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ORIGINAL PAPER

Blood concentrations of lead, cadmium, mercury and their association with biomarkers of DNA oxidative damage in preschool children living in an e-waste recycling area

Xijin Xu · Weitang Liao · Yucong Lin · Yifeng Dai · Zhihua Shi · Xia Huo

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Abstract Reactive oxygen species (ROS)-induced DNA damage occurs in heavy metal exposure, but the simultaneous effect on DNA repair is unknown. We investigated the influence of co-exposure of lead (Pb), cadmium (Cd), and mercury (Hg) on 8-hydroxy-deoxyguanosine (8-OHdG) and human repair enzyme 8-oxoguanine DNA glycosylase (hOGG1) mRNA levels in exposed children to evaluate the imbalance of DNA damage and repair. Children within the age range of 3–6 years from a primitive electronic waste (e-waste) recycling town were chosen as participants to represent a heavy metal-exposed population. 8-OHdG in the children's urine was assessed for heavy metal-induced oxidative effects, and the hOGG1 mRNA level in their blood represented the DNA repair ability of the children. Among the children surveyed, 88.14% (104/118) had a blood Pb level >5 μg/dL, 22.03% (26/118) had a blood Cd level >1 μg/dL, and 62.11% (59/95) had a blood Hg level >10 μg/dL. Having an e-waste workshop near the house was a risk factor contributing to high blood Pb ($r_s = 0.273$, $p < 0.01$), while Cd and Hg exposure could have come from other contaminant sources. Preschool children of fathers who had a college or university education had significantly lower 8-OHdG levels (median 242.76 ng/g creatinine, range 154.62–407.79 ng/g creatinine) than did children of fathers who had less education ($p = 0.035$). However, we did not observe a significant difference in the mRNA expression levels of *hOGG1* between the different variables. Compared with children having low lead exposure (quartile 1), the children with high Pb exposure (quartiles 2, 3, and 4) had significantly higher 8-OHdG levels ($\beta_{Q2} = 0.362$, 95% CI 0.111–0.542; $\beta_{Q3} = 0.347$, 95% CI 0.103–0.531; $\beta_{Q4} = 0.314$, 95% CI 0.087–0.557). Associations between blood Hg levels and 8-OHdG were less apparent. Compared with low levels of blood Hg (quartile 1), elevated blood Hg levels (quartile 2) were associated with higher 8-OHdG levels ($\beta_{Q2} = 0.236$, 95% CI 0.039–0.406). Compared with children having low lead exposure (quartile 1), the children with high Pb exposure (quartiles 2, 3, and 4) had significantly higher 8-OHdG levels.

X. Xu · W. Liao · Y. Dai · Z. Shi
Laboratory of Environmental Medicine and Developmental Toxicology, and Guangdong Provincial Key Laboratory of Infectious Diseases and Molecular Immunopathology, Shantou University Medical College, Shantou, China

X. Xu
Department of Cell Biology and Genetics, Shantou University Medical College, Shantou, China

Y. Lin
Tabor Academy, Marion, MA, USA

Y. Lin · X. Huo (✉)
School of Environment, Guangzhou Key Laboratory of Environmental Exposure and Health, Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, China
e-mail: xhuo@jnu.edu.cn

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Introduction

Informal and nonstandard electronic waste (e-waste) recycling activities discharge hazardous chemicals into the environment, including heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), nickel (Ni), and mercury (Hg) (Zeng et al. 2016). Guiyu, a town located in southeast China, is possibly the largest e-waste recycling location in the world, where about 100,000 people are employed as e-waste recyclers (Landrigan and Suk 1998). The concentrations of Pb and Cd, and the $PM_{2.5}$ mass in Guiyu have been and remain higher than those in other Asian sites (Zheng et al. 2016). However, the extent of child exposure to Hg in an e-waste recycling area remains unknown. There are different heavy metal exposure sources, routes, and periods of possible inhibitory, synergistic, or additive effects of chemicals (Grant et al. 2013). Because of the unique ways in which children interact with the environment, they are likely to receive larger doses of toxicants than adults (Heacock et al. 2016).

Among heavy metals, Pb, Cd, and Hg are the most commonly encountered toxic substances that can inflict harm on humans and the ecosystem according to the Agency for Toxic Substances and Disease Registry (Cheremisinoff 2016). Heavy metals influence a number of diverse systems and organs, resulting in both acute and chronic effects on children's health. The Pb is a persistent and pervasive environmental pollutant that is known to have adverse neurological, hematological, gastrointestinal, and immunological effects (Bellinger 2004; Patrick 2006). Even low-level Pb exposure is associated with lower intelligence in children independent of associations with attentional and behavioral problems (Hong et al. 2014). Additionally, Pb can induce DNA damage in PC12 cells and mouse blood cells (Devi et al. 2000; Xu et al. 2006).

The Cd is a toxic and carcinogenic heavy metal, which has been associated with kidney disease, bone disease, and cardiovascular disease (Maddaloni et al. 2011). Dietary Cd is a risk factor for breast, endometrial, or ovarian cancers in postmenopausal woman (Adams et al. 2014). Exposure to Cd during pregnancy may have a detrimental effect on the head circumference of newborns and subsequent child growth (Lin et al. 2011). Additionally, early-life, low-level exposure to Cd through breast milk induces oxidative stress (Kippler et al. 2012). At the same time, Cd is a long-lived, multi-organ toxicant that remains in a child's body until adulthood (Sughis et al. 2011).

The Hg has a wide range of adverse health effects on humans, and the sources of exposure are markedly different for the three chemical forms: organic, inorganic, and elemental (Al-Saleh et al. 2013). Children are potentially more susceptible than adults to Hg because of differences in the stages of brain development and organ growth (Olin and Sonawane 2003). Some studies have also shown that the levels of mitochondrial DNA damage exhibited weak correlations with total blood Hg levels in bats (Karouna-Renier et al. 2014).

Genotoxicity is among the many biochemical effects of heavy metals including Pb, Cd, and Hg. DNA is susceptible to oxidative damage, and many studies have demonstrated an increase in oxidative DNA lesions attributable to Pb, Cd, or Hg exposure (Potts et al. 2001; Nzengue et al. 2008). Among the diverse oxidative DNA lesions, 8-hydroxy-deoxyguanosine (8-OHdG) is commonly considered a biomarker of oxidative stress as well as a risk factor for many diseases, including cancer (Potts et al. 2003; Lu et al. 2007). Human 8-oxoguanine DNA glycosylase 1 (hOGG1) is the DNA repair enzyme that recognizes and excises 8-OHdG, playing an important role in maintaining genomic stability (Paz-Elizur et al. 2007). Furthermore, other studies suggest that hOGG1 mRNA expression might be useful in accessing oxidative stress induced by toxicants and can serve as a biomarker for ROS generation (Mo et al. 2006; Ke et al. 2009). However, some previous studies indicate that there is no significant association between heavy metals (such as Pb, Cd, and Hg) and oxidative stress (Hossain et al. 2014). Nevertheless, evidence for chronic Pb, Cd, and Hg co-exposure and oxidative stress is still limited, and the mechanism underlying heavy metal toxicity is also obscure.

In light of the information described above, children in e-waste recycling areas face the immediate environmental impact of heavy metal pollution.

Studies have shown that the exposure to persistent organic pollutants such as bisphenol A, bisphenol S and polycyclic aromatic hydrocarbons at the e-waste dismantling site may have an effect on oxidative damage to DNA among selected participants (Lu et al. 2016; Zhang et al. 2016). Therefore, the present study was conducted to investigate the potential effect of environmental exposure in preschool children to heavy metals and its impact on DNA oxidative damage and repair.

Materials and methods

Study population and sample collection

A total of 118 children who were living in the three villages of Guiyu, aged 3–6 years, were recruited in this study. Written informed consent was obtained from the parents of all participants. The study had been approved by the Human Ethics Committee of Shantou University Medical College, China. Samples of venous blood were obtained from each volunteer by well-trained nurses, and urine samples were obtained from the children. Two milliliters of blood was collected into an EDTA anticoagulant-containing vacuum tube and stored at −70 °C until measured for blood Pb, Cd, or Hg and analyzed for gene expression. Urine samples measuring 15 mL were collected into a disposable polypropylene tube and stored at −20 °C until analyzed for 8-OHdG.

Evaluation of physical development indices and questionnaire

Child physical growth and development indices including height, weight, and head and chest circumferences were measured by trained study members. Each subject's parents or guardians were interviewed by trained healthcare personnel, and a detailed questionnaire about the child's lifestyle and residential environment was completed. The questionnaire addressed factors that might influence a child's blood heavy metal levels, including questions relating to physical activity, eating habits, diet and nutrition, behavioral habits, residence, parent occupation, parent smoking habits, parent education level, and family socioeconomic status. A medical and health history was also considered.

Measurement of blood concentrations of heavy metals

Measurement of blood Pb and Cd was performed by graphite furnace atomic spectrophotometry (ZEEnit-650 GFAAS, Analytik-Jena, Germany). Details of the measurement procedures were as previously described (Yang et al. 2013). The recovery achieved from spiked blood samples in these experiments was 95–107% and 100–103% for Pb and Cd, respectively. Peripheral blood Hg level was determined by atomic fluorescence spectrometry (AFS-8130, Jitian, Beijing, China). The main parameters used for Hg determination were a native high voltage of 270 V, lamp current of 30 mA, atomizer height of 8 mm, and a carrier gas flow of 300 mL/min. The linear correlation coefficient of the Hg standard calibration curve was 0.9990. The accuracy of the method was controlled by recoveries between 82 and 105%. The limit of detection (LOD) for the method, calculated as three times the standard deviations of the blank sample, was 0.20 μg/L in blood. No sample was below the LOD for blood Hg.

Biochemical assays for urinary creatinine and 8-OHdG levels

We used commercial enzyme-linked immunosorbent assay kits (ELISA) to measure the excretion of 8-OHdG (Huijia Biomedical Technology, Xiamen, China) according to the manufacturer's instructions. The reaction was terminated by the addition of a sulfuric acid solution, and the color change was measured spectrophotometrically (Thermo Electron Corp.) at a wavelength of 450 nm. The concentration of 8-OHdG in the urine was determined by comparing the optical density (OD) value of the sample with the standard. Urinary creatinine levels were measured using an assay kit (Nanjing, Jiancheng Bioengineering Institute, China). The concentration of 8-OHdG was expressed as ng/L and then converted to ng/g creatinine.

Determination of blood *hOGG1* expression by real-time RT-PCR

Total RNA was isolated using a TRIzol reagent (Invitrogen) and reverse transcribed to cDNA with the PrimeScript RT reagent kit (Takara Biotechnology, Otsu, Shiga, Japan) according to the manufacturer's

instructions with some modifications. The primer sequences for *hOGG1* were 5′-ACA CTG GAG TGG TGT ACT AGC G-3′ and 5′-GCC GAT GTT GTTGTT GGA GG-3′. The PCR reaction was carried out in a final volume of 20 μL, containing 0.4 μL of 10 μM *hOGG1* primers, 10 μL SYBR Premix Ex Taq, 0.4 μL of 10 μM ROX reference dye, and 2 μL cDNA templates for a total volume of 20 μL. Quantification of *hOGG1* expression using the Takara RT-PCR assay kit (Takara Biotechnology, Otsu, Shiga, Japan) was performed on an ABI 7300 Sequence Detection System (Ambion, Austin TX, USA). PCR amplification of the housekeeping gene *GAPDH* was performed using the same conditions as those for *hOGG1*. The mRNA expression of *hOGG1* was normalized to that of GAPDH.

Statistical analyses

Data are expressed as median and ranges or mean and standard deviation. Demographic and other characteristic differences between the groups defined by the residence region were evaluated using Pearson's Chi-square tests or an independent sample *t* test. Spearman's correlation analysis was performed to access the relative contribution of the children's lifestyle and family status to heavy metal exposure.

Because both the exposure and outcome variables were not normally distributed, a nonparametric test was employed to test the differences in blood heavy metals and 8-OHdG or *hOGG1* levels, and we used a natural log transformation to analyze the data. We examined the association of blood Pb, Cd, or Hg level with urinary 8-OHdG level by using separate regression models for each exposure–outcome association. Next, we categorized exposures into quarters and estimated the difference in the mean value for the 2nd, 3rd, and 4th quarters compared with the 1st quarter. Regression diagnostics were conducted for all models, including examination of fit, heteroscedasticity, and influence. Through the stratified analyses, the urinary 8-OHdG and blood heavy metal levels were determined for different variables.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) Version 19.0 (IBM Corp., Armonk, NY, USA) and Stata Release 13.0 software (Stata Corp, College Station, TX, USA). A level of two-sided $p < 0.05$ was considered to be statistically significant.

Results

Demographic characteristics of the participants

The distribution of characteristics among the study population regarding age; gender; physical development indices; blood Pb, Cd, and Hg levels; and urinary 8-OHdG level as well as *hOGG1* expression level is presented in Table 1. The mean age of the children was 4.85 years, and the percentage of boys was greater than that of girls (64.41 vs. 35.59%). The children's mean body mass index (BMI) was 16.96 kg/cm^2, and the head and chest circumferences were 49.70 and 8.24 cm, respectively.

Blood heavy metal concentrations in children

According to the diagnostic criteria for child blood Pb levels, as defined by the US Centers for Disease Control and Prevention (Betts 2012), the children having blood Pb levels ≥5.0 μg/dL were considered to have elevated blood Pb levels. Among Guiyu children, 88.14% of them (104/118) had blood Pb concentrations greater than 5 μg/dL. The threshold value of blood Cd concentrations reported in the literature for risk of intoxication is 5 μg/dL (Jin et al. 2002). Because all the children had blood Cd levels less than 5 μg/L, we regarded 1 μg/L as the cutoff point for blood Cd levels. Among the children, 22.03% (26/118) had blood Cd levels greater than 1 μg/L. Blood Hg levels above 5.8 μg/L in children result in appreciable harm, as indicated by the US Environmental Protection Agency. In this area, all the children had blood Hg levels greater than 5.8 μg/dL; therefore, we considered 10 μg/L as the cutoff value for blood Hg levels. The proportion of children having blood Hg levels above 10 μg/L was 62.11% (Table 1).

As shown in Table 2, we found that the blood Cd levels of children for the different ages of 3, 4, 5, and 6 years showed a significant difference ($p < 0.05$). When the children were 6 years old, their blood Cd levels (median 0.82 μg/L, range 0.35–2.83 μg/L) were higher than the other children. However, the differences in blood Pb and Hg levels at different ages were not significant ($p > 0.05$). There were no significant differences in blood Pb, Cd, and Hg levels between boys and girls ($p > 0.05$). Blood Pb concentrations were categorized by the e-waste workshop near the child's house. We observed that the children

Table 1 Characteristics of the study population

Demographic variables	*n*	Values [*n* (%)]
Age (years)	118	4.85 ± 1.01
Gender	118	
Male	76	64.41
Female	42	35.59
Body mass index (BMI kg/m^2)	112	16.96 ± 2.62
Head circumference (cm)	110	49.70 ± 1.55
Chest circumference (cm)	110	8.24 ± 3.49
8-OHdG (ng/g Cr)	118	407.79 (152.05, 876.26)
hOGG1	118	0.038 (0.009, 0.542)
Blood Pb levels (μg/dL)	118	7.43 (3.70, 21.64)
>5 μg/dL	104	88.14
≤5 μg/dL	14	11.86
Blood Cd levels (μg/L)	118	0.72 (0.27, 6.86)
>1 μg/L	26	22.03
≤1 μg/L	92	77.97
Blood Hg levels (μg/L)	95	11.13 (7.17, 29.82)
>10 μg/L	59	62.11
≤10 μg/L	36	37.89

Values are arithmetic mean ± SD; median (minimum, maximum)

whose homes were near an e-waste workshop had higher blood Pb levels than the other children (median 7.86 μg/dL, range 3.70–21.64 μg/dL versus median 6.31 μg/dL, range 4.20–16.55 μg/dL, $p < 0.01$). The blood Cd levels of preschool children were categorized by the length of outdoor playing time and vitamin intake. We found that blood Cd levels in children were significantly higher in the 1.0–2.0 h per day outdoor playing group (median 0.79 μg/L, range 0.35–2.83 μg/L) than for the other groups whose playing time was different. The blood Cd concentrations (median 0.89 μg/L, range 0.49–6.86 μg/L) for children having no vitamin intake was also higher than in the other children. In our investigation, we only found differences in blood Hg levels between variables such as the frequency of calcium tablet intake ($p < 0.05$). Those children who had no intake of calcium tablets showed blood Hg levels (median 15.25 μg/L, range 9.71–20.66 μg/L) higher than those of the other children.

Correlation between blood heavy metal concentrations and other variables

Spearman's correlation analyses were performed to access the factors related to the three kinds of heavy metals in blood (Table 3). Blood Pb levels were found to be positively related to an e-waste workshop near the house ($r_s = 0.273$, $p < 0.01$); blood Cd levels were observed to be positively associated with age and BMI ($r_s = 0.274$, $p < 0.01$ and $r_s = 0.291$, $p < 0.05$, respectively) and negatively related to dairy and vitamin intake ($r_s = -0.217$, $p < 0.05$ and $r_s = -0.247$, $p < 0.05$, respectively). Nevertheless, the increase in blood Hg concentrations was only negatively correlated with the intake of calcium tablets ($r_s = -0.241$, $p < 0.05$).

Relationships between toxic heavy metals and urinary 8-OHdG levels and blood *hOGG1* expression

The median urinary 8-OHdG concentration of the surveyed preschool children was 407.79 ng/g creatinine (range 152.05–876.26 ng/g creatinine). The median mRNA expression level of *hOGG1* in children was 0.038 (range 0.009, 0.542) (Table 1). The preschool children of fathers who had a college or university education had significantly lower 8-OHdG levels (median 242.76 ng/g creatinine, range 154.62–407.79 ng/g creatinine) than did the children of fathers who had less education ($p = 0.035$)

Table 2 Relationships between related factors and the Pb, Cd, and Hg levels in the blood samples

Variables	Pb (μg/dL)		Cd (μg/L)		Hg (μg/L)	
	n	Median (range)	*n*	Median (range)	*n*	Median (range)
Age (years)						
3 ~	14	7.97 (4.23, 18.18)	14	0.59 (0.27, 1.56)*	10	10.44 (7.17, 24.33)
4 ~	28	7.68 (4.77, 21.64)	28	0.67 (0.30, 6.86)	18	15.06 (8.13, 29.82)
5 ~	37	7.50 (3.70, 16.55)	37	0.74 (0.34, 1.45)	34	10.12 (7.49, 26.24)
6 ~	38	7.02 (4.20, 9.72)	38	0.82 (0.35, 2.83)	33	11.38 (8.00, 20.66)
Outdoor playing (hours per day)						
<0.5	18	7.21 (4.20, 18.18)	18	0.71 (0.27, 1.79)*	12	11.58 (9.37, 26.24)
0.5–1.0	32	6.76 (4.37, 17.21)	32	0.74 (0.34, 6.86)	29	11.23 (8.37, 24.33)
1.0–2.0	23	8.22 (4.84, 21.64)	23	0.79 (0.35, 2.83)	22	10.50 (8.00, 29.82)
2.0–3.0	10	8.01 (4.89, 8.97)	10	0.55 (0.36, 0.71)	6	9.93 (7.17, 15.36)
>3.0	5	7.44 (4.23, 9.99)	5	0.55 (0.47, 1.15)	5	10.75 (8.67, 17.39)
Dairy intake						
No	3	5.95 (5.71, 19.72)	3	0.98 (0.55, 1.56)	2	9.90 (8.67, 11.13)
Occasionally	10	8.79 (4.92, 21.64)	10	6.91 (0.52, 1.31)	7	11.33 (8.68, 12.80)
Often	46	7.40 (4.20, 17.61)	46	0.79 (0.34, 6.86)	38	11.34 (7.57, 24.33)
Always	34	7.36 (4.37, 18.18)	34	0.62 (0.27, 1.56)	31	10.73 (7.17, 29.82)
Vitamin intake						
No	23	8.36 (4.20, 19.72)	23	0.89 (0.49, 6.86)*	18	13.06 (8.00, 24.72)
Occasionally	39	6.39 (4.37, 21.64)	39	0.66 (0.27, 1.56)	31	11.30 (8.66, 29.82)
Often	24	7.47 (5.71, 17.61)	24	0.66 (0.35, 1.79)	23	11.13 (7.17, 18.90)
Always	3	8.53 (4.43, 9.77)	3	0.76 (0.43, 1.00)	2	20.75 (15.25, 26.24)
Calcium tablet intake						
No	10	8.73 (4.23, 10.94)	10	0.94 (0.56, 1.45)	7	15.25 (9.71, 20.66)*
Occasionally	27	7.50 (4.20, 19.72)	27	0.75 (0.27, 6.86)	23	13.61 (8.37, 29.82)
Often	50	7.40 (4.37, 21.64)	50	0.68 (0.35, 2.83)	42	10.50 (7.17, 24.72)
Always	4	6.31 (4.43, 8.34)	4	0.57 (0.43, 0.98)	4	15.01 (9.69, 26.24)
E-waste workshop near the house						
No	43	6.31 (4.20, 16.55)**	43	0.72 (0.36, 6.86)	35	10.18 (7.17, 26.24)
Yes	55	7.86 (3.70, 21.64)	55	0.74 (0.27, 1.48)	48	11.35 (8.16, 29.82)

* $p < 0.05$

** $p < 0.01$

Table 3 Spearman's correlations between the levels of blood Pb, Cd, and Hg in all children and other factors

Related factors	Blood Pb levels		Blood Cd levels		Blood Hg levels	
	r_s	*p*	r_s	*p*	r_s	*p*
Age (years)	−0.131	0.159	0.274	0.003	−0.091	0.378
BMI (kg/cm^2)	−0.129	0.174	0.219	0.021	−0.068	0.527
Outdoor playing (hours per day)	0.025	0.818	−0.051	0.639	−0.175	0.136
Dairy intake	−0.069	0.511	−0.217	0.037	0.087	0.450
Vitamin intake	0.021	0.848	−0.247	0.020	−0.070	0.555
Calcium tablet intake	−0.110	0.298	−0.159	0.132	−0.241	0.036
E-waste workshop near the house	0.273	0.007	−0.032	0.752	0.149	0.178

r_s Spearman's correlation coefficient

Values of $p < 0.05$ were considered statistically significant

Table 4 Urinary 8-OHdG (ng/g creatinine) concentrations stratified by different variables

Variable	n	Median (range)	Statistics	*p*
Gender				
Male	76	408.82 (152.05, 863.70)	$U = 1260.500$	0.057
Female	42	407.79 (155.23, 876.26)		
Age (years)				
3 ~	14	407.79 (195.37, 755.15)	$\chi^2 = 1.145$	0.766
4 ~	28	407.79 (154.62, 668.91)		
5 ~	37	407.79 (152.05, 863.70)		
6 ~	38	407.79 (167.09, 876.26)		
Outdoor playing (hours per day)				
<0.5	18	407.79 (154.62, 698.61)	$\chi^2 = 1.353$	0.852
0.5–1.0	32	407.79 (152.05, 772.44)		
1.0–2.0	23	407.79 (179.99, 876.26)		
2.0–3.0	10	404.32 (206.20, 526.02)		
>3.0	5	407.79 (247.01, 625.72)		
Dairy intake				
No	3	316.08 (306.42, 514.78)	$\chi^2 = 2.637$	0.451
Occasionally	10	407.79 (206.20, 617.31)		
Often	46	407.79 (179.99, 772.44)		
Always	34	394.52 (152.05, 876.26)		
Vitamin intake				
No	23	407.79 (160.51, 625.72)	$\chi^2 = 2.478$	0.479
Occasionally	39	407.79 (152.05, 772.44)		
Often	24	407.79 (272.71, 876.26)		
Always	3	251.72 (197.93, 668.91)		
Calcium tablet intake				
No	10	405.84 (155.23, 863.70)	$\chi^2 = 0.844$	0.839
Occasionally	27	407.79 (167.09, 612.48)		
Often	50	407.79 (160.51, 876.26)		
Always	4	361.16 (251.71, 560.08)		
E-waste workshop near the house				
No	43	407.79 (152.05, 772.44)	$U = 1181.000$	0.991
Yes	55	407.79 (154.62, 876.26)		
Paternal education levels				
Primary school	9	407.79 (258.24, 772.44)	$\chi^2 = 10.318$	0.035
Junior high school	56	407.79 (152.05, 876.26)		
Vocational school	10	456.35 (236.03, 677.92)		
Senior high school	7	407.79 (179.99, 426.03)		
College/university	10	242.76 (154.62, 407.79)		
Paternal smoking habits				
Non-smoker	24	407.79 (160.51, 677.92)	$\chi^2 = 1.273$	0.866
1–2 cigarettes/day	7	233.81 (189.75, 668.91)		
5–10 cigarettes/day	21	407.79 (152.05, 698.61)		
1 pack/day	28	407.79 (167.09, 717.99)		
>1 pack/day	12	407.79 (206.20, 876.26)		

Values of $p < 0.05$ were considered statistically significant

(Table 4). No significant differences were found according to the other investigated factors. To investigate whether exposure to Pb, Cd, and Hg altered the expression levels of detoxifying genes, the mRNA expression levels of *hOGG1* in the children were quantified using RT-PCR. However, we did not observe a significant difference in the expression levels of *hOGG1* mRNA between the different variables (data not shown).

To estimate the adjusted mean difference in urinary 8-OHdG associated with blood Pb, Cd, and Hg levels, we used the quartiles of the three metals as independent variables on urinary 8-OHdG after covariate adjustment in a multiple regression analysis (Fig. 1). Covariates for model 1 were gender, age, blood Cd levels, and blood Hg levels. Covariates for model 2 were gender, age, blood Pb levels, and blood Hg levels. Covariates for model 3 were gender, age, blood Pb levels, and blood Cd levels. Compared with low exposure (quartile 1), the high Pb exposure children (quartiles 2, 3, and 4) had significantly higher 8-OHdG ($\beta_{Q2} = 0.362$, 95% CI 0.111–0.542; $\beta_{Q3} = 0.347$, 95% CI 0.103–0.531; $\beta_{Q4} = 0.314$, 95% CI 0.087–0.557). Associations between blood Hg and 8-OHdG were less apparent. Compared with low levels (quartile 1), higher blood Hg levels (quartile 2) were associated with higher 8-OHdG levels ($\beta_{Q2} = 0.236$, 95% CI 0.039–0.406). There was no difference in fit between the models with blood Cd parameterized as quartiles and urinary 8-OHdG levels. However, there were no fit models to investigate the association between the three kinds of toxic heavy metal exposure and *hOGG1* expression (data not shown).

Discussion

Considerable research has shown that long-term exposure to heavy metals including Pb, Cd, and Hg can cause adverse health effects on multiple organs such as the nervous system, kidney, bone structure, reproductive organs, and heart (Waalkes 2000; Ros and Mwanri 2003; Thomas et al. 2009). Most of the damage is direct or indirect through elevated levels of oxidative stress. Currently, Pb, Cd, and Hg pollution has become one of the most important environmental health problems, especially for preschool children who are considered especially vulnerable to environmental threats. There are specific periods in their development when the exposure to chemical, physical, or biological agents may result in adverse health outcomes (Weiss 2000; Jarosinska and Gee 2007). Blood Pb levels above 5 μg/dL in children were defined as dangerous by the Centers for Disease Control and Prevention (CDC) in 2012 (Betts 2012). Blood Hg levels in children above 5.8 μg/L have been defined as causing appreciable harm by the US Environmental Protection Agency (Rice et al. 2003). Many studies suggest that even a small accumulation of Pb, Cd, or Hg in the human body can be harmful (Shen et al. 2001; Canfield et al. 2003; Thijssen et al. 2007). In our previous studies in Guiyu, from 2004 to 2006, the median blood Pb levels of children were 12.3–14.0 μg/dL. The percentage of children whose

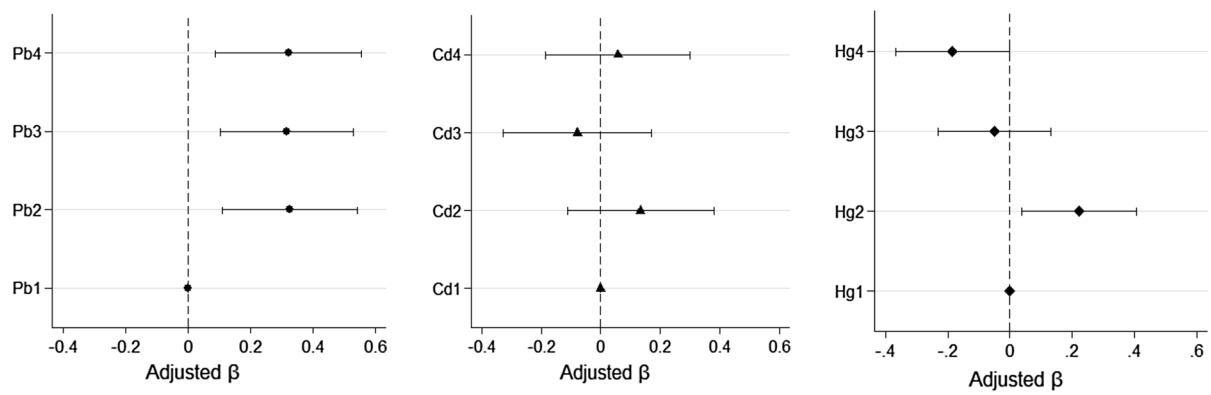

Fig. 1 Effect estimates and 95% confidence intervals for quartiles of blood Pb, Cd, and Hg with 8-OHdG levels. *Dashed horizontal line* represents null association

blood Pb levels were above 5 μg/dL was 100%, and the mean blood Cd level was 1.3–1.73 μg/L (Huo et al. 2007; Guo et al. 2010). In the present study, the median blood levels of Pb and Cd in preschool children were 7.43 and 0.72 μg/L, respectively. The percentage of children whose blood Pb levels were above 5 μg/dL was 88.14% as shown in Table 1. All children were recruited from the same kindergarten, which therefore indicates that the blood Pb and Cd levels of Guiyu children had somewhat declined. Reasons for the descending trend in blood Pb and Cd levels in the local children might be the government regulation of e-waste processing, our work in improving the environmental and health awareness of the local population, and most of all, the closing of many home workshops due to the world economic recession in 2009. A study conducted in 2009 shows that the mean blood Pb levels of Chinese children from 2001 to 2007 were 8.07 μg/dL, with children living in industrial areas having higher mean blood Pb levels than children living in urban and suburban areas, and with suburban levels being higher than urban levels (He et al. 2009). Although the children have experienced a gratifying decline in Pb exposure in recent years, heavy metal poisoning is still a serious public health problem in Guiyu. Moreover, child blood Pb levels in Guiyu, a rural area, are also higher than in Shantou City (Luo et al. 2003). Thus, environmental Pb and Cd contamination in Guiyu is still high and might cause adverse health effects in the local children. However, this is the first time that child blood Hg has been measured in an e-waste recycling area. In our study, the blood Hg levels in preschool children ranged from 7.17 to 29.82 μg/L, with a median level of 11.13 μg/L, which is higher than the concentrations in the US children (Gallagher et al. 2013). Blood Hg levels in every child were above the US Environmental Protection Agency reference dose of 5.8 μg/L, and 62.11% of children had blood Hg levels above 10 μg/L. The soaring level of blood Hg in these children may be partly attributed to consuming higher amount of seafood. The results of one survey performed in US demonstrated that women living in coastal regions have the highest fish consumption in the past 30 days and the highest blood Hg levels compared to the women residing inland. Women living in the Northeast were at the greatest risk for having blood Hg concentrations greater than 5.8 μg/L (6.5%), compared to the Midwest (0.78%) (Cusack et al. 2017). Additionally, another study carried out in Taiwan reported that pregnant women with high seafood consumption had a 2.91-fold greater risk of high blood Hg levels (5.8 μg/L) (Huang et al. 2017). Though the large amounts of seafood consumption may contribute partly to the elevation of blood Hg level, the most important cause should be the exposure to heavy metals. Urgent prevention measures are needed to protect children from Hg pollution. We found that blood Pb level is positively related to the factor of having an e-waste workshop near the house. Blood Cd is considered an indicator of recent exposure, and we noticed that it increases along with age, which may also include a contribution from the long-term body burden (Satarug et al. 2010; Adams and Newcomb 2013). At the same time, we also observed that Cd seems to play a less significant role in child body development. Luo et al. (2015) found that low-dose Cd exposure had no adverse effects on the physical development of pups. However, factors correlating with lower blood Cd levels are vitamin and calcium tablet intake. Factors correlating with reduced blood Hg levels are calcium tablet intake in agreement with a prior study on the kidney (Hossain et al. 2014). The reason for this finding could be that calcium supplement intake can accelerate the excretion of Hg from blood.

ROS are often implicated in Pb-, Cd-, and Hg-induced oxidative stress. DNA is susceptible to oxidative damage, and several studies have demonstrated that Pb and Cd cause oxidative DNA damage to cultured cells and animals (Li et al. 2009; Du et al. 2012; Skipper et al. 2016). Measurements of 8-OHdG provide information on the oxidation of DNA and are frequently used in genotoxicity studies (Valavanidis et al. 2009). In our study, nonparametric analyses showed different concentrations of 8-OHdG in blood Pb quartiles, and 8-OHdG concentrations in both the 2nd and 4th quartile blood Pb levels were higher than in the 1st quartile blood Pb level. The levels of 8-OHdG in blood Hg quartiles were also different, but only the 8-OHdG concentrations in the 2nd quartile blood Hg level were higher than the 4th quartile. However, the concentrations of 8-OHdG did not differ in the quartiles of blood Cd levels. We demonstrated that the degree of DNA damage varied for different Pb and Hg exposure levels in preschool children from Guiyu. Separate regression model analyses showed that children with higher blood Pb had greater urinary

8-OHdG than did those with lower blood Pb. The Pb can generate ROS by itself and induce oxidative stress (Liu et al. 2010). It was also found in mice experiments that Pb exposure significantly increased the levels of ROS and malondialdehyde (MDA), which are indicators of oxidative stress (Xu et al. 2008). The present findings are not similar to those of previous studies, which showed that umbilical cord blood and urinary Pb was not associated with umbilical cord blood and urinary 8-OHdG in neonates and pregnant women, respectively (Engström et al. 2010; Ni et al. 2014). We hypothesize that the increase in oxidative stress with increasing Pb exposure in preschool children can be related to DNA damage in other organs that are susceptible to Pb toxicity, such as the developing brain, bone, and erythrocytes (Liu et al. 2011, 2015; Yang et al. 2013). Further studies with additional markers of organ toxicity in young children are needed to confirm this hypothesis.

It is rather unexpected that Cd, which induces ROS and has been suggested as a factor for carcinogenesis (Vainio et al. 1993), showed no relationship with urinary 8-OHdG. In agreement with this observation, Wang et al. (2015) reported that no significant associations were found between urinary Cd and 8-OHdG among coke-oven workers. However, Kippler et al. (2012) observed that Cd exposure in predominantly breast-fed Bangladeshi infants is associated with induced oxidative stress, and Ni et al. (2014) showed that neonate exposure to Cd, as measured by the levels in umbilical cord blood, is strongly associated with increased umbilical cord blood 8-OHdG levels (Kippler et al. 2012; Ni et al. 2013).

DNA damage in animals exposed to Hg has been implicated in immune and nervous system dysfunction, where the molecular mechanisms include the generation of free radicals and oxidative stress, effects on microtubules, influence on DNA repair mechanisms, and direct interaction with DNA (Taddei et al. 2001; Kim et al. 2003; Crespo-López et al. 2009). One study found that Hg affected the excretion of urinary 8-OHdG in a dose-related pattern that was mostly associated with long-term exposure to low Hg levels (Al-Saleh and Elkhatib 2012). From our results, we noted that the association between the levels of Hg and 8-OHdG is not in a dose-dependent manner in preschool children, and the 8-OHdG levels in the 2nd quartile blood Hg level are higher than in the other quartiles. A possible reason is that for the local children living in a long-term, co-exposure environment, blood Hg concentration is relatively high such that all the children have a blood Hg level that is above 5.8 μg/L.

We also considered the other possibility that urinary 8-OHdG levels may be confounded by a variety of factors such as physiological, nutritional, lifestyle, and socioeconomic status (Wong et al. 2005; Mori et al. 2011). The difference in 8-OHdG concentration between e-waste recycling area and reference area was not consistent in different previous study results. In the study carried by Ni et al. (2014), there was no significant difference in umbilical cord blood 8-OHdG concentration between neonates from Guiyu and reference area, though the umbilical cord blood 8-OHdG concentrations were positively associated with blood Cd, Cr, and Ni concentrations. Zhang et al. (2016) also reported that there was no significant difference in urinary 8-OHdG concentration between e-waste recycling area and reference area. However, in another study carried in Longtang town, Qingyuan City, China, the urinary 8-OHdG concentration in participants living in e-waste recycling area was significantly higher than that in reference area (Lu et al. 2016). To eliminate the influencing factors such as life style and dietary, all the children were recruited from Guiyu in this study. Through the stratified analyses, we only found that paternal education level, used as a proxy for socioeconomic status, was related to children's oxidative DNA damage. Paternal education may convey information that influences the patterns of potential metal exposure as well as healthcare for the children. Furthermore, we also investigated the association between 8-OHdG and children exposed to environmental tobacco smoke; however, Guiyu children may not be exposed to tobacco smoke on regular occasions from their family members. The effects of passive smoking on 8-OHdG in preschool children were less likely to appear in our results. On the other hand, unstable statistical results due to sub-grouping of subjects, based on the availability of information on the children's lifestyle, were found such that the present results should be validated in future studies with suitable research designs.

There is accumulating evidence that metals such as Pb and Cd can not only induce oxidative DNA damage, but can also interfere with distinct steps in diverse DNA repair systems (Hartwig and Schwerdtle

2002). It is plausible that the toxic effects of these metals can impair the balance between oxidants and antioxidants (Wang et al. 2015). In this study, we found that there was no evidence of a correlation between Pb, Cd, and Hg exposure and the expression of the DNA repair gene *hOGG1* in the local preschool children. On the one hand, these results are different from animal and cell experiments as well as adult studies, which suggest that Cd exposure can increase cell apoptosis and DNA damage and decrease DNA repair capacity (Al Bakheet et al. 2013; Listed 2014; Lei et al. 2015). On the other hand, the present findings are consistent with a previous study, which showed that long-term exposure to Pb and Hg does not influence *hOGG1* expression (Al Bakheet et al. 2013). The co-exposure situation in these children might be a possible explanation for this observed lack of association. In addition, it should be considered that there might be some uncertainties regarding the assessment of biomarkers for DNA repair genes.

A weakness of this study could be the relatively small sample size, which may have compromised the statistical prediction and statistical inference. Thus, further clarification in future studies is required. Furthermore, considering that the e-waste area is part of a co-exposure field, there might be a release of various pollutants including heavy metals and persistent organic pollutants that might together, through the process of recycling e-waste, play an important role in DNA damage. Therefore, we need to measure a larger number of chemical pollutants in future studies.

Conclusion

Our results suggest that increased levels of DNA damage and no influence on the DNA repair capacity may result from Pb and Hg exposure early in life, potentially increasing the risk for mutations and cancer later in life. Thus, environmental metal exposure to Pb may play an important role in oxidative DNA damage to preschool children who are living in an e-waste area. In addition, the study shows that the present Cd exposure level may not induce DNA damage and affect DNA repair in preschool children.

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