Environmental Research 150 (2016) 653–662

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Environmental exposure to arsenic and chromium in children is associated with kidney injury molecule-1

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ARTICLE INFO

Article history:
Received 22 April 2016
Received in revised form
8 June 2016
Accepted 19 June 2016
Available online 15 July 2016

Keywords:
Children
Chromium
Arsenic
KIM-1
miRNAs

ABSTRACT

Environmental hazards from natural or anthropological sources are widespread, especially in the north-central region of Mexico. Children represent a susceptible population due to their unique routes of exposure and special vulnerabilities. In this study we evaluated the association of exposure to environmental kidney toxicants with kidney injury biomarkers in children living in San Luis Potosi (SLP), Mexico. A cross-sectional study was conducted with 83 children (5–12 years of age) residents of Villa de Reyes, SLP. Exposure to arsenic, cadmium, chromium, fluoride and lead was assessed in urine, blood and drinking water samples. Almost all tap and well water samples had levels of arsenic (81.5%) and fluoride (100%) above the permissible levels recommended by the World Health Organization. Mean urine arsenic (45.6 ppb) and chromium (61.7 ppb) were higher than the biological exposure index, a reference value in occupational settings. Using multivariate adjusted models, we found a dose-dependent association between kidney injury molecule-1 (KIM-1) across chromium exposure tertiles [(T1: reference, T2: 467 pg/mL; T3: 615 pg/mL) (p-trend = 0.001)]. Chromium upper tertile was also associated with higher urinary miR-200c (500 copies/μl) and miR-423 (189 copies/μL). Arsenic upper tertile was also associated with higher urinary KIM-1 (372 pg/mL). Other kidney injury/functional biomarkers such as serum creatinine, glomerular filtration rate, albuminuria, neutrophil gelatinase-associated lipocalin and miR-21 did not show any association with arsenic, chromium or any of the other toxicants evaluated. We conclude that KIM-1 might serve as a sensitive biomarker to screen children for kidney damage induced by environmental toxic agents.

1. Introduction

Worldwide, one-third of disease burden among children is due to preventable environmental risk factors (Landrigan and Etzel, 2014; WHO, 2006a). Environmental exposures in early life are not only associated with diseases in childhood but they also heavily influence irreversible health effects later in life (Barker, 2004; Boekelheide et al., 2012). Higher, and sometimes unique exposure as well as uptake routes along with immature metabolic and excretory pathways make children particularly susceptible to environmental exposure risks (WHO, 2006b). Moreover, during maturation, differentiation and growing stages, their organs and systems may be more vulnerable to damage (WHO, 2006b).

In Mexico, the environmental hazards are complex and widespread. Water supply for about 75% of the total population relies on groundwater abstraction, which is heavily contaminated with

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http://dx.doi.org/10.1016/j.envres.2016.06.032
0013-9351/

arsenic and fluoride, particularly in the north-central region of Mexico (CONAGUA, 2010; Armienta and Segovia, 2008). In San Luis Potosi, a north-central state in Mexico, the risk of exposure to heavy metals such as arsenic, cadmium, chromium and lead is high as a result of mining and industrial activities (Calderon et al., 2001; Carrizales et al., 2006; Dominguez-Cortinas et al., 2013; Trejo-Acevedo et al., 2009). In animal models, these kidney toxicants have been shown to target predominantly the proximal tubule causing reactive oxygen species generation followed by endoplasmic reticulum stress and mitochondrial damage, culminating in cellular necrosis/apoptosis (Barbier et al., 2005; Sabolic, 2006). In humans, chronic exposure to heavy metals and fluoride has been associated with kidney disease (Soderland et al., 2010; Weidemann et al., 2015). However, most of these studies have been conducted in adults that are occupationally exposed and therefore, the effects of environmental exposure in children largely remains uninvestigated. Another limiting factor is the use of in-sensitive and non-specific biomarkers, such as serum creatinine (SCr), for evaluating kidney injury. Non-invasive and sensitive biomarkers such as kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) have demonstrated to be not only more sensitive than SCr, but also specific in detecting tubular damage (Vaidya et al., 2008; Mishra et al., 2005). Yet another class of promising biomarkers are microRNAs (miRNAs) (Pavkovic and Vaidya, 2016) and in particular, miR-21, miR-200c and miR-423 that showed high sensitivity and specificity to distinguish patients with acute kidney injury (AKI) as compared to patients without any clinical evidence of kidney injury (Ramachandran et al., 2013; Pavkovic et al., 2016).

The use of sensitive and specific biomarkers that associate with environmental kidney toxicants exposure may allow early diagnosis and facilitate prompt intervention strategies that can prevent irreversible health effects in children later in life. Therefore, the aim of this study was to evaluate the association of exposure to environmental kidney toxicants and kidney injury biomarkers in children living in San Luis Potosi, Mexico.

2. Material and methods

2.1. Description of the study area

The present study was carried out in Villa de Reyes county, a rural population (10,383 inhabitants) in San Luis Potosi, Mexico. The residents of this locality have reported high incidence of chronic kidney disease (CKD) with unknown etiology, especially in young adults. The Mexican Ministry of Health reported that genitourinary diseases are the third leading cause of death in Villa de Reyes, just after accidents and diabetes-mellitus-related outcomes (SEDESOL, 2010). Additionally, ground water represents the main source of water for domestic purposes but remains heavily contaminated with natural occurring elements such as arsenic and fluoride (Ortiz-Pérez et al., 2006). Twenty-five % of the residents in this community live in extreme poverty, 22.6% do not have basic domestic water supply system and 66% lack access to primary health insurance – factors that significantly increases the vulnerability of this population (CONEVAL, 2010).

2.2. Study design and participants

We performed a cross-sectional sampling of children attending two public elementary schools in Villa de Reyes, in June 2014. The study was approved by the institutional review board at the School of Medicine, Universidad Autónoma de San Luis Potosi. Parents were previously informed about the study and provided a written informed consent form prior to children's enrollment. Children participating were among 107 boys and girls 5–12 years old) attending first to sixth grade with uninterrupted residency in the study area since birth. Girls with menarche were excluded. Children who reported congenital kidney diseases or urinary tract infections or those taking nonsteroidal anti-inflammatory drugs or antibiotics were also. Data on residency, drinking and cooking water sources and consumption, marine food consumption as well as, parent's occupation, grade of education, smoking and alcohol consumption habits; were gathered at the time of enrollment using a questionnaire. Body weight and height were recorded as well. A single spot urine sample was collected in sterile plastic cups and placed immediately on ice. Aliquots (2 ml) of the urine supernatant were prepared in sterile polypropylene tubes after centrifugation ($3000 \times g$, 10 min) and stored at $-80\,^{\circ}C$. A single blood sample of approximately 12 h fasting was drawn in two different tubes: one for lead analysis (metal-free vacationer EDTA) and another tube for serum isolation. Samples were placed on ice immediately after collection, then blood and serum samples were stored at 4 and $-20\,^{\circ}C$, respectively. On March 2015, the water sampling was performed. From 107 original participants, 63 donated both a tap water (domestic water supplies) and bottled water samples ($n=126$). Additionally, samples from tube well water were collected directly from the three local water systems at three different depths ($n=9$): a superficial (1 m), middle (100 m) and deep level (130 m). All tubes containing samples for heavy metals analysis (urine and water) were previously rinsed in 0.1% HCl (v/v).

2.3. Measurements of kidney toxicants

Quantitation of arsenic, cadmium and, chromium in urine and water samples was performed using a PerkinElmer ELAN DRC-II inductively coupled plasma-mass spectrometer (PerkinElmer Waltham, MA). Lead levels in whole blood were quantified by atomic absorption spectrophotometry using graphite furnace using a Perkin-Elmer 3110 at. absorption spectrophotometer (PerkinElmer Inc., Houston, TX). Quantification of fluoride in urine and water samples was performed using an ion selective electrode as described previously (Cardenas-Gonzalez et al., 2013). All samples were analyzed in duplicates ($\%CV < 10$) with a recovery rate above 95%, for all the assays.

2.4. Determination of kidney injury biomarkers

Urinary KIM-1 and NGAL were measured using micro-bead assays as described previously (Vaidya et al., 2008). Urinary albumin and urine creatinine and SCr were measured using Daytona auto-analyzer (Randox Laboratories, Virginia). All samples were analyzed in duplicates and the intra-assay CV was below 15%.

2.5. RNA isolation and measurement of microRNAs

2.5.1. RNA isolation

200 µl urinary supernatant was used for isolation with the miRNeasy Serum/Plasma Kit from Qiagen (Valencia, CA) according to manufacturer's instructions.

2.5.2. Reverse transcription (RT) and pre-amplification

1.5 µL of the eluted RNA was revers transcribed into cDNA using the miScript RTII kit (Qiagen). The prepared cDNA was diluted five-fold, and 5 µL of the diluted cDNA was then pre-amplified with Qiagen's miScript PreAMP kit. The pre-amplified cDNA was diluted five-fold prior to qPCR detection.

2.5.3. qPCR

A SYBR Green-based qPCR was performed with specific primer probes for miR-21, −200c, −423 and a positive RT control miRTC