



Association between human exposure to heavy metals/metalloid and occurrences of respiratory diseases, lipid peroxidation and DNA damage in Kumasi, Ghana[☆]

Nesta Bortey-Sam^a, Yoshinori Ikenaka^{a, b}, Osei Akoto^c, Shouta M.M. Nakayama^a, Kwadwo A. Asante^d, Elvis Baidoo^c, Christian Obirikorang^e, Hazuki Mizukawa^a, Mayumi Ishizuka^{a, *}

^a Laboratory of Toxicology, Department of Environmental Veterinary Science, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita Ku, Sapporo, 060–0818, Japan

^b Water Research Group, Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa

^c Department of Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

^d CSIR Water Research Institute, P. O. Box AH 38, Achimota, Accra, Ghana

^e Department of Molecular Medicine, School of Medical Sciences, Kwame Nkrumah University of Science & Technology, Kumasi, Ghana

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ABSTRACT

Heavy metals and metalloids contamination in soils, water, food and livers of wild rats have been studied in Kumasi, Ghana and despite the estimated risks to residents, there is no epidemiological study to ascertain these projections. In addition, the World Health Organization and International Agency for Research on Cancer have reported an increase in respiratory diseases and cancers, in Ghana. The study's purpose was therefore to explore the potential associations between metal exposure and occurrences of respiratory diseases, lipid peroxidation and/or DNA damage to different age groups and sexes in Kumasi. Human urine was collected from the general population in urban and control sites in Kumasi and nine metals were measured in each sample. Results showed that although Zn was the most abundant total urinary As concentration was higher in 83% of samples compared to reference values. Urinary concentrations of metals, malondialdehyde (MDA) and 8-hydroxy-2-deoxy-guanosine (8-OHdG) were higher in urban sites compared to the control site. Based on the results obtained, there was no significant correlation between urinary metals and age. However, urinary Cd and MDA were highest in age groups 61–85 and 3–20 years, respectively. Significantly higher levels of urinary Co, As and Cd were detected in female participants. The study revealed that exposure to As was significantly associated with increased odds of asthma (odds ratio (OR) = 2.76; CI: 1.11–6.83) and tachycardia (OR = 3.93; CI: 1.01–15.4). Significant association was observed between urinary metals and MDA and 8-OHdG indicating possibility of lipid peroxidation and/or DNA damage in Kumasi residents.

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1. Introduction

Heavy metals and metalloids are among the most toxic substances (ATSDR, 2015) and despite their natural abundance, they are formed mainly from human activities such as mining, smelting,

combustion, tannery or fertilizer applications. Humans and animals could be exposed to metals via inhalation, consumption and/or dermal contact (Saoudi et al., 2012). Despite the importance metals (iron, zinc, copper and manganese) play in maintaining normal physiological functions, excessive intake could result in health implications (Magge et al., 2013). In addition, exposure to cadmium, nickel, lead and arsenic could generate reactive oxygen species (ROS) leading to modifications of DNA and lipids (Stoys and Bagchi, 1995). These modifications have been reported to contribute to the incidence of cancers and cardiovascular diseases (Shi et al., 2004). Indicators of DNA damage, oxidative stress and lipid peroxidation

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* Corresponding author. Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, N18, W9, Kita-ku, Sapporo, 060–0818, Japan.

E-mail address: ishizum@vetmed.hokudai.ac.jp (M. Ishizuka).

such as 8-hydroxy-2-deoxyguanosine (8-OHdG) and malondialdehyde (MDA) have widely been used to determine the health effects of human exposure to metals (Chen et al., 2005).

Besides DNA damage and lipid peroxidation, various epidemiological studies have also found and reported associations between heavy metal/metalloid (including arsenic, cadmium, copper, manganese, nickel, lead) exposure and the occurrence of respiratory effects such as asthma, rhinitis, wheeze, bronchitis, and allergies (Gehring et al., 2015; Huang et al., 2016).

The growing rate of industrialization (including mining), and resulting increases in economic activity and population growth in Kumasi, Ghana, has led to increased pollution of the environment (Bortey-Sam et al., 2014). Studies of environmental contamination and possible health risks due to metal exposure via medicinal herbs (Nkansah et al., 2016a), geophagic white clay (Nkansah et al., 2016b), food (Nkansah et al., 2016c), dust (Nkansah et al., 2015), soils (Akoto et al., 2016, 2017) and streams (Akoto et al., 2010) within Kumasi metropolis have been reported. Furthermore, levels of zinc, arsenic, copper and nickel in livers of wild rats sampled in Kumasi (Bortey-Sam et al., 2015a) were higher compared to the levels in wild rats sampled around mining sites in Kabwe, Zambia (Nakayama et al., 2013). Despite these reports and estimated risks, there is no study to assess the impact of metal exposure to Kumasi residents.

In Ghana, there are an estimated 16,000 cancer cases annually and also an increase in occurrence of respiratory disease (GLOBACAN, 2008; WHO, 2011). In 2012, the estimated cancer incidence in Kumasi was 11.9 per 100,000 and was higher in females (15.7 per 100,000) than males (7.3 per 100,000) (Laryea et al., 2014). Due to the high cancer incidence and respiratory symptoms in Kumasi (GLOBACAN, 2008; Laryea et al., 2014; WHO, 2011), and unavailability of research on the epidemiology and risks of metal exposure to residents, the objectives of this study were to: explore the potential associations between metal exposure and occurrence of respiratory diseases; assess the relationship between metal exposure and incidence of oxidative stress; find the association between urinary concentrations of metals, MDA, 8-OHdG with age and sex.

2. Materials and methods

2.1. Sampling

Urine is considered the main excretory pathway for metals and a better medium for biomonitoring metal exposure (Smolders et al., 2014). Heavy metals and metalloid concentrations in urine could be an indication of both long and short term exposures (Crinnion, 2010). In view of this, human urine ($n = 190$; 57 males and 133 females) was collected in the morning from the general population of three urban sites (Atonsua, Manhyia and Tafo) in Kumasi (Fig. 1). Samples were collected into corning tubes (Corning Incorporated, New York, USA) in January to February of 2015. Manhyia is in close proximity to Kejetia (1.1 km apart), Adum (1.5 km apart) and Romanhill (1.2 km apart), where soils were polluted with metals (Akoto et al., 2017). In previous studies, concentrations of metals were highest in the livers of wild rats trapped in Adum compared to other sites in Kumasi (Bortey-Sam et al., 2015a). Tafo is also 2.3 and 2.6 km from Suame and Mbrom, respectively, whose soils were polluted with metals (Akoto et al., 2017).

Moreover, 12 human urine samples (7 males and 5 females) were collected from Kwame Nkrumah University of Science and Technology campus (KNUST) and used as reference/control samples, even though metal exposure via consumption or inhalation was possible. KNUST, a university in Kumasi, has minimal vehicular motion and no industrial activities. In previous studies, heavy metals and metalloid levels in KNUST soils were low compared to

recommended levels (Akoto et al., 2016, 2017). In addition, particulate matter and soil samples from KNUST have been used as controls in previous studies of environmental contaminants (Bortey-Sam et al., 2013, 2014; Bortey-Sam et al., 2015b).

For quality control purposes, urine was collected from 4 children in residential areas of KNUST to form a composite. Composite samples were used to give a more representative measure and also to account for any variabilities in heavy metals and metalloid concentrations. Since humans could be exposed to metals through various sources, the sample was measured several times to confirm the concentration.

During the sampling process, participant's information, including age, gender, body weight, height, place of residence, occupation, and personal lifestyle including smoker/non-smoker, were obtained through face-to-face interviews. Further, information on respiratory symptoms related to metals such as asthma, wheeze, tachycardia, bronchitis and rhinitis (Gehring et al., 2015; Huang et al., 2016) were collected. The Ethical/Institutional Review Board of Ghana Health Service (GHS) and Council for Scientific and Industrial Research (CSIR), Accra, Ghana, approved this study. Written and informed consent was obtained from each participant and parents gave consent and completed questionnaires on behalf of their children. The samples collected were kept frozen at the Department of Chemistry, KNUST, Ghana. Later the samples were transported to the Toxicology laboratory of the Graduate School of Veterinary Medicine, Hokkaido University, Japan, and stored at -30°C until analysis.

2.2. Sample extraction and analysis

2.2.1. Heavy metals and a metalloid

Method described by Yabe et al., (2015) was used for the extraction of heavy metals and a metalloid from the urine samples collected. Briefly, 1 mL of each urine was transferred into a digestion vessel and 5 mL of 60% nitric acid (Kanto Chemical) and 1 mL of 30% hydrogen peroxide (Kanto Chemical) were added. Sample digestion (Speedwave MWS-2; Berghof) was for 52 min and up to 190°C . The digested samples were transferred into corning tubes and diluted to 10 mL with de-ionized water (Milli-Q). Concentrations of arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), nickel (Ni) and zinc (Zn) in each urine were measured by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS; 7700 series, Agilent technologies, Tokyo, Japan).

2.2.2. Malondialdehyde, MDA (elisa kit)

Concentrations of urinary MDA were measured (based on instructions from manufacturer) using a UV-VIS Spectrophotometer (UV-2600 Shimadzu Corporation, Kyoto, Japan). Briefly, 10 μL of butylated hydroxytoluene (BHT) reagent was transferred into a vial and 250 μL of calibrator (0, 1, 2, 3 and 4 μM) or urine was added. After the addition of 250 μL each of 1 M phosphoric acid and 2-thiobarbituric acid (TBA) reagent, the solution was vortexed vigorously and incubated at 60°C for 1 h. The mixture was transferred into a cuvette and spectra was recorded from 400 to 700 nm after it was centrifuged at $10,000\times g$ for 2–3 min. 3rd derivative analysis was performed at 514 nm.

2.2.3. 8-Hydroxy-2-deoxy-guanosine (8-OHdG)

Extraction and analysis of urine sample for 8-OHdG followed the method described by Bortey-Sam et al., (2017). Briefly, urine (1 mL) was diluted with HPLC grade water (2 mL) after spiking with 25 ng/mL of (15N5) 8-OHdG (internal standard). Prior to sample loading, the Oasis HLB cartridge (3 cc, 60 mg; Waters Corporation, Milford, MA, USA) was primed with 1 mL each of methanol and water. The solid-phase extraction cartridge was then washed with 3 mL of water and the target analyte (8-OHdG) eluted with 3 mL of water:

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