International Journal of Hygiene and Environmental Health 220 (2017) 227–234

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

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

Reference levels and relationships of nine elements in first-spot morning urine and 24-h urine from 210 Chinese children

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ARTICLE INFO

Article history:
Received 23 July 2016
Received in revised form 25 October 2016
Accepted 25 October 2016

Keywords:
Urine
Metal
Arsenic
Creatinine
Reference

ABSTRACT

Toxic elements have caused great concern due to their adverse health effects. Biological monitoring is generally considered to be a useful tool to assess human exposure to these elements during risk assessment. To better interpret the biological monitoring data, elemental reference values are critical. The major objectives of this study were to clarify whether first-spot morning urine can serve as a surrogate for 24-h urine and to determine the concentration reference interval of some elements in Chinese children's urine. In total, 259 first-spot morning urine samples and 24-h urine samples were collected from 210 2- to 12-year-old healthy children in China and analyzed for creatinine, aluminum, barium, manganese, titanium, cerium, scandium, vanadium, yttrium, and arsenic. The results showed that the creatinine-adjusted concentrations of aluminum, barium, manganese, titanium, cerium, scandium, vanadium, yttrium, and arsenic in the first-spot urine were significantly correlated with those in the 24-h urine. This showed that first-spot morning urine is a favored matrix for monitoring element exposure due to its easy collection and low collection cost. The reference interval using ng/mg creatinine as the unit for Chinese children's urine was 1.63–2653 for aluminum, 3.71–116.8 for barium, 0.67–91.77 for titanium, 0.20–53.42 for arsenic, 1.36–25.29 for manganese, 0.24–8.59 for vanadium, 0.02–2.27 for cerium, 0.01–0.65 for yttrium, and 0.002–0.483 for scandium. These reference intervals may provide reference levels to assess Chinese children's exposure to these elements.

1. Introduction

Human exposure to heavy metals and metalloids is becoming a serious problem, especially for children, as they are considered as the most vulnerable population. Human biological monitoring (HBM) is generally acknowledged to be a useful tool to assess human systemic exposure to chemicals at both the occupational and environmental levels (Iversen et al., 2003; Gao et al., 2011; Zhang et al., 2012; Hoet et al., 2013; Khlifi et al., 2014; Gil and Hernández, 2015). HBM is defined as the measurement of concentrations of chemicals or their metabolites or reaction products in human biological median such as blood, urine, or breast milk (Sabbioni et al., 1992; Schulz et al., 2011). Measuring the concentrations in human specimens reflects the total exposure from all possible sources while environmental analyses are helpful to identify contaminant sources. Urine is one of the most commonly used matrices for HBM because it can be collected easily in a noninvasive manner (Minoia et al., 1990, 1992; Molina-Villalba et al., 2015).

HBM is a useful tool to monitor human exposure to metal elements. However, the knowledge of reference intervals (RIs) is important for interpretation of biological monitoring (BM) data and assessment of whether particular exposure levels are higher than would normally be expected. RIs are those values that lie between the lower reference limits (LRLs) and upper reference limits (URLs) (CLSI/IFCC Guideline C28-A3, 2008). Reference limits (RLs) are calculated from reference values which are defined as those concentrations of certain substances that are expected to be present in the unexposed population (Zeiner et al., 2004). Those values are influenced by environment and lifestyle factors and may differ between countries/regions, so they should be established at a national/regional level.

Currently, several countries such as the USA, Canada and Germany have established HBM programs for the exposure assessment of elements (and other chemicals) in the general population. In the US in 2015, the updated tables for the Fourth National Report

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http://dx.doi.org/10.1016/j.ijheh.2016.10.013
1438-4639/

on Human Exposure to Environmental Chemicals provide basic statistical parameters for 14 elements in urine for the American population (approximately 398 children aged 6–11 years, 390 children aged 12–19 years and 3500 adults) (CDC, 2015). The Report on Human Biomonitoring of Environmental Chemicals in Canada presents basic statistical parameters for 13 elements in urine based on samples collected between March 2007 and February 2009 from nearly 5600 Canadians (approximately 2000 children) (Health Canada, 2010). The German Environmental Survey (GerES III) reported RVs for arsenic, cadmium, lead, and mercury in urine from approximately 4822 adults aged 18–69 years (1997–1999) and GerES IV reported RVs for those four elements in urine from approximately 1800 children aged 3–14 years (2003–2006) (UBA, 1998, 2008). The EURO TERVIHT project (Trace Elements Reference Values in Human Tissues) was started in 1980s for evaluating, checking, and suggesting harmonized procedures for the establishment of trace element reference intervals in body fluids and tissues (Minoia et al., 1990; Iversen et al., 2003). Therefore, RVs or basic statistical parameters of trace elements in urine samples collected from healthy adults have been published for many European countries, such as Italy (Minoia et al., 1990), Denmark (Poulsen et al., 1994; Kristiansen et al., 1997), Hungary (Zeiner et al., 2004), Czech and Slovak (Kučera et al., 1995), France (Goullé et al., 2005), Austria (Zeiner et al., 2006), Belgium (Cornelis et al., 1994; Hoet et al., 2013), the UK (Hamilton et al., 1994; White and Sabbioni, 1998; Morton et al., 2014), and Norway (Birgisdottir et al., 2013). Compared to adults, there is less literature about RVs or basic statistical parameters for children. Alimonti et al. (2000) reported reference values of chromium, nickel, and vanadium for 131 primary school children in Rome. Heitland and Köster (2006) published a comprehensive list of the BM values for 30 elements in urine samples collected from 72 healthy children aged 2–17 years in Germany in 2006. Molina-Villalba et al. (2015) reported BM values for arsenic, cadmium, lead, manganese, and mercury in urine samples collected from 261 non-exposed children aged 6–9 years in Spain in 2015. Roca et al. (2016) published BM values for 20 elements in urine samples from 125 children aged 6–11 years in Spain in 2016. To date, the background reference concentrations for metal elements in urine from the Chinese population are limited. Feng et al. (2015) determined the concentrations of 23 elements in urine collected from adults aged 28–74 years in Wuhan, China. Lu et al. (2016) presented urinary BM values for 17 elements in 53 adults aged 20–26 years.

Almost all of the studies mentioned previously reporting RVs or basic statistical parameters of elemental concentrations in urine have used spot urine samples (including first-spot morning urine) except for Minoia et al. (1990), who present reference values of 46 elements in 24-h urine collected from more than 350 healthy Italian adults. In contrast to 24-h urine samples, the collection of spot urine is more time and cost effective but has more uncertainty due to urinary dilution effects. Thus, whether the spot urine can serve as a surrogate for a daily sample depends on how well the chemical concentrations in it approximate those in 24-h urine (Scher et al., 2007; Wang et al., 2016).

In this paper, we collected urine samples from 210 healthy children in three provinces of China. The concentrations of aluminum (Al), barium (Ba), manganese (Mn), titanium (Ti), cerium (Ce), scandium (Sc), vanadium (V), yttrium (Y), and arsenic (As) were determined by ICP-MS and LC-AFS. The major objectives of this study were to determine reference concentrations of these elements in the urine of Chinese children, to explore the relationship of elemental concentration between first-spot morning urine and 24-h urine, and to investigate the relationship among elemental concentrations in the first-spot morning urine.

2. Materials and methods

2.1. Study areas and selection of participants

This study selected a population sample of 210 children as participants, including 60 children from Guangdong province (southeastern China), 90 children from Hubei province (central China), and 60 children from Gansu province (northwestern China). Therefore, the area where the selected children lived generally covers the geographic zones from the southeastern humid subtropical zone to the northwestern arid temperate zone. Subjects were selected according to stratified random principle: first, three provinces were selected according to geographic and economic conditions; then, cities (capital of each province) and rural sites (counties near the cities) were selected; third, one primary school and one kindergarten were randomly selected from each city and county; fourth, every grade for each school or kindergarten was selected; finally, children were randomly selected from each grade. The environments where children live were not contaminated by obviously point sources.

A questionnaire survey was conducted to obtain information about children's basic situation (such as gender, age, height, and weight), family background (such as education levels and occupation of their parents), daily activity patterns (such as daily time children spent on indoor and outdoor activities), lifestyle and eating habits. The children's ages ranged from 2.5 to 11.9 years old, with an average of 6.5. The average child's weight was 25.1 kg (12.0–75.0 kg), while the average child's height was 119.5 cm (80–175 cm). The children were divided into two age groups, namely, 2–6 years (nursery school group) and 7–12 years (primary school group). During the questionnaire survey we obtained informed written consent from parents and children and distributed all sampling tools to them.

2.2. Sample collection

First-spot morning urine and 24-h urine were collected for one day (186 children) and three consecutive days (24 children). Parents and teachers (for small children) and children were asked to collect all urine into the plastic toilet device used for each urine collection. Then, first-spot morning urine was transferred to 1-L polyethylene bottles, while the remaining urine was transferred to 2-L polyethylene bottles. These polyethylene bottles were cleaned with 5% (*V*/*V*) ultrapure HNO_3 before using. After finishing collection, parents or teachers gave two bottles of urine samples (one bottle for first-spot morning urine and one bottle for the remaining urine) marked by the child's name to us. One milliliter of 5% (*V*/*V*) HNO_3 was added to the bottles, and then they were transported to the laboratory in a refrigerated truck and stored at −20 °C until they were analyzed within 3 months.

2.3. Sample pretreatment

Urine samples were defrosted at room temperature and mixed on a rotary mixer for a minimum of 5 min. Fifteen milliliters of urine was added in a pre-cleaned digestion tube, and 3 mL of concentrated HNO_3 and 2 mL of H_2O_2 were added to the tube. Then, the tube was placed in a microwave digestion reactor for digestion. The digestion program was as follows: the temperature was gradually increased to 120 °C and maintained for 5 min. It was then increased to 160 °C and maintained for 5 min. Finally, it was increased to 180 °C and maintained for 15 min (GB5009.15-2014, 2014). After the digestion, the solutions were diluted to the final volume of 30 mL with ultrapure water and then stored at 4 °C for measuring Al, Ba, Ce, Mn, Sc, Ti, V, and Y. Urine samples were directly diluted 5-fold with ultrapure water and stored at 4 °C for measur-