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Anti-smoking legislation and its effects on urinary cotinine and cadmium levels

Jinny E. Sánchez-Rodríguez [a], Mónica Bartolomé [a], Ana I Cañas [a], Olga Huetos [a], Carmen Navarro [a], A. Carolina Rodríguez [a], Misericordia Arribas [b], Marta Esteban [a], Ana López [a], Argelia Castaño [a,*]

[a] Environmental Toxicology, Centro Nacional de Sanidad Ambiental (CNSA), Instituto de Salud Carlos III, Carretera Majadahonda-Pozuelo Km. 2, Majadahonda, 28220 Madrid, Spain

[b] Servicio de Prevención, Instituto de Salud Carlos III, Majadahonda, 28220 Madrid, Spain

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ABSTRACT

Anti-smoking legislation has been associated with an improvement in health indicators. Since the cadmium (Cd) body burden in the general population is markedly increased by smoke exposure, we analyzed the impact of the more restrictive legislation that came into force in Spain in 2011 by measuring Cd and cotinine in first morning urine samples from 83 adults in Madrid (Spain) before (2010) and after (2011) introduction of this law. Individual pair-wise comparisons showed a reduction of creatinine corrected Cotinine and Cd levels for non-active smokers, i. e. those which urinary cotinine levels are below 50 μg/L. After the application of the stricter law, cotinine levels in urine only decreased in non-active smokers who self-reported not to be exposed to second-hand smoke. The reduction in second hand smoke exposure was significantly higher in weekends (Friday to Sunday) than in working days (Monday to Thursday). The decrease in U-Cd was highly significant in non-active smokers and, in general, correlated with lower creatinine excretion. Therefore correction by creatinine could bias urinary Cd results, at least for cotinine levels higher than 500 μg/L.

The biochemical/toxicological benefits detected herein support the stricter application of anti-smoking legislation and emphasize the need to raise the awareness of the population as regards exposure at home.

1. Introduction

Tobacco use contributes to respiratory, renal and cardiovascular problems, increases the risk of cancer, and is considered to be the single most significant cause of preventable diseases, disability and premature mortality in most developed countries (CDC, 2013).

The implementation of comprehensive national anti-smoking legislation (banning smoking in indoor public spaces or in all workplaces) has been associated with improvements in indoor air quality and in a number of health outcomes in non-smoking workers and the general population, including fewer respiratory and sensory symptoms and improvements in lung function (Hahn, 2010; CDC, 2013). Moreover, such legislation has been associated with reductions in the overall number of heart attacks and asthma-related hospital admissions (Institute of Medicine, 2009; Sims et al., 2013), and of preterm births (Kabir et al., 2009).

Spain became a party to the WHO Framework Convention on Tobacco Control in 2005. The first legislation governing smoking in public places and tobacco advertising, promotion and sponsorship (Law 28/2005) achieved a considerable reduction in exposure to environmental tobacco smoke in the workplace and, to a lesser extent, in bars and restaurants (Galán et al. 2007). In January 2011, new legislation (Law 42/2010) substantially amended the previous law by mandating an additional ban on smoking in indoor public places and workplaces, and repealed provisions that permitted designated smoking rooms, which had been used to continue to allow smoking in most bars, restaurants and airports. According to Spain's National Statistics Institute, in 2001 31.7% of the population

Abbreviations: ; SHS, second-hand smoke; SHSe, SHS exposure; U-Cd_{cr}, urinary creatinine-corrected cadmium; U-cot_{cr}, urinary creatinine-corrected cotinine; ICC, the intra-class correlation coefficient; BMI, body mass index

* Corresponding author.

E-mail addresses: jinnysanchez@isciii.es, jinnysanchez@gmail.com (J.E. Sánchez-Rodríguez), mbj@isciii.es (M. Bartolomé), acanas@isciii.es, anaicanas@gmail.com (A. Cañas), olgahh@isciii.es (O. Huetos), carnavarro@isciii.es (C. Navarro), acrodriguez@isciii.es, ac.rodriguez@csic.es (A.C. Rodríguez), marribas@isciii.es (M. Arribas), m.esteban@isciii.es (M. Esteban), alopezh@isciii.es (A. López), castano@isciii.es (A. Castaño).

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smoked daily, whereas this prevalence dropped to 24.0% in 2012, the lowest for the past 25 years (INE, 2013).

Cigarette smoke contains more than 7000 chemical substances, including nicotine, cotinine and toxic metals such as cadmium (Cd) (CDC, 2013). Cotinine is also the major metabolite of nicotine, with a longer half-life, and, as such, is frequently used to distinguish smokers from non-smokers and in studies of passive smoking (O′ Connor et al., 1995). For the general population, cigarette smoking may represent a major source of chronic exposure to Cd. The amount of this metal absorbed upon smoking one pack of cigarettes per day is about 1–3 μg/day (ATSDR, 2012). Smoking roughly doubles the Cd body burden in comparison to not smoking, mainly in the kidney, as reflected by urinary Cd (U-Cd) (ATSDR, 2012). Furthermore, a very large fraction of Cd is released into sidestream smoke, which is the main component of second-hand smoke (SHS) (Wu et al. 1995). Passive smoking has been related to increased Cd levels in blood (Shaham et al., 1996) and children's hair (Razi et al., 2012), although other authors have found no such association (Becker et al., 2007; McElroy et al., 2007; Richter et al., 2009).

Both Cd and tobacco smoke are classified by the International Agency for Research on Cancer as carcinogenic for humans, Group 1 (IARC, 2013). Long-term human exposure to Cd has been associated with renal, cardiovascular, lung and bone diseases (Järup and Akesson, 2009; ATSDR, 2012), reproductive, fetal-growth and child-development problems (Satarug and Moore 2004; Kippler et al. 2012; Ciesielski et al. 2012) and other diseases (Han et al., 2009; Satarug et al., 2010). Indeed, Cd may mediate the effect of smoking on the development of tobacco-related lung disease (Mannino et al., 2004; Lampe et al., 2008) and peripheral artery disease (Navas-Acien et al., 2004).

In light of the above, it is important to establish whether a stricter smoking ban has an impact on the reduction of cotinine and Cd load in the general population. To this end, we have evaluated whether urinary cotinine and Cd excretion in a group of adults living in Madrid (Spain) was modified after application of the above-mentioned law.

2. Material and methods

2.1. Recruitment

During their annual medical check-up, workers from the Instituto de Salud Carlos III (ISCIII) in Madrid (Spain) were asked to participate in a biomonitoring study running from 2007 to 2011 (Castaño et al., 2012). Each year, participants were requested to sign an informed consent, complete a self-administered questionnaire and provide a first morning urine sample. The questionnaire included lifestyle habits, such as smoking and smoke-exposure patterns. Verification of the subjects' smoking habits and body mass index (BMI) was obtained from the occupational health physician's report. Both the questionnaire and the study were approved by the ISCIII's Prevention Service to comply with the Declaration of Helsinki for human subject research (WMA, 2008).

A total of 83 non-occupationally exposed participants (59 women and 24 men) aged 25–62 years, whose urine samples were collected in both 2010 and 2011 and had creatinine values of between 0.3 and 3 g/L, were selected for inclusion in this study. This cohort constitutes a very stable population with no great variations in lifestyle habits and diet, as seen from the questionnaires and a preliminary comparison of the main dietary food groups (vegetables, pasta, bread/cereals, legumes; data not shown). Ferritin serum levels were relatively constant for each participant in the study period and higher than 6.5 μg/L for women and 16.6 μg/L for men.

2.2. Chemical analysis

Urine samples were aliquoted and stored at −20 °C in polypropylene tubes prewashed with 10% HNO_3 until analysis. Creatinine concentrations were determined for 1:40 dilutions using the Jaffé alkaline picrate method (Spinreact Kit, Spain).

Urinary cotinine (U-cot) was first determined by HPLC using a Diode Array Detector (Bartolomé et al., 2014). Briefly, 5 ml urine plus 2-phenylimidazol, as internal standard, was extracted on a solid phase using Oasis HLB cartridges (Waters, USA), evaporated to dryness under nitrogen and reconstituted with mobile phase solution (milli-Q water, acetonitrile). A 25 μL aliquot was injected into an HPLC system (Agilent Technologies, 1200), with detection at 259 nm (LOQ 15 μg/L).

For U-cot concentrations below 25 μg/L, the urine extract was reconstituted with 50 μL toluene and a 2 μL aliquot was injected into a single quadrupole, selected ion monitoring, electron impact gas chromatography–mass spectrometer (Agilent Technologies 5975C), with an LOQ of 1 μg/L. The results are expressed as creatinine-corrected cotinine (U-cot_{cr}).

Urinary cadmium (U-Cd) analyzes were performed in the same run, from October to December 2012, using a Dynamic Reaction Cell (DRC)-ICP-MS (PerkinElmer ELAN DRC-e), as described by Cañas et al. (2013). Briefly, mixed urine samples were diluted 1:5 in 10% HNO_3 containing 20 μg/L rhodium as internal standard (Merck KgaA, Germany) and the DRC parameters were a flow rate of 1.7 mL/min oxygen (Air Liquide) and 0.7 Rpq. External calibration ranges were 0.025–10 μg/L (Cd standard, Perkin Elmer, USA) and the limit of quantification (LOQ) was 0.025 μg/L. As an internal quality control check, the accuracy of the results was checked by analysis of the certified reference material Clincheck® urine (Recipe, Germany) analyzed every ten samples. The values obtained (2.33 ± 0.19 μg/L, N=17, and 1.81 ± 0.06 μg/L, N=16) were in good agreement with the certified values (1.94–2.90 μg/L and 1.71–2.57 μg/L, respectively).

During the measurements we participated in three Quebec Multielement External Quality Assessment Schemes (QMEQAS) as an external quality control. The values obtained (0.84 ± 0.06, 2.29 ± 0.07 and 14.03 ± 0.13 μg/L) were in good agreement with the consensus values (1.07 ± 0.15, 2.50 ± 0.16 and 14.06 ± 0.90 μg/L, respectively). The coefficient of analytical variation for U-Cd was 10%.

2.3. Statistical analysis

All analyzes were performed using PAWS Statistics 19 and STATA 11. Urine levels below the LOQ were replaced with LOQ/$\sqrt{2}$ (Hornung and Reed, 1990). Two groups were established for active / passive smoking behavior, thus, in the active smokers group were included those individuals with U-cot levels above or equal 50 μg/L (SRNT, 2002). The urinary biomarker levels estimated before and after introduction of the stricter anti-smoking legislation, i.e. in 2010 and 2011, were compared using Wilcoxon test for related samples. Comparisons between groups of different U-cot level or SHSe in non-active smokers were performed using Mann Whitney U-test for biomarker concentrations and a z-test for proportions. Statistical significance was established at $p < 0.05$.

The intra-class correlation coefficient (ICC), i.e. the inter-participant variance divided by the sum of inter- and intra-participant variance, was used to compare the agreement between repeated measurements of continuous variables. Additionally, Spearman's or Pearson's correlations were used to estimate associations between concentration measures and between the analytes quantified.