

Correlation between Metals in the Urine Samples from Smokers and Non-smokers

Among the smokers, we observed relationships between Fe and Zn ($R = 0.73$, $p < 0.001$), Fe and Cu ($R = 0.63$, $p < 0.001$), and Zn and Cu ($R = 0.65$, $p < 0.001$). Among the non-smokers, a clear, significant correlation was observed between Cr and Ni ($R = 0.72$, $p < 0.001$) in the urine.

In accordance with CA and the Sneath criterion, the metals were framed into different clusters in the groups studied (Fig. 1). In non-smokers, there were three clusters: Cr-Ni-Co-Cd-Pb, Cu-Zn, and Fe, while in smokers, there were four clusters: Cr-Ni-Cu-Zn, Pb, Cd-Co, and Fe. In both groups, only one cluster, Fe, was framed in the same way.

Discussion

We found that Cd, Co, and Cr concentrations in the urine were higher in smokers and Cu, Fe, and Zn concentrations were higher in non-smokers. We also observed that smokers in the public sector had significantly elevated levels of Cd and Pb in the urine when compared with other occupations among both the smokers and the non-smokers. The age factor was a significant influence only regarding concentrations of Zn and Cu in smokers and non-smokers.

The Levels of Metals in the Urine of Smokers and Non-smokers

Smoking is regarded as the main source of Cd in humans and a major source of Pb exposure for humans, which explains increased Cd and Pb levels in the smokers we studied. While the increase was statistically insignificant for Pb, probably due to high variance, the increasing tendency was clear to see [8, 18, 20]. The levels of Cr we measured in both smokers and non-smokers were in turn several times higher than those observed by other scientists [21]. This suggests our subjects' constant exposure to some Cr source, possibly water. High levels of toxic metals lead to metal-induced oxidative stress and to a subsequent decrease in the levels of trace elements, also noted in smokers [22]. This is crucial, since an increased Fe level improves the rate of proliferation of lymphocytes and determines the body's sensitivity to immunotoxicity from xenobiotic metals [23]. Smoking is also related to an increase in the concentrations of Cu and the intensification of lipid peroxidation [24]. However, we noted a higher level of Cu in non-smokers, suggesting other sources of Cu exposure in that group.

In our study, the concentrations of Ni and Pb among both smokers and non-smokers were the only ones we found to exceed the reference values (4.4 µg/L and 1.9 µg/L, respectively) for these metals' levels in urine [25]. Even levels we measured in non-smokers were higher than observed by other

Table 3 Concentrations of heavy metals (µg/L) in the urine of non-smokers ($n = 35$) and smokers ($n = 64$)

Metal	Non-smokers			Smokers			Mann-Whitney p
	Median	Q_1	Q_3	Median	Q_1	Q_3	
Cd	0.03	0.02	0.04	0.12	0.02	0.9	0.003
Co	0.6	0.32	0.9	1.22	0.72	1.65	< 0.001
Cr	14.00	11.85	19.31	18.17	15.03	23.23	0.015
Cu	25.51	20.09	29.95	20.98	16.85	26.88	< 0.001
Fe	61.48	45.85	75.32	32.56	23.53	40.14	< 0.001
Ni	6.79	4.45	13.4	10.26	4.17	19.64	0.274
Pb	3.43	1.68	5.21	7.03	1.13	18.75	0.059
Zn	24.25	19.18	38.08	17.96	13.22	23.22	< 0.001

Table 4 The levels of metals in the urine (median ± IQR) in relation to age. Data for non-smokers below data for smokers, followed by a statistical comparison between the groups

Age	N	Cd	Co	Cr	Cu	Fe	Ni	Pb	Zn
< 35 (young)	29	0.8 ± 1.0	1.0 ± 0.8	18.2 ± 7.4	20.4 ± 10.2	28.4 ± 13.7	6.6 ± 11.4	15.5 ± 22.3	16.8 ± 8.8[A]
	19	0.04 ± 0.02	0.6 ± 0.5	14.0 ± 8.3	19.6 ± 8.1[A]	42.5 ± 42.9	6.4 ± 4.6	1.0 ± 4.6	18.9 ± 20.7
35–50 (middle-aged)	24	0.03 ± 0.5	1.3 ± 1.1	18.4 ± 12.0	19.0 ± 8.1	33.0 ± 15.2	11.8 ± 18.2	5.5 ± 16.7	15.9 ± 8.8[B]
	9	0.03 ± 0.03	0.5 ± 0.8	12.9 ± 5.8	21.5 ± 5.9[B]	51.8 ± 17.9	9.7 ± 14.3	4.4 ± 2.1	23.6 ± 7.3
> 50 (old)	11	0.07 ± 0.7	1.3 ± 1.1	17.8 ± 14.4	26.6 ± 11.1	36.1 ± 34.0	11.9 ± 21.1	3.0 ± 13.4	21.8 ± 10.0[AB]
	7	0.02 ± 0.04	0.6 ± 0.5	16.3 ± 10.1	29.9 ± 3.6[AB]	59.8 ± 6.3	12.3 ± 5.9	3.2 ± 2.3	33.2 ± 8.5
Kruskal-Wallis p		$p = 0.063$	$p = 0.192$	$p = 0.838$	$p = 0.249$	$p = 0.127$	$p = 0.097$	$p = 0.276$	$p = 0.016$
		$p = 0.661$	$p = 0.661$	$p = 0.464$	$p = 0.023$	$p = 0.733$	$p = 0.203$	$p = 0.426$	$p = 0.273$

Italicized values indicate statistically significant

The same letters in the column indicate the difference between age groups

scientists [26, 27]. We did find no reference values, however, for levels of Co, Cr, and Fe in the urine.

Relationship between Urine Metals Levels and Age

Available data indicated that homeostasis of Zn is relatively stable throughout life until the age of 65, when the aging process leads to the nutritional deficiency of micronutrients [28, 29]. Our data are consistent with that and additionally indicate that smoking habits have a greater influence than age on levels of Zn, since only among smokers we did observe statistically significant differences in levels of Zn. An inverse relationship between Zn and smoking-derived Cd also supports the above-mentioned dependence [30]. Statistically significant differences in Cu levels were observed in non-smokers of different ages and are consistent with studies on human nutrition and metabolism [31]. Generally, the absorption of Cu ions occurs mainly in the small intestine, thus lowering the metabolic rate as observed in older people and may reduce absorption intensity and simultaneously increase Cu excretion in urine [31]. We may also suspect that older people consume more vitamin C, which inhibits the absorption of Cu [32]. We also observed that levels of Pb tend to be highest among young smokers. This is consistent with the US study, which determined that youth, low BMI, and low PIR (poverty to income ratio) lead to an increase in levels of Pb [33].

Relationship Between Levels of Metal in Urine and Occupation

Our analysis of smokers with regard to their professions indicated public sector workers were the most vulnerable to high

Table 5 The levels of metals in the urine (median ± IQR) in relation to socioeconomic status. Data for non-smokers below data for smokers, followed by a statistical comparison between groups

	n	Cd	Co	Cr	Cu	Fe	Ni	Pb	Zn
Laborer	10	0.01 ± 0.03[AB]	1.1 ± 0.7	19.9 ± 5.2	23.2 ± 5.1	33.5 ± 5.9	8.6 ± 6.5	2.0 ± 8.1[A]	18.8 ± 4.9
	3	0.02 ± 0.2	0.6 ± 0.3	22.0 ± 12.2	29.9 ± 9.4	62.3 ± 15.4	12.6 ± 7.2	2.5 ± 3.5	33.4 ± 11.6
Public sector	26	0.7 ± 1.0[A]	0.9 ± 1.0	16.7 ± 6.7	20.2 ± 7.8	28.6 ± 13.7	6.9 ± 10.1[A]	21.4 ± 26.7[AB]	14.8 ± 8.8
	22	0.03 ± 0.03	0.7 ± 0.6	12.5 ± 8.1	24.8 ± 11.1	62.0 ± 42.9	4.9 ± 3.8[AB]	4.0 ± 3.8	21.8 ± 21.7
Self-employed	21	0.2 ± 1.2[B]	1.3 ± 2.4	20.4 ± 11.3	18.4 ± 11.0	37.15 ± 20.4	20.6 ± 16.5[AB]	3.0 ± 10.3[B]	18.7 ± 10.3
	4	0.04 ± 0.02	0.7 ± 1.5	13.7 ± 2.6	17.3 ± 4.7	70.2 ± 24.0	22.5 ± 9.7[A]	3.5 ± 1.0	31.0 ± 16.7
Home-maker	3	0.12 ± 0.1	1.7 ± 4.2	15.1 ± 7.4	20.8 ± 7.5	23.0 ± 15.1	1.3 ± 4.2[B]	1.8 ± 18.2	15.0 ± 5.7
	1	0.04	0.7	17.9	27.0	62.8	9.6	0.9	23.9
Retirees	4	0.03 ± 0.4	1.4 ± 2.5	28.6 ± 14.0	23.1 ± 19.7	34.4 ± 34.2	15.4 ± 20.0	3.1 ± 5.9	22.0 ± 10.8
	5	0.03 ± 0.02	0.3 ± 0.2	19.3 ± 6.6	28.9 ± 0.9	60.5 ± 2.3	14.3 ± 2.7[B]	3.3 ± 1.2	32.2 ± 7.3
Kruskal-Wallis p		$p = 0.028$	$p = 0.269$	$p = 0.069$	$p = 0.491$	$p = 0.229$	$p = 0.001$	$p = 0.001$	$p = 0.281$
		$p = 0.932$	$p = 0.284$	$p = 0.106$	$p = 0.289$	$p = 0.935$	$p = 0.0003$	$p = 0.623$	$p = 0.816$

* The group of non-smoking home-makers consisted only of one subject

Italicized values indicate statistically significant

The same letters in the column indicate the difference between occupation groups

Fig. 1 Cluster analysis of the levels of metals in urine among smokers and non-smokers. Boxes around elements indicate clusters framed according to the Sneath criterion at 33%. Vertical dashes present the Sneath criterion at 33% and 66%. Significant correlations according to Spearman's analysis are presented in the right corners
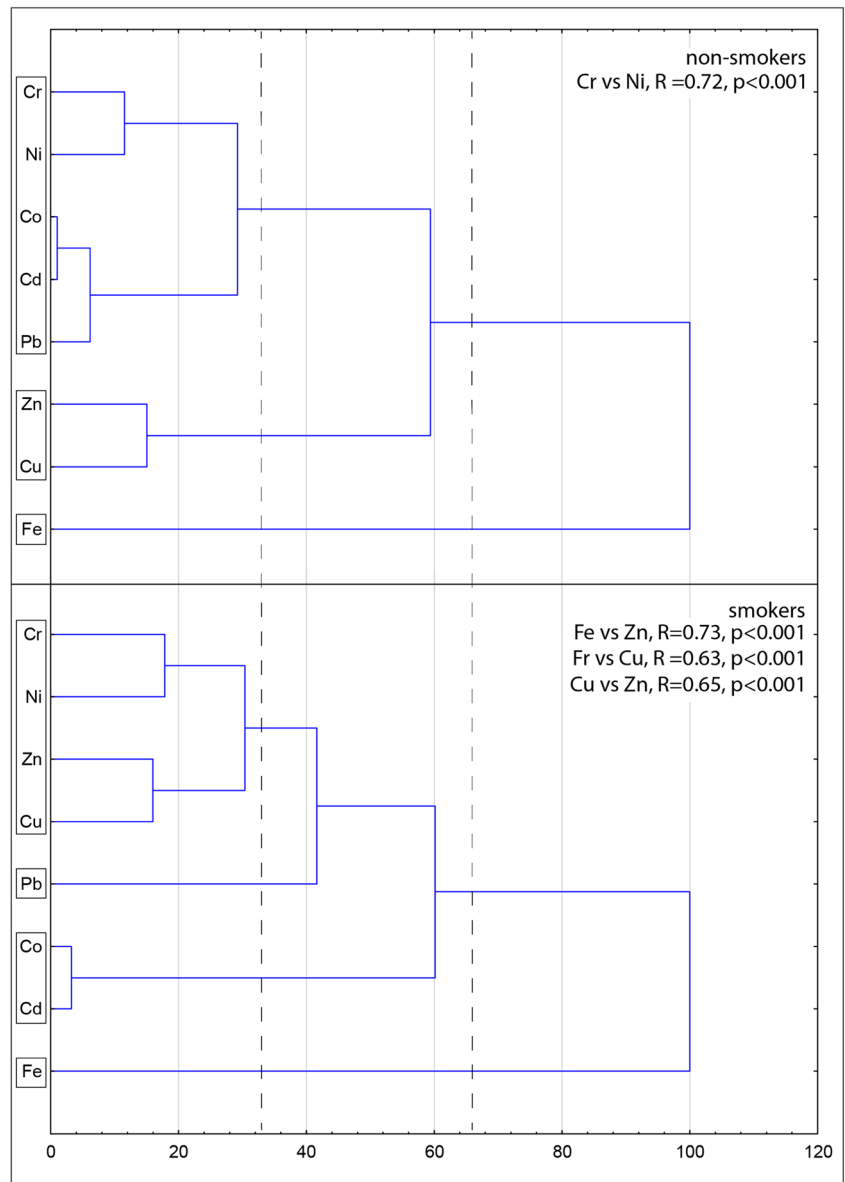
levels of Cd and Pb. Smoking is implicated in inducing significantly high levels of Cd [34]. In addition to smoking, traffic fumes and the incineration of urban waste were, however, mentioned as sources of Cd; thus, those we observed may also have come from different places [35]. That group also presented significantly higher levels of Pb, which may be due to smoking, but may also be linked to a different diet and water sources than in those with other kinds of jobs [36, 37]. The self-employed also appear to be susceptible to toxic metals, since that group demonstrated significantly higher levels of Ni in urine among both smokers and non-smokers. This suggests sources of exposure to Ni other than smoking and may include diet (e.g., a high consumption of nickel-rich products such as spinach, lettuce, or cocoa powder) and frequent use of stainless-steel kitchen utensils or cheap jewelry [38].

Correlation Between Levels of Metals in Urine Samples from Smokers and Non-smokers

Fe and Zn are found in similar food sources and thus have the potential to be positively correlated [39]. We observed this relation only in smokers and this may be related to a different diet in the groups studied, as some dietary compounds reduced absorption [40]. An Fe-deficient diet generates Cu deficiency, which may explain the clear correlation between those metals we noted [41]. In our study, Cu was found to be correlated with Zn and this suggests common sources of exposure to them [42]. Ni tends to correlate with Cr in smokers, which is pivotal since their accumulation was observed in lung cancer tumors suggesting that both metals may play a role in the development of lung cancer [6, 43]. We

found that correlation to be stronger in non-smokers and that may be related to second-hand smoke [44].

CA analysis revealed relationships between essential metals as well as between essential and xenobiotic metals. The first of these relationships indicates the participation of essential metals in maintaining homeostasis, while the second suggests antagonism between the above-mentioned groups of metals [45, 46]. This is crucial, since it indicates that regardless of smoking habits, the metabolism is still working to reduce the adverse health effects of toxic metals [47]. Clusters of metals may suggest their similar metabolic pathways [48] or their mutual origin, e.g., environmental pollution or occupational exposure [49]. For both issues, age and diet, however, may have an impact on the relationships [50].

Our study is one of only a few carried out in Eastern Iran to assess the levels of metals in human samples and at the same time to take into consideration several exposure factors. We managed to match the study and the control groups with the same basic characteristic to facilitate unbiased comparisons. As a limitation, however, we need to point out the study population, while sufficient to discuss Birjand's population, should be increased if the results are to be applied to the general population of Iran [51]. The specific approach of this study is to use urine as a biomonitoring tool. This matrix through non-invasive, easy, and abundant collection has its advantages over popular in vivo human studies (blood, hair, and nails which are harder to collect as well as the fact that samples are smaller and often contaminated), but it should be used in inferences on short-term exposure, since it is associated with a rapid metabolism and element and compound turnover [52, 53]. This limitation may be minimized by using timed urine samples, which allow us to observe changes in metabolites concentrations in each sample collected in the following 24, 48, or 72 h, or by combining urine studies with blood content analysis, for instance.

Conclusions

Smoking leads to an increase in levels of Cd, Co, and Cr in smokers' urine samples compared with the samples supplied by non-smokers. Age and occupation were also shown to influence the levels of metals in the urine. Age, however, affects the concentrations of Cd, Ni, and Pb, while profession only affects the concentrations of Cu and Zn. A growing body of scientific evidence shows that smoking is connected with the development of cancer, even if subjects are passive smokers. It is thus necessary to monitor the levels of metals in smokers and non-smokers and to identify the relationships between smoking habits and carcinogenesis, especially in those countries in which smoking is still in fashion and popular.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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