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Environmental cadmium exposure induces alterations in the urinary metabolic profile of pregnant women

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ABSTRACT

Cadmium (Cd) is a well-recognized, hazardous toxic heavy metal, and the adverse effects of high-level Cd exposure on human health have been well documented. However, little is known about the health effects of low-level environmental Cd exposure on pregnant women. The objective of this study was to assess urinary metabolic alterations in pregnant women exposed to environmental Cd, and to identify informative biomarkers. Urine samples from 246 pregnant women in the first trimester of pregnancy were collected, and urinary Cd concentrations were quantified using inductively coupled plasma mass spectrometry (ICP-MS). Urinary metabolomics was analyzed by ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS). Cd-related metabolic biomarkers were examined by comparing the samples of the first and third tertiles of Cd exposure classifications, using a partial least-squares discriminant (PLS-DA) model. Five putative biomarkers were identified, including L-cystine, L-tyrosine, dityrosine, histamine, and uric acid, all of which were related to oxidative stress and nephrotoxic effects induced by Cd exposure. The results show that low-level environmental Cd exposure could induce metabolite profile alterations in pregnant women, which might be associated with adverse health effects. Our findings provide new insights into the early molecular events following Cd exposure, and may be valuable for the health risk assessment of Cd exposure during pregnancy.

1. Introduction

Cadmium (Cd) is a well-known toxic heavy metal, and Cd pollution due to human activities, such as mining and smelting, has become a major environmental threat to human health (Anetor, 2012). It is now well recognized that Cd exposure has adverse effects on the lungs, kidneys, and bones, as well as the cardiovascular system, and it increases the risk of a number of different types of cancer (Adams et al., 2012; Peters et al., 2010; Satarug and Moore, 2004; Suwazono et al., 2006). Since Cd is known to accumulate in the placenta, there is growing interest in investigating Cd-related developmental toxicities (Korpela et al., 1986; Osman et al., 2000). Several epidemiological studies have shown significant associations between prenatal Cd exposure and adverse birth outcomes, such as preterm birth, low birth weight, and small for gestational age (Johnston et al., 2014; Kippler et al., 2012; Menai et al., 2012; Nishijo et al., 2002). However, most of these studies focused on people exposed to high-levels of Cd. Since pregnant women and their fetuses are especially susceptible to environmental pollutant exposure, it is a particularly important public health issue to explore the risk of exposure to relatively low-level Cd in pregnant women.

Because physiological responses to low level environmental exposures are often subtle and occur early in the process, it has been difficult to directly estimate the effects of environmental toxicants in

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human populations (Ellis et al., 2011). As a sensitive and high-throughput analytical technique, metabolomics enables the assessment of biological effects associated with environmental toxicant exposures (Dudka et al., 2014). Recently, metabolomics methods have been applied in epidemiological and toxicological studies to analyze the metabolic responses of organisms to environmental pollutants, including endocrine disruptors and heavy metals (Bundy et al., 2009; Lankadurai et al., 2013). Ellis et al. investigated the metabolomic effects on a human population exposed to environmental Cd by using nuclear magnetic resonance (NMR), and found that six mitochondrial and one carbon metabolism-related urinary metabolites, including citrate and dimethylglycine, were associated with Cd exposure (Ellis et al., 2011). Most recently, two other studies reported alterations in urine metabolic profiles of women exposed to Cd, and low-molecular weight metabolites in urine, identifying L-cystine, L-tyrosine, urea, and glycine, as potential biomarkers of exposure to Cd (Gao et al., 2014; Xu et al., 2016). However, to the best of our knowledge, there are currently no metabolomics studies focused on the metabolic effects of ambient Cd exposure on pregnant women.

In this study, we aimed to investigate the urinary metabolic profile in pregnant women with low-level environmental Cd exposure, and to identify metabolites that could serve as biomarkers for Cd exposure. In brief, 246 urine samples were collected from women in early pregnancy, which is a critical period for embryonic development. Both Cd exposure levels and metabolic profiles were analyzed in the same urine samples, using inductively coupled plasma mass spectrometry (ICP-MS) and ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS), respectively. The obtained UPLC-QTOF-MS data were processed by multivariate analysis to identify potential biomarkers for environmental Cd exposure. Because the samples were collected from pregnant women with no clear adverse health outcomes, the detected biomarkers have the potential to mark any early Cd effects, providing a valuable measure to better characterize the toxicities associated with prenatal Cd exposure. In addition, the metabolic pathways associated with these putative metabolic biomarkers are also discussed.

2. Material and methods

2.1. Study population and sample collection

This study was approved by the institutional ethics committee and conducted under the Helsinki Declaration. Informed consent was obtained from all participants. Two hundred and forty-six Chinese pregnant women were enrolled from the Women and Children Medical and Healthcare Center of Wuhan (Hubei province, China) from 2013 to 2014. All of the participants were ethnically Han Chinese. Relevant information, including maternal age, height, weight, education level, household income, pregnancy history, and lifestyle characteristics (e.g., smoking and alcohol consumption) during pregnancy were collected by questionnaire. Information about the participants' health and gestational age were obtained from medical records. All selected subjects had very similar demographics in order to reduce confounding factors.

The first morning void spot urine was collected from each subject at the end of the first trimester (mean ± SD 13.20 ± 1.23 weeks). All participants were asked to provide mid-stream urine samples after fasting for at least 12 h. The urine samples were stored at −80 °C until analysis.

2.2. Chemicals and reagents

The products used in this study were analytical pure nitric acid (Fisher Optima, Thermo Fisher Scientific Inc.), ICP-MS tuning solution 5190–465 (Agilent Technologies Inc.), mixed standard IV-ICP-MS-71A (Agilent Technologies Inc.), mixed internal standard IV-ICP-MS-71D (Agilent Technologies Inc.), LC-MS grade water, methanol and formic acid (Fisher Optima, Thermo Fisher Scientific Inc.). The L-cystine, L-tyrosine, histamine, and uric acid standards were purchased from Sigma (Sigma-Aldrich Corporation). The dityrosine standards were purchased from TRC (Toronto Research Chemicals Inc.).

2.3. Urinary cadmium analysis

The urinary Cd concentrations were detected by ICP-MS (Agilent 7700, Agilent Technologies Inc.) according to our previously reported method (Yang et al., 2016). The limit of detection (LOD) for Cd was 0.003 μg/L. The limit of quantitation (LOQ) was calculated by 10 × LOD/3. The average LOQ of Cd was 0.01 μg/L, and no sample was below the LOQ. The intra-day coefficient of variation (CV) was 1.32% and inter-day CV was 2.05%. Urinary Cd concentrations were expressed as μg/g creatinine, and urine creatinine concentrations were detected by a creatinine kit (BS-200 CREA Kit, Shenzhen Mindray Bio-medical Electronics Corporation).

In order to investigate the Cd induced metabolome alteration, the 246 pregnant women were divided into three groups based on the tertile distribution of Cd concentrations in the urine. The 1st tertile was defined as the low Cd exposure (LCE) group, while the 2nd tertile and 3rd tertile were defined as the middle Cd exposure (MCE) group and the high Cd exposure (HCE) group, respectively.

2.4. UPLC-QTOF-MS analysis

The Cd concentrations and metabolic profiles were analyzed in the same urine samples. The untargeted metabolomic analysis was carried out by UPLC-QTOF-MS (ACQUITY UPLC and Xevo G2 Q-TOF, Waters Corporation). Each urine sample was thawed at 4 °C and vortex-mixed for 3 min, then centrifuged at 21000 g for 10 min at 4 °C. A 100 μL sample of supernatant was carefully transferred into another tube followed by dilution with 100 μL ultrapure water. A 50 μL of diluted sample was transferred into a autosampler vial for UPLC-QTOF-MS analysis.

The chromatographic separation was performed on an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm, Waters Corporation) with a flow rate of 0.5 mL/min, and the column was maintained at 40 °C, with a sample injection volume of 5 μL. The mobile phase was a mixture of (A) water with 0.1% (v/v) formic acid and (B) methanol with 0.1% (v/v) formic acid. The programmed gradient was 0 min, 1% B; 1 min, 1% B; 3 min, 15% B; 6 min, 50% B; 9 min, 95% B; 10 min, 95% B; 10.1 min, 1% B; 12 min, 1% B. The mass spectrometer was operated under positive ionization mode. The scan range was 50–1500 *m/z*. Data were collected in MS^E continuum mode. The capillary voltage and sampling cone voltage were set at 3200 V and 40 V, respectively. The MS^E energy ramp was 20–45 V. The source temperature was set at 120 °C, and the cone gas flow at 25 L/h. A desolvation gas temperature of 350 °C and a desolvation gas flow of 900 L/h were used.

All urinary samples were analyzed at random. Moreover, a quality control (QC) sample was prepared by pooling aliquots of each sample. The QC sample was analyzed at the beginning, the end, and periodically throughout the whole analytical workflow (one QC sample was analyzed after every ten sample injection), to evaluate the stability of analytical systems for the large-scale sample analysis.

2.5. Urinary metabolomics analysis and biomarker identification

The UPLC-QTOF-MS data were processed using the MassLynx V4.3 software (Waters Corporation) for peak detection, peak alignment, and peak area normalization in each data set. The peaks with $S/N < 100$ were excluded. Then a table containing retention time-exact mass pairs (metabolic features) and normalized peak intensity were extracted from raw data. The preprocessed data were entered into the MarkerLynx software (Waters Corporation) for partial least-squares discriminant analysis (PLS-DA). The PLS-DA was applied for group differentiation, and for obtaining the scores plot and the greatest variable importance in projection (VIP) values. The generated PLS-DA models were validated by the 200-time permutation test. The biomarkers were screened from the most robust model, in which the variables (metabolic features) that made significant contributions to discriminating between groups were considered as the potential biomarkers. Therefore, variables with VIP values > 1 in the model were further evaluated as potential biomarkers. In addition, *P* values of the variables between the groups were less than 0.05 for the putative biomarkers. The metabolites that met these two criteria were further analyzed through structural identification.

The chemical structures of the potential biomarkers were identified based on the accurate *m/z* and fragment ions that were acquired by $\mathrm{MS^E}$ analysis. The accurate theoretical *m/z* values and structural information of the potential biomarkers were searched using online databases, including the Human Metabolome Database (HMDB) (http://www.hmdb.ca) and the METLIN database (http://metlin.scripps.edu). When identifying the potential biomarkers, the measured *m/z* values were matched with the theoretical values, and 10 ppm was set as the accepted mass error. Biomarker identities were finally confirmed by comparison with commercial standards.

2.6. Statistical analysis

Univariate statistical tests were performed by the SPSS 20.0 software (IBM Corporation). Categorical variables were tested using the χ^2 test, and continuous variables were tested using Mann-Whitney or Kruskal-Wallis tests. *P* values less than 0.05 were considered significant. The correlation between urinary Cd and metabolites were analyzed by linear regression models. In linear regression models, Cd concentrations were transformed by natural logarithm (ln-) to satisfy statistical normality.

3. Results

3.1. Descriptive statistics of the study population

The demographic characteristics of study population are listed in Table 1. There were no significant differences in age, height, weight, BMI, multiparity, and gestational age among the three groups. In addition, there were no significant differences in lifestyle characteristics (e.g., smoking, passive smoking, and alcohol consumption) during pregnancy among the three groups. The concentration of urinary Cd was significantly higher ($P < 0.01$) in the HCE group compared to the LCE group and MCE group, and urinary Cd concentration of the MCE group was significantly higher ($P < 0.01$) than the LCE group.

Table 1
Characteristics of the study population.

Characteristics	LCE	MCE	HCE
	n = 82	n = 82	n = 82
Multiparity	5 (6.1%)	10 (14.6%)	14 (17.1%)
Smoking	1 (1.2%)	1 (1.2%)	0 (0%)
Passive smoking	12 (14.6%)	15 (18.3%)	15 (18.3%)
Drinking	0 (0%)	0 (0%)	0 (0%)
Folic acid supplement	49 (59.8%)	51 (62.2%)	54 (65.9%)
Multivitamin supplements	44 (53.7%)	37 (45.1%)	33 (40.2%)
Household income			
≥50,000 yuan per year	62 (75.7%)	58 (70.7%)	66 (80.5%)
< 50,000 yuan per year	20 (24.4%)	24 (29.3%)	16 (19.5%)
Height (cm)	161.4 ± 3.7	161.2 ± 4.5	160.8 ± 3.8
Weight (kg)	54.3 ± 8.1	54.5 ± 7.0	53.3 ± 6.7
BMI	20.8 ± 2.8	21.0 ± 2.6	20.6 ± 2.6
Maternal age (years)	28.1 ± 2.8	28.5 ± 3.7	28.8 ± 3.2
Gestational age (weeks)	13.1 ± 1.2	13.1 ± 1.2	13.5 ± 1.3
Cadmium in urine (μg/g creatinine)	0.3 ± 0.1	$0.5 \pm 0.08^{**}$	$1.1 \pm 0.5^{**\#\#}$

Data are expressed as mean ± SD for continuous variables, n (%) for categorical variables.
** represents $P < 0.01$ compared with LCE group.
$^{\#\#}$ represents $P < 0.01$ compared with MCE group.
P values were calculated using the χ^2 test (categorical variables) or Mann-Whitney test (continuous) between each group.

3.2. Reliability of measurement methods

The QC sample was used to examine and evaluate the stability of UPLC-MS analytical system. The spectral data of QC samples and all the urine samples were analyzed by principal component analysis (PCA). A tight QC clustering was observed in the PCA scores plot (Fig. S1). Moreover, the variations of retention time (RT) and intensity of the peaks across the QC samples were evaluated. The results showed that the RT shift was less than 0.2 min; and 78% of the peaks in QC samples exhibited a relative standard deviations (RSD) of peak intensity below 20%. Therefore, the QC data verified that the analytical method was stable and reliable, and the quality of the data was suitable for further analysis.

3.3. Metabolomic analysis

The PLS-DA was applied to examine the variances of the metabolic profiles among groups, and identify differential metabolites related to Cd exposure. The PLS-DA score plot (Fig. 1A) showed that the LCE group had the largest separation from the MCE and HCE groups, but MCE group could not be distinguished from HCE group. In order to further differentiate the groups and identify potential biomarkers, the PLS-DA analyses were performed separately on combinations of two groups (LCE *vs* MCE and LCE *vs* HCE). As shown in Fig. 1B, the LCE group and the MCE group could be separated into distinct clusters, based on the distinct metabolic alterations between them. Similar results were obtained when comparing the LCE group with the HCE group (Fig. 1C). The quality of the PLS-DA model was first evaluated by R^2Y (cum) and Q^2 (cum) parameters, which indicated the goodness-of-fit and prediction capability, respectively. The PLS-DA model built on the LCE and MCE group comparison showed a R^2Y value of 0.776 and Q^2 value of 0.450, and the model built on the LCE and HCE group comparison showed a R^2Y value of 0.858 and Q^2 value of 0.658. Therefore, the PLS-DA model built on the LCE and HCE group analytics was selected to screen for potential biomarkers, as this was the group that exhibited the most robust differences. Moreover, the 200-time permutation test was used to validate the developed PLS-DA model, in order to investigate the over-fitting of data in the model. The R^2Y and Q^2 values in the permutation test were lower than the original ones; and the Q^2 Y-axis intercept (-0.242) was below 0, which showed that the PLS-DA model was not over-fitting (Fig. S2).

3.4. Biomarker identification

The metabolites responsible for the class separation were considered as potential Cd exposure related biomarkers. A total of 79 differential metabolites were identified based on the accurate MS data and MS/MS fragmentation pathway. Among them, 5 metabolites were confirmed by available commercial standards, including L-cystine, L-tyrosine, dityrosine, histamine, and uric acid. The identified metabolites and their selection parameters are listed in Table 2, and the mass fragment information of the metabolites is shown in Fig. S3. L-cystine, L-tyrosine, and dityrosine were upregulated with Cd exposure, while histamine and uric acid were downregulated with Cd exposure. The correlation between the identified biomarkers and Cd was also evaluated by the linear regression models (Fig. 2). The results showed that the maternal urinary Cd concentrations were positively associated with L-cystine ($P = 0.003$), L-tyrosine ($P = 0.016$), and dityrosine ($P = 0.001$) levels.

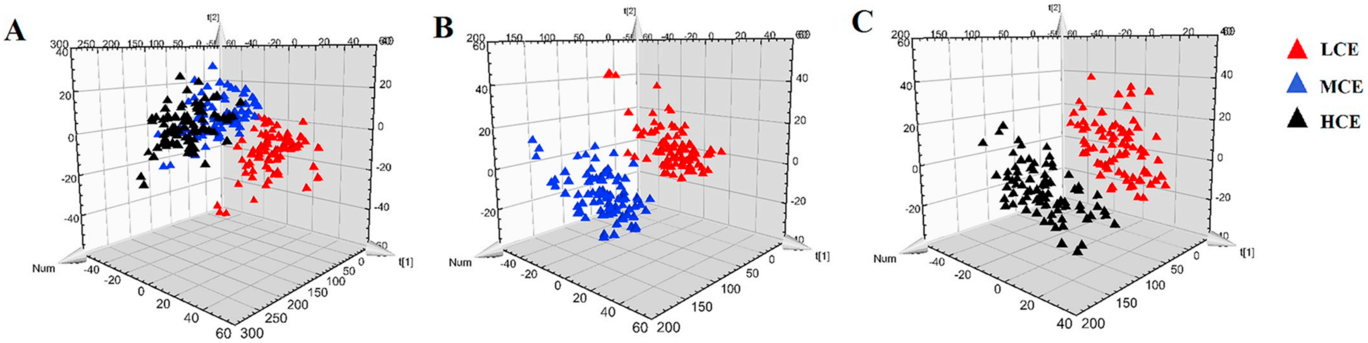

Fig. 1. PLS-DA score plot of the urinary metabolic profiles. The PLS-DA models were built on (A) LCE, MCE and HCE groups ($R^2Y = 0.322$, $Q^2 = 0.176$); (B) LCE and MCE groups ($R^2Y = 0.776$, $Q^2 = 0.450$); and (C) LCE and HCE groups ($R^2Y = 0.858$, $Q^2 = 0.658$).

Meanwhile, the concentrations of Cd in maternal urine were negatively related to histamine ($P = 0.009$) and uric acid ($P = 0.004$) levels.

4. Discussion

Urine samples contain a myriad of endogenous metabolites which can effectively reflect the metabolic state of the human body in pathophysiological conditions. In this study, UPLC-QTOF-MS based metabolomics was used to evaluate the metabolic responses induced by prenatal Cd exposure. Our results suggest that environmental Cd exposure may associates with urine metabolome disruption in pregnant women. In addition, five urinary metabolites were identified as biomarkers for Cd exposure.

Urinary Cd concentration is commonly recognized as a valid indicator of Cd body burden, and it is also widely used as a marker to evaluate Cd exposure (Adams and Newcomb, 2013; Buchet et al., 1990). The World Health Organization (WHO) has suggested a health-based exposure limit of urinary Cd at 5 μg/g creatinine (WHO, 1992). However, increasing evidence indicates that maternal exposure to Cd at levels much lower than the reference value of 5 μg/g creatinine is related to adverse obstetric and perinatal outcomes, such as gestational diabetes mellitus (Xing et al., 2018), pregnancy-induced hypertension (Liu et al., 2018), impaired fetal growth (Wai et al., 2017; Zhang et al., 2018), and preterm birth (Yang et al., 2016). In the present study, the urinary Cd levels of all the participants were within the limit defined by WHO. However, we found that low-level environmental Cd exposure was associated with significant alterations of metabolite markers related to oxidative stress and nephrotoxicity. These findings suggest that prenatal low-level cadmium exposure may also cause physiological or pathological variations, and have adverse effects on mothers and fetuses.

Metabolomics is an emerging technology which focuses on the systematic study of metabolites within the biosystem (including the cell, tissue, and organism) in response to a stressor (Ogura et al., 2013). Recently, researchers began applying metabolomics to identify Cd exposure biomarkers and delineate the metabolic responses of organisms to Cd. Differential urinary metabolic profiles related to Cd exposure have been characterized for individuals living near a point source of Cd pollution (Ellis et al., 2011), women exposed to environmental Cd (Xu et al., 2016), and human with long-term Cd exposure (Gao et al., 2014). As a result, some significantly changed metabolites in those individuals were identified as biomarkers, which suggest that Cd exposure may be associated with abnormal amino acid metabolism and tricarboxylic acid cycle. These abnormal changes in metabolism might relate to adverse effects, such as nephrotoxicity, under long-term Cd exposure. However, these studies investigated the metabolic profile alteration of populations living in highly Cd polluted areas (Ellis et al., 2011) or populations with high Cd exposure (Gao et al., 2014; Xu et al., 2016), but little is known about pregnant women with low-level environmental exposure.

In the present study, the urinary metabolic changes in pregnant women with low level environmental Cd exposure were assessed. An environmental exposure-oriented metabolome strategy was employed, where one third of the samples with the lowest Cd levels (0.3 ± 0.1 μg/g creatinine) and one third of the samples with the highest Cd levels (1.1 ± 0.5 μg/g creatinine) were used to establish the multivariant statistical models. By using the PLS-DA method, the two groups were clearly differentiated, suggesting that Cd exposure was correlated with specific metabolic variations in pregnant women. The participants of this study were similar in age, BMI, pregnancy history, and lifestyle characteristics, reducing the uncertainty induced by individual metabolic variations among participants to a certain extent. Furthermore, the Cd exposure-oriented PLS-DA analysis, which was based on hundreds of samples, eliminated any features associated with individual metabolic variations. Ultimately, five potential biomarkers were putatively identified in the PLS-DA model, as L-cystine, L-tyrosine,

Table 2
Identified Cd-related urinary metabolic biomarkers.

Compound	VIP[a] values	RT (min)	Selected ion	Measured mass (Da)	Calculated mass (Da)	Mass error (ppm)	Major MS/MS fragment ions (*m/z*)	Normalized peak intensity (relative contents)[b]	
								LCE	HCE
L-Cystine	1.75	0.82	$[M+H]^+$	241.0320	241.0317	1.24	120.0125, 151.9828	4.51 ± 1.76	5.47 ± 2.11**
L-Tyrosine	1.76	2.63	$[M+H]^+$	182.0829	182.0817	6.59	123.0442, 165.0522	2.33 ± 1.63	3.03 ± 1.30**
Dityrosine	1.93	3.65	$[M+H]^+$	361.1417	361.1400	4.71	269.1285, 298.1094, 315.1361	0.63 ± 0.23	0.75 ± 0.19**
Histamine	1.92	0.70	$[M+H]^+$	112.0880	112.0875	4.46	–	0.51 ± 0.72	0.28 ± 0.44*
Uric acid	1.60	1.88	$[M+H]^+$	169.0376	169.0362	8.28	124.0145, 126.0310, 152.0106	97.60 ± 67.67	75.27 ± 43.87*

Values are mean ± SD.

VIP values, the greatest variable importance in projection values; RT, retention time.

[a] VIP values was obtained from the PLS-DA model.

[b] *P* value were calculated from *t*-test between the LCE group and HCE group; * represents $P < 0.05$ compared with LCE group,** represents $P < 0.01$ compared with LCE group.

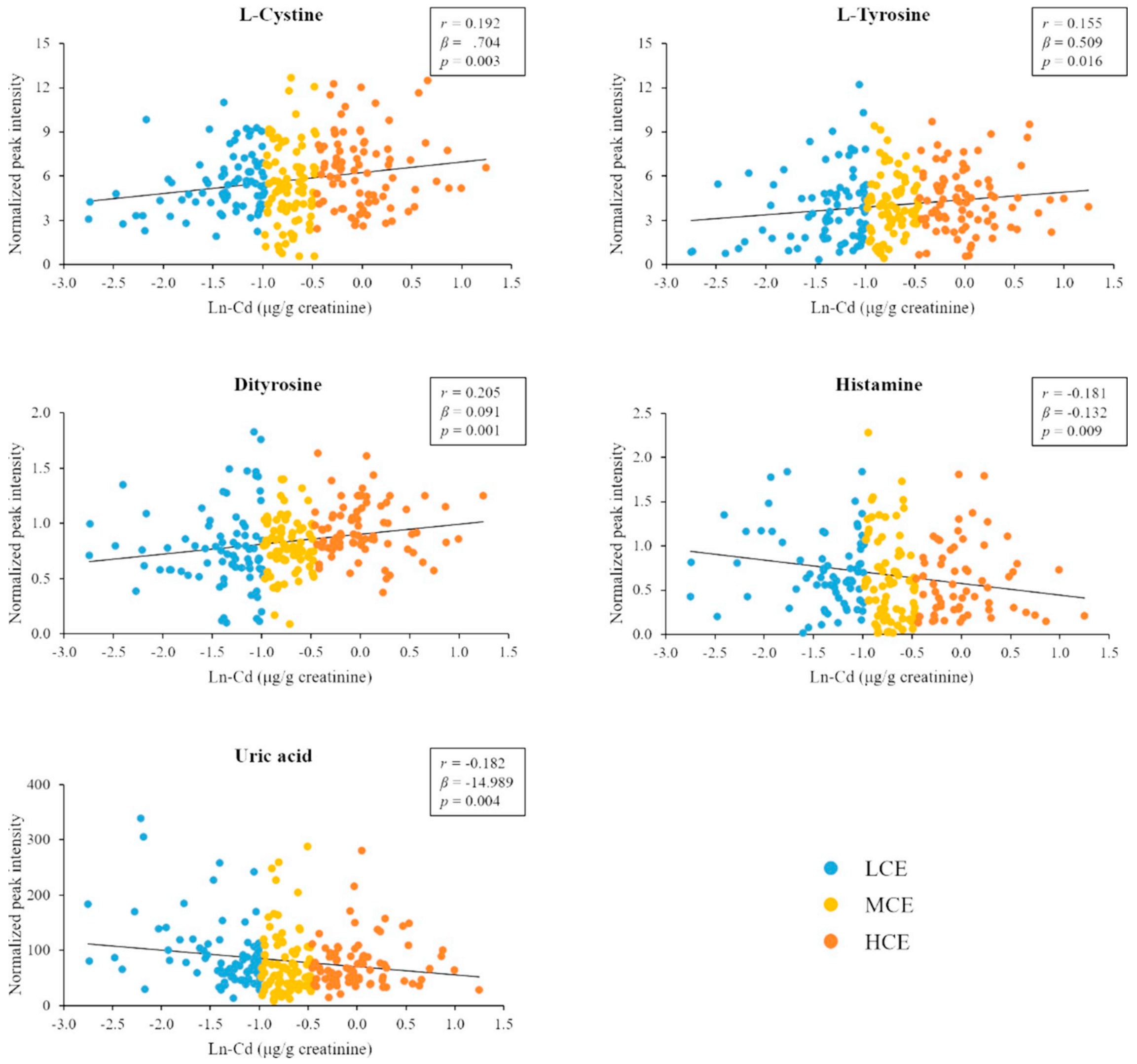

Fig. 2. Associations of maternal urinary Cd concentrations with the biomarkers using linear regression models. Graphs are the scatter-plots of Cd and biomarkers. β represents the change of normalized peak intensity of each biomarker per unit increase in the natural logarithm transformed maternal urinary Cd concentration (μg/g creatinine).

dityrosine, histamine, and uric acid. These biomarkers provide clues that suggest Cd exposure is linked to changes in amino acid metabolism (L-cystine, L-tyrosine, dityrosine, and histamine), and purine metabolism (uric acid), and that metabolic alterations are biologically dependent (Fig. S4). Therefore, the biological significance of these Cd-related biomarkers requires further evaluation.

Cd is not a redox-reactive heavy metal, so it cannot generate reactive oxygen species (ROS) directly (Al-Saleh et al., 2015); but Cd can induce oxidative stress by increasing the production of cellular ROS and disrupting the antioxidant defense systems (Stohs et al., 2001). The biomarkers L-tyrosine and dityrosine (a stable cross-linkage product of L-tyrosine), are indicators of oxidation. When a biosystem is exposed to oxidative stress, L-tyrosine is oxidized to dityrosine by peroxidase-catalyzed mechanisms (DiMarco and Giulivi, 2007). Elevated levels of urinary dityrosine have been observed in aging animals, as well as patients with systemic inflammation (Bhattacharjee et al., 2001; Leeuwenburgh et al., 1997, 1999), confirming its importance as a biomarker for oxidative stress. In this study, both L-tyrosine and dityrosine levels were found to be increased in the HCE group compared with the LCE group. Similar to our findings, elevated L-tyrosine levels were observed in urine samples from females with increased long-term environmental Cd exposure (urinary Cd ≥ 5 μg/L) (Gao et al., 2014), while dityrosine was not identified in that study. The results of our study also supported the earlier findings by Dudka et al. in workers occupationally exposed to heavy metals (Dudka et al., 2014). Using NMR, their study identified ten metabolites, including L-tyrosine, that were associated with arsenic, Cd and lead exposure, and were mostly related to oxidative stress.

Along with these metabolic changes, homeostatic mechanisms are presumably induced to protect the body from Cd-induced oxidative damage, by upregulating the levels of antioxidants and their metabolites (Liu et al., 2009). Glutathione is one of the most effective intracellular antioxidants characterized so far (Whillier et al., 2009). The increase in L-cystine could be an indication of increased intracellular glutathione levels in the state of oxidative stress, since it is the preferred form of L-cysteine for the synthesis of glutathione (Gukasyan et al., 2003). Although glutathione and L-cysteine were not directly identified in the urine in this study, the significantly high level of L-cystine found in the HCE group compared to the LCE group, strongly points to oxidative stress caused by Cd.

It is well-established that the kidney is a critical target organ for Cd-induced toxicity (Renugadevi and Prabu, 2009). In the present study, two biomarkers related to renal function were detected. Histamine is a biogenic amine, and has been shown to play a critical role in renal pathophysiology, through influencing the glomerular function of human kidneys (Ichikawa and Brenner, 1979; Sedor and Abboud, 1984;

Trachtman et al., 1987). A decrease in urinary histamine has been observed in patients with proteinuria, which may be a result of renal mesangial cell injury (Trachtman et al., 1987). In this study, the level of histamine in the HCE group was significantly decreased compared to the LCE group, which suggests a potential Cd-induced kidney dysfunction. Uric acid is produced by the enzyme xanthine oxidase, which oxidizes oxypurines such as xanthine into uric acid, and is excreted in the urine as the end-product of purine metabolism. A large number of animal experiments have demonstrated that Cd can significantly decrease the levels of uric acid in urine (Milton Prabu et al., 2010; Renugadevi and Prabu, 2009, 2010). The researchers speculated that this decrease may be associated with Cd induced nephrotoxicity (Milton Prabu et al., 2010). As a result, the reabsorption of uric acid is increased and its excretion is decreased in the kidney. It is therefore possible that the decreased urinary uric acid levels observed in pregnant women from the HCE group may be related to a form of Cd-induced metabolic disorder of the kidney. However, further in vivo and in vitro studies are needed to determine the specific cellular and systemic targets of these Cd-induced biochemical changes.

The significance of this study is that it assessed maternal urinary Cd concentrations and urinary metabolomics in a large sample size, and suggest that Cd exposure associates with urinary metabolome disruptions in pregnant women. Furthermore, a number of metabolite markers were identified, including dityrosine, an important biomarker of oxidative stress, providing compelling evidence for Cd-induced oxidative damage. This study also has some limitations: (1) Information about renal function/damage was not collected. (2) Many covariates, such as diet, may affect urine metabolomics. Although the biomarkers were screened with strict procedure, we could not completely eliminate the uncertainty induced by the covariates. (3) Several metabolites were detected, but remain unidentified at present. Their metabolic pathways might include other metabolic changes induced by Cd exposure. (4) Only the urine samples in first trimester were collected and analyzed. Although first trimester is the window period of Cd exposure, it is also important to consider that Cd exposure during the second and third trimester of pregnancy could lead to different metabolic variations due to general metabolic changes in the maternal body with the progression of pregnancy. We will address these possible variations in future studies.

5. Conclusions

In summary, the UPLC-QTOF-MS-based untargeted metabolomics approach was successfully applied to analyze the urinary metabolite profile alterations of pregnant women with environmental Cd exposure, and to screen for potential Cd exposure related biomarkers. As a result, five specific metabolites with significantly altered levels related to oxidative stress and nephrotoxicity were identified as putative biomarkers of Cd exposure during pregnancy. These results also provide valuable insights for understanding the Cd-induced adverse effects in pregnant women. Moreover, this study offers a general approach to evaluating the metabolism disrupting effects of many other environmental toxins, which could be useful in personalized risk assessments.

Conflict statement

The authors declare there is no conflict of interest in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.02.007.

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