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Low cadmium exposure in males and lactating females–estimation of biomarkers☆

Anja Stajnko[a,b], Ingrid Falnoga[a,*], Janja Snoj Tratnik[a], Darja Mazej[a], Marta Jagodic[a,b], Mladen Krsnik[c], Alfred B. Kobal[d], Marija Prezelj[c], Lijana Kononenko[e], Milena Horvat[a,b]

[a] Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, Ljubljana, Slovenia
[b] Jožef Stefan International Postgraduate School, Jamova 39, Ljubljana, Slovenia
[c] Institute of Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana, Njegoševa 4, Ljubljana, Slovenia
[d] Department of Occupational Health, Idrija Mercury Mine, Arkova 43, Idrija, Slovenia
[e] Chemical Office of RS, Ministry of Health of RS, Ajdovščina 4, Ljubljana, Slovenia

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ABSTRACT

Background: Urine cadmium (Cd) and renal function biomarkers, mostly analysed in urine spot samples, are well established biomarkers of occupational exposure. Their use and associations at low environmental level are common, but have recently been questioned, particularly in terms of physiological variability and normalisation bias in the case of urine spot samples.

Aim: To determine the appropriateness of spot urine and/or blood Cd exposure biomarkers and their relationships with renal function biomarkers at low levels of exposure. To this end, we used data from Slovenian human biomonitoring program involving 1081 Slovenians (548 males, mean age 31 years; 533 lactating females, mean age 29 years; 2007–2015) who have not been exposed to Cd occupationally.

Results: Geometric means (GMs) of Cd in blood and spot urine samples were 0.27 ng/mL (0.28 for males and 0.33 for females) and 0.19 ng/mL (0.21 for males and 0.17 for females), respectively. Differing results were obtained when contrasting normalisation by urine creatinine with specific gravity. GMs of urine albumin (Alb), alpha-1-microglobulin (A1M), N-acetyl-beta-glucosaminidase (NAG), and immunoglobulin G (IgG) were far below their upper reference limits. Statistical analysis of unnormalised or normalised urine data often yielded inconsistent and conflicting results (or trends), so association analyses with unnormalised data were taken as more valid. Relatively weak positive associations were observed between urine Cd (ng/mL) and blood Cd (β=0.11, p=0.002 for males and β=0.33, $p < 0.001$ for females) and for females between urine NAG and blood Cd (β=0.14, p=0.04). No associations were found between other renal function biomarkers and blood Cd. Associations between Cd and renal function biomarkers in urine were stronger ($p < 0.05$, β=0.11–0.63). Mostly, all of the associations stayed significant but weakened after normalisation for diuresis. In the case of A1M, its associations with Cd were influenced by current smoking and blood Pb in males and by pre-pregnancy smoking and blood Se in females (β up to 0.34, $p < 0.001$). Statistical analysis of unnormalised or normalised urine data often yielded inconsistent and conflicting results (or trends), so association analyses data with unnormalised were taken as more valid.

Conclusions: The observed uncertainties introduced by urine normalisation, particularly by creatinine, confirm blood Cd as a superior low-Cd exposure biomarker versus urine Cd in cases when 24 h urine is unattainable. Evidence that A1M can be positively related to Cd, smoking (current or pre-pregnancy), Pb, and Se status, points to the versatile biological functions of A1M.

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* Corresponding author.
E-mail address: ingrid.falnoga@ijs.si (I. Falnoga).

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1. Introduction

Cadmium (Cd) is a non-essential toxic metal that occurs in the environment naturally and as a pollutant from industrial and agricultural activities. Lifelong human exposure in the general population occurs primarily through diet, water, and/or tobacco smoke. Cd accumulates primarily in the kidneys, with a long biological half-life (> 10 years), as well as in the liver, pancreas, bones, and lungs in case of smokers (Nordberg et al., 2012, 2015). Gastrointestinal (GI) absorption from dietary exposure is 3–5% and that from inhalation 10–50%. GI absorption is related to iron status and is generally higher in females than in males. Kidney Cd is often stated as Cd body burden, and the kidneys are the major target for Cd toxicity, especially epithelial cells of proximal tubules in the cortex (Åkerström, 2014; Nordberg et al., 2015).

Environmental Cd exposure in the general human population is primarily estimated by Cd levels in urine (U-Cd) and blood (B-Cd). Typically, B-Cd is an indicator of Cd exposure in recent months, given the short half-life of Cd in blood (2–3 months), whereas U-Cd better reflects Cd body burden (especially kidney deposits) accumulated and retained over the long term–of course, in the absence of renal damage (Agency for Toxic Substances and Disease Registry (ATSDR), 2012; Nordberg et al., 2015). However, for low levels of environmental exposure, the observed low levels of Cd in urine as well as those in blood could reflect both recent and past exposure (Akerstrom et al., 2013a; Bernard, 2016), and the two types of exposure cannot be distinguished. For urine, the most reliable samples are daily cumulative samples (24-h urine), but owing to challenging collection, they are most often substituted by spot samples, especially in large human biomonitoring (HBM) studies. For inter-individual comparability, spot urine levels are regularly normalised for variations in diuresis, most often by creatinine (Crea, method regularly available in clinical labs), but also by specific gravity (SG), although neither route fully adjusts against variations in diuresis (Hoet et al., 2016; Santonen et al., 2015; Weaver et al., 2016).

'Normal' or reference Cd levels in urine are generally below 1 μg/g Crea, although they vary with age, area, and smoking habits. Blood reference levels can differ more drastically, especially due to smoking habits; general levels in non-smokers are 0.3–1.2 ng/mL, while those in smokers and heavy smokers are 0.6–3.9 ng/mL and up to 7 ng/mL, respectively (Nordberg et al., 2015).

Notably, urine Cd is a more specific measure of Cd exposure than other markers of renal function. However, Cd risk assessment in humans continues to rely on the relationship between urinary Cd and renal function biomarkers, tubular or glomerular (Bernard, 2016). A widely accepted indicator of the adverse effect of Cd on the kidneys is increased urinary excretion of low molecular weight proteins (LMWP, < 40 kDa) such as α_1-microglobulin (A1M, also called protein HC, 31 kDa), β_2-microglobulin (B2M, 11.8 kDa), metallothionein (MT, < 10 kDa), and retinol-binding protein (RBP, 22 kDa), and of enzymes such as N-acetyl-β-glucosaminidase (NAG, 130–140 kDa, particularly isoenzyme B). If they exceed their respective critical levels, the above-mentioned substances serve as biomarkers of impaired reabsorption of proximal tubules. Levels of high-molecular-weight protein (HMWP, > 40 kDa) such as albumin (66 kDa) and immunoglobulin G (IgG, 150 kDa) in urine primarily serve as biomarkers of glomerular damage (Delaney et al., 2012; Barregard and Elinder, 2015).

Current health risk assessments based on U-Cd and renal function biomarkers association have shown adverse effects of Cd when urinary levels of it exceed 4 μg/g Crea, which is rarely seen in the general population (Nordberg et al., 2015; Bernard, 2016). At lower levels, particularly below 1 μg/g Crea, the meanings of observed associations are much less clear, as explained below.

Various studies have reported the possibility of early kidney effects induced by Cd at lower exposure levels (U-Cd < 1 μg/g Crea) within the general population. These reports were based on the results of positive associations between U-Cd and various urine renal function biomarkers (Bernard et al., 1995; Yamanaka et al., 1998; Jarup et al., 2000). More recently, the causality of these associations has been seriously questioned, sometimes by the same authors, but most thoroughly and convincingly by Bernard (2016). Clearly, both the level of U-Cd and renal function biomarkers in urine could be confounded by physiological sources of variability, by smoking, or silent pathological processes with no etiologic relationship with Cd (Nordberg et al., 2015; Bernard, 2016; Byber et al., 2016). Cd in blood plasma, mostly bound to low-molecular-weight MTs, but also to albumin, follows the same urinary excretion pathways (glomerular filtration and proximal tubule reabsorption) as do other plasma proteins that are used as renal function biomarkers. Therefore, variations in kidney reabsorption function owing to normal physiological processes or owing to renal damage caused by other malfunctions (mostly unidentified cardiovascular diseases and diabetes) can lead to co-excretion of protein-bound Cd (Cd-MT, Cd-Alb) and renal function biomarkers, resulting in their positive correlations, regardless of Cd exposure. Clearly, smokers can have higher U-Cd and B-Cd levels than non-smokers, but "if smoking causes proteinuria independently of Cd", then it may act as a confounder (Nordberg et al., 2015).

Additional uncertainties could be (are) introduced by normalisation of differences in urine concentration with creatinine (Crea) and/or specific gravity (SG); problems with 'incomplete corrections' and doubts pertaining to value for systematic creatinine normalisation in environmental and clinical settings are important limitations (Bernard, 2016; Hoet et al., 2016; Nordberg et al., 2015; Tang et al., 2015; Weaver et al., 2016; Zhang et al., 2015). Consequently, normalisation problems contribute to uncertainties in the association between U-Cd or B-Cd and urine renal function biomarkers, especially at levels lower than 1 or 0.5 μg/g Crea (Nordberg et al., 2015). To overcome all of the abovementioned issues, B-Cd levels and their association to urine Cd and renal function biomarkers in blood and urine should be studied as well (Barregard and Elinder, 2015; Byber et al., 2016; Weaver et al., 2016).

The aim of present study was to assess the correlations among various biomarkers, such as B-Cd and U-Cd exposure biomarkers and four renal proteins representing renal function biomarkers (Albumin, A1M, NAG and IgG) in a Slovenian study sample comprising 533 lactating females and 548 males, aged 18–49 years and with no known occupational exposure to Cd. The focus was to identify factors that might influence these associations to estimate the appropriateness and reliability of U-Cd and/or B-Cd as biomarker(s) of choice for low levels of environmental exposure.

2. Materials and methods

2.1. Study population

For the present study, we used data on 1081 occupationally non-exposed individuals aged 18–49 years, including 533 primiparous lactating females and 548 males, who participated in a wider Slovenian Human Biomonitoring survey (HBM, 2007–2015), recruited from 12 geographically different regions covering rural, urban, and industrialized areas. All participants filled out a questionnaire covering their socio-economic status, medical history, lifestyle, and nutritional habits. Some basic personal data of the study population, stratified by gender, are summarized in Table 1. Regarding smoking, the data on current smoking and pre-pregnancy smoking are provided. Females quitting smoking due to pregnancy were classified as 'pre-pregnancy' smokers. The questionnaire did not provide any information about the life-time status of former smoking, and we could only identify current smokers and women who had stopped smoking at the beginning of pregnancy.

There was no report of diabetes or renal disease among the participants of the present study. The study population had relatively