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RESEARCH ARTICLE

# The relationship between plasma and urinary 8-hydroxy-2-deoxyguanosine biomarkers measured by liquid chromatography tandem mass spectrometry

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**Abstract** Although 8-hydroxy-2-deoxyguanosine (8-OHdG) is a widely used promising biomarker of DNA damage, there are concerns about which tissues or body fluids should be sampled. The objective of this study is to evaluate the correlation of DNA oxidative damage biomarkers, 8-OHdG, between blood and urine and risk factors associated with 8OHdG. The study population was recruited from a baseline survey of a worksite lifestyle study including 92 office workers aged 23 to 60 years. A self-administered questionnaire was used to collect information on personal characteristics. The plasma and urinary 8-OHdG was measured by liquid chromatography tandem mass spectrometry (LC-MS/MS). In linear regression, a positive relation was found ($p < 0.01$) between the log-transformed plasma and urinary 8-OHdG levels adjusted for gender, age, BMI, and smoking status. Our findings showed that age, gender and smoking were significantly associated with plasma 8-OHdG, but not with urinary 8-OHdG. Our results suggest that there is a positive relation between the biomarkers of plasma (steady state DNA damage) and urinary 8-OHdG (total DNA damage). However, the plasma 8-OHdG is more sensitive than urinary 8-OHdG to detect increased oxidative damages induced by risk factors.

**Keywords** 8-hydroxydeoxyguanosine · Urine · Plasma · LC-MS/MS

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## Introduction

Reactive oxygen species (ROS) may be produced by environmental agents such as radiation and chemical carcinogens, as well as by endogenous oxygen metabolism (Klaunig et al. 1998; Pilger and Rudiger 2006; Halliwell 2000). ROS can induce a series of DNA damage, including single and double strand breaks, abasic sites and base modification, which has been considered to play an important role in the mechanism of aging and carcinogenesis (Pilger and Rudiger 2006; Halliwell 2000; Federico et al. 2007; Evans et al. 2004; Kawanishi and Hiraku 2006a; Kawanishi et al. 2006b; Karihtala and Soini 2007; Schleicher and Friess 2007). Among the diverse oxidative lesions, 8-hydroxy-2′-deoxyguanosine (8-OHdG) is one of the most abundant base modifiers and has gained much attention because of its mutagenic potential for G to T transversion (Pilger and Rudiger 2006; Halliwell 2000).

8-OHdG is a modified base that occurs in DNA after an attack by hydroxyl radicals that are formed as products and intermediates of aerobic metabolism and during oxidative stress. In addition, the repair process for 8-OHdG-inflicted

damages results in excised 8-OHdG adduct being circulated in blood, which is then excreted into the urine via the blood circulation (Pilger and Rudiger 2006; Halliwell 2000). 8-OHdG detected in the urine and plasma can serve as biomarkers of oxidative DNA stress reflecting DNA repair (Lai et al. 2005; Chuang et al. 2003; Kristenson et al. 2003; Kuo et al. 2003). Biomonitoring in humans has demonstrated that 8-OHdG levels may be correlated with oxidative stress and DNA damage (Lai et al. 2005; Chuang et al. 2003; Kristenson et al. 2003; Kuo et al. 2003). Due to easy collection, urinary 8-OHdG has been widely studied as a biomarker of oxidative stress (Pilger and Rudiger 2006; Halliwell 2000). In contrast, the data on plasma and serum 8-OHdG as a marker of oxidative damage is very limited (Chen et al. 2007; Liu et al. 2004; Alper et al. 2005).

Although 8-OHdG is a popular and promising biomarker of DNA damage, there are increasing concerns about which tissues or body fluids should be sampled to measure the levels of this modified nucleoside (Pilger and Rudiger 2006; Halliwell 2000). To our knowledge, the relations between levels of 8-OHdG in different compartments or body fluids have rarely been studied (Liu et al. 2004; Hu et al. 2004). The objective of this study is to evaluate the correlation of 8-OHdG between blood and urine. In addition, the risk factors for both blood and urinary 8-OHdG levels are elaborated and compared.

## Materials and methods

### Study population

We carried out a baseline survey of a worksite lifestyle study and recruited 92 office workers aged 23 to 60 years (including 69 males and 23 females). This study has been approved by the institutional review board and all the participants signed informed consents prior to enrollment into this study. A self-administered questionnaire was used to collect information on personal characteristics, such as age, education, marital status, and life style factors such as smoking habits, alcohol drinking, and history of diseases. Participants were instructed not to eat for at least 6 hours prior to blood sampling. Participants were also asked to collect a spot morning urine sample in a container. Venous blood was obtained from the cubital vein in the same morning and collected in a vacutainer.

### Measurements of urinary 8-OHdG levels by LC-MS/MS

The urinary 8-OHdG was measured using a validated method of liquid chromatography/tandem mass spectrometry (LC-MS/MS) (API 3000, Applied Biosystems, Foster City, CA, USA) with on-line solid-phase extraction as reported by Hu et al. (Hu et al. 2004). Briefly, 20 μL of urine was diluted 10-fold with 5 % methanol containing 0.1 % formic acid. After the addition of 40 μL of $^{15}N_5$-8-OHdG solution (20 μg/L in 5 % methanol/0.1 % formic acid) as internal standard, a 100 μL of prepared urine sample was directly injected into the on-line solid-phase extraction LC-MS/MS. After automatic sample cleanup, LC-MS/MS analysis was done using a PE Series 200 HPLC system interfaced with a PE Sciex API 3000 triple-quadrupole mass spectrometer with electrospray ion source. The samples were analyzed in the positive ion multiple reaction monitoring mode, and the transitions of the precursors to the product ions were as follows: $m/z$ 284 → 168 (quantifier ion) and 284 → 140 (qualifier ion) for 8-OHdG, and $m/z$ 289 → 173 (quantifier ion) and 289 → 145 (qualifier ion) for $^{15}N_5$-8-OHdG. With the use of isotopic internal standards and on-line solid-phase extraction, this method had a high sensitivity with limit of detection of 5.7 ng/L (2.0 fmol) on column. The recovery of the total procedure was 92 ± 2.5 % in five standard solutions processed in the same way as the urine samples.

### Measurements of plasma 8-OHdG levels by LC-MS/MS

The analysis of plasma 8-OHdG was performed in the same laboratory and supervised by one of our coauthors (SHL), which has been described elsewhere (Wang et al. 2011a; Chen et al. 2007). Briefly, using isotope-dilution LC-MS/MS, 500 μL of plasma was first diluted with 500 μL of water, followed by the addition of 20 μL of a $^{15}N_5$-8-OHdG solution (42.6 pg/mL) as the internal standard. After the addition of 150 μL of 1 M ammonium acetate buffer (pH 5.25) and vigorous vortexing, the sample was loaded into a Sep-Pak C18 cartridge (100 mg/1 mL; Waters) preconditioned with 1 mL methanol and 1 mL distilled water. The column was then washed with 1 mL of water. The fraction containing 8-OHdG was eluted with 1 mL of 40 % (*v*/*v*) methanol, collected, dried under a vacuum for 2 h, and dissolved in 500 μL of 80 % acetonitrile containing 0.1 % formic acid.

Twenty microliters of the sample solution was injected into the HPLC/MS/MS instrument. The HPLC system consisted of a PE 200 autosampler, two PE 200 micropumps (Perkin Elmer, Norwalk, CT, USA), and a polyamine-II endcapped HPLC column (150 × 2.0 mm, 5 μm, YMC) with an identical guard column (10 × 2 mm, YMC). The mobile phase was 80 % acetonitrile with 0.1 % formic acid, delivered at a flow rate of 300 μL/min. The eluent of the HPLC system was connected to a triple-quadrupole mass spectrometer (API 3000, Applied Biosystems, Foster City, CA, USA) equipped with a TurboionSpray™ source. Electrospray ionization was performed in the positive mode. For all samples, the $[M+H]^+$ ion was selected by the first mass filter. After collisional activation, the $[M+H-116]^+$ ions, corresponding to $BH^{+2}$, were selected by the last mass filter. The optimized source parameters multiple reaction monitoring mode (MRM) transition

pairs of 8-OHdG, $^{15}N_5$-8-OHdG, dG and $^{15}N_5$-dG were set as *m/z* 284 → 168, *m/z* 289 → 173, *m/z* 268 → 152, and *m/z* 273 → 157 for the quantitative pair and *m/z* 284 → 140 and *m/z* 289 → 145 for the 8-OHdG and $^{15}N_5$-8-OHdG qualitative pair, respectively. Nitrogen was used as the nebulizing, curtain, heater (6 L/min), and collision gases. The TurboionSpray™ probe temperature was set to 300 °C.

The recovery ranged from 95.1 to 106.1 % at trace levels, with a CV lower than 5.4 %. The limits of detection (LOD) and LOQ were 0.008 ng/mL and 0.02 ng/mL, respectively.

### Statistical analysis

All the analyses were performed with the SPSS statistical package. We compared the distributions using a non-parametric Wilcoxon rank-sum test for continuous variables and Fisher's exact or chi-square test for categorical variables. The concentration of urinary 8-OHdG was expressed as ng/mL and adjusted to the urinary concentration of creatinine (μg/g creatinine) to control for variation in urinary output. The concentration of plasma 8-OHdG were expressed as pg/mL or as ng/g creatinine after adjusted for blood creatinine. Logarithmic transformation was performed for plasma and urinary 8-OHdG in order to approximate to normal distribution. We estimated the relation between the urinary and plasma 8-OHdG concentrations in linear regression analysis adjusted for confounders and determinants of interest. The risk factors of plasma and urinary 8-OHdG were analyzed by multiple linear regression.

## Results

### The distribution of characteristics in the study population

The characteristics of total study population and comparison between males and females is listed in Table 1. Most of the study population were young age (<30 years old, 75 %), married (69.7 %), high education (68.1 % higher than college), non-smokers (67.4 %), and non-drinkers (83.5 %).

When comparison between males and females was done, marital status, smoking status and BMI were shown to be significantly different. Males had more singles, more smokers, and higher BMI than females. The prevalence of current smoking in males was 44 %, but none of females was smoker. Among male current smokers, the mean daily smoking rate was 11 cigarettes/day. The mean BMI was 24.1 kg/m$^2$ (SD 3.5) in males and 22.1 kg/m$^2$ (SD 3.4) in females.

**Table 1** Distribution of characteristics in the study population

| Characteristic | Males | | Females | | Total | | *P* value[a] |
|---|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | *n* | % | |
| Number of subject | 69 | 75.0 | 23 | 25.0 | 92 | 100 | |
| Age[a] | 37.83 ± 9.32 | | 38.55 ± 7.63 | | 38.01 ± 8.89 | | 0.41 |
| BMI (kg/m$^2$)[a] | 24.1 ± 3.5 | | 22.1 ± 3.4 | | 23.6 ± 3.5 | | <0.05 |
| Age[b] | | | | | | | |
| >30 | 19 | 27.5 | 4 | 17.4 | 23 | 25.0 | |
| <30 | 50 | 72.5 | 19 | 82.6 | 69 | 75.0 | |
| Marital status[c] | | | | | | | <0.05 |
| Married | 41 | 62.1 | 21 | 91.3 | 62 | 69.7 | |
| Single | 25 | 37.9 | 2 | 8.7 | 27 | 30.3 | |
| Education[c] | | | | | | | 0.20 |
| High school | 19 | 27.9 | 10 | 43.5 | 29 | 31.9 | |
| College | 49 | 72.1 | 13 | 56.5 | 62 | 68.1 | |
| Current smoking habits[c] | | | | | | | <0.01 |
| Smokers | 30 | 43.5 | 0 | 0 | 30 | 32.6 | |
| Non-smokers | 39 | 56.5 | 23 | 100 | 62 | 67.4 | |
| Current alcohol consumption habits[b] | | | | | | | 0.10 |
| Yes | 14 | 20.6 | 1 | 4.3 | 15 | 16.5 | |
| No | 54 | 79.4 | 22 | 95.7 | 76 | 83.5 | |
| Tea-drinking habits | | | | | | | 0.13 |
| Yes | 26 | 38.2 | 12 | 57.1 | 38 | 42.7 | |
| No | 42 | 61.8 | 9 | 42.9 | 51 | 57.3 | |
| Coffee-drinking habits | | | | | | | 1.00 |
| Yes | 14 | 20.9 | 4 | 19.0 | 18 | 20.5 | |
| No | 53 | 79.1 | 17 | 81.0 | 70 | 79.5 | |

Values shown are mean ± standard deviation (SD)

*BMI* body mass index

[a] Comparison between the males and females

[b] Fisher's exact test

[c] $\chi^2$ test

### Comparison of plasma and urinary 8-OHdG levels between males and females

Since smoking had found to be risk factor of 8-OHdG and males had higher proportion of smokers than females, the analysis was stratified by gender. The mean concentration of plasma 8-OHdG was 7.53 ± 2.79 pg/mL ($n = 69$) in the males and 4.23 ± 1.38 pg/mL ($n = 23$) in the females ($p < 0.01$) (Table 2). After adjusting for blood creatinine, the plasma 8-OHdG levels were 0.691 ± 0.26 μg/g creatinine and 0.506 ± 0.17 μg/g creatinine ($n = 24$) among the males and females, respectively ($p < 0.05$).

The mean concentration of urinary 8-OHdG was 4.73 ± 3.67 ng/mL ($n = 69$) among the males and 2.90 ± 1.86 ng/mL ($n = 23$) in the females ($p < 0.05$). After adjusting for urinary creatinine, the urinary 8-OHdG levels were 4.55 ± 4.44 μg/g creatinine and 4.34 ± 3.85 μg/g creatinine among the males and females, respectively ($p = 0.84$).

Further stratified the males and females by smoking status (Table 2), there was no significant difference of 8-OHdG levels between smokers and non-smokers in males. Neither creatinine-adjusted plasma nor creatinine-adjusted urinary 8-OHdG was found significantly different between smoking and non-smoking males.

**Table 2** Concentrations of plasma 8OHdG and urinary 8-OHdG measured by LC-MS/MS and stratified by gender and smoking status

| | Males | | Females | | *P* value[a] |
|---|---|---|---|---|---|
| | *N* | Mean ± SD | N | Mean ± SD | |
| Plasma 8-OHdG (pg/mL) by LC-MS/MS | | | | | |
| Smoker | 30 | 7.55 ± 3.04 | 0 | – | – |
| Non-smokers | 39 | 7.52 ± 2.62 | 23 | 4.23 ± 1.38 | <0.01 |
| Subtotal | 69 | 7.53 ± 2.79 | 23 | 4.23 ± 1.38 | <0.01 |
| *P* value[b] | – | 0.97 | – | – | – |
| Blood creatinine (mg/dL) | | | | | |
| Smoker | 30 | 1.08 ± 0.09 | 0 | – | – |
| Non-smokers | 39 | 1.11 ± 0.12 | 23 | 0.84 ± 0.08 | <0.01 |
| Subtotal | 69 | 1.10 ± 0.11 | 23 | 0.84 ± 0.08 | <0.01 |
| *P* value[b] | – | 0.23 | – | – | – |
| Plasma 8-OHdG (ng/g creatinine) by LC-MS/MS | | | | | |
| Smoker | 30 | 703.2 ± 286.3 | 0 | – | – |
| Non-smokers | 39 | 680.7 ± 241.0 | 23 | 506.6 ± 170.3 | <0.05 |
| Subtotal | 69 | 690.5 ± 259.9 | 23 | 506.6 ± 170.3 | <0.05 |
| *P* value[b] | – | 0.72 | – | – | – |
| Urinary 8-OHdG (ng/mL) by LC-MS/MS | | | | | |
| Smoker | 30 | 4.75 ± 3.71 | 0 | – | – |
| Non-smokers | 39 | 4.71 ± 3.68 | 24 | 2.90 ± 1.86 | 0.01 |
| Subtotal | 69 | 4.73 ± 3.67 | 24 | 2.90 ± 1.86 | <0.05 |
| *P* value[b] | – | 0.96 | – | – | – |
| Urinary creatinine (mg/dL) | | | | | |
| Smoker | 30 | 131.7 ± 69.3 | 0 | – | – |
| Non-smokers | 39 | 124.2 ± 66.0 | 23 | 93.3 ± 58.0 | 0.07 |
| Subtotal | 69 | 127.5 ± 67.0 | 23 | 93.3 ± 58.0 | 0.03 |
| *P* value[b] | – | 0.68 | – | – | – |
| Urinary 8-OHdG (μg/g creatinine) by LC-MS/MS | | | | | |
| Smoker | 30 | 4.47 ± 3.67 | 0 | – | – |
| Non-smokers | 39 | 4.61 ± 4.99 | 23 | 4.34 ± 3.85 | 0.82 |
| Subtotal | 69 | 4.55 ± 4.44 | 23 | 4.34 ± 3.85 | 0.84 |
| *P* value[b] | – | 0.89 | – | – | – |

Values shown are mean ± standard deviation (SD)

*8-OHdG* 8-hydroxy-2′deoxyguanosine

[a] Comparison between the male and female groups

[b] Comparison between the smokers and non-smokers

### Relation between plasma and urinary 8-OHdG levels measured by LC/MS/MS

The correlation between urinary creatinine-adjusted 8-OHdG and plasma 8-OHdG by LC-MS/MS with log transformation is 0.31 ($p < 0.01$) (Fig. 1). In linear regression, a positive relation was found between the log-transformed plasma 8-OHdG and urinary creatinine-adjusted 8-OHdG levels [The equation is: urinary 8-OHdG creatinine-adjusted (ug/g) = 0.10 + 0.54 × plasma 8-OHdG (pg/mL)]. The correlation between urinary and plasma 8-OHdG was statistically significant.

### Risk factors affecting plasma and urinary 8-OHdG levels

In order to elaborate the factors associated with either plasma or urinary 8-OHdG, a multiple regression model was used to uncover the significant risk factors. Tables 3 and 4 show the regression models for plasma 8-OHdG and urinary 8-OHdG,

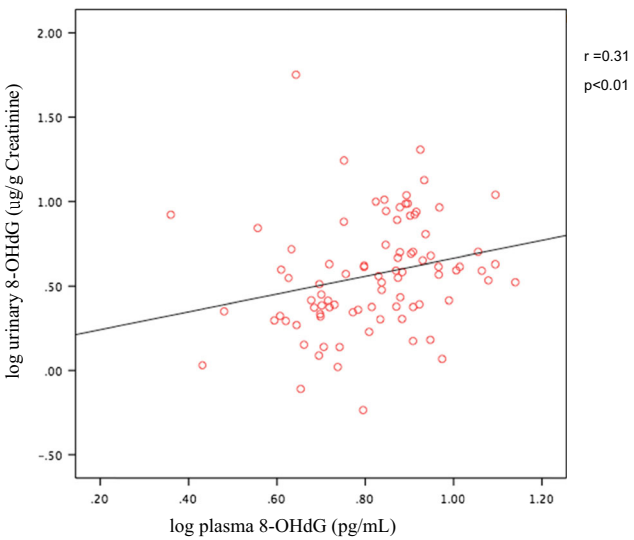


**Fig. 1** Correlation between urinary creatinine-adjusted 8-OHdG and plasma 8-OHdG by LC-MS/MS with log transformation

**Table 3** Multiple linear regression of plasma log (8-OHdG) measured by LC-MS/MS on risk factors

| Variable | Model 1[a] Coefficient (β) | SE (β) | *P* value | Model 2[a] Coefficient (β) | SE (β) | *P* value |
|---|---|---|---|---|---|---|
| Age (years) | 0.005 | 0.002 | <0.05 | 0.006 | 0.002 | 0.02 |
| Gender (male/female) | 0.24 | 0.04 | <0.001 | – | – | – |
| Smoking (yes/no) | 0.005 | 0.04 | 0.91 | 0.10 | 0.05 | 0.05 |
| Alcohol drinking (yes/no) | 0.02 | 0.05 | 0.63 | 0.04 | 0.06 | 0.51 |
| Coffee (yes/no) | 0.04 | 0.04 | 0.42 | 1.33E-005 | 0.05 | 1.00 |
| Tea (yes/no) | 0.02 | 0.03 | 0.63 | −0.02 | 0.04 | 0.59 |

[a] Model 1 includes males and females; Model 2 only includes males since no smokers in females

respectively. Age and gender were positively significantly associated with plasma 8-OHdG (Table 3, model 1). Since all smokers are males and the gender effect may be accounted by smoking, another model (model 2) was used to exclude gender effect as shown in the right-side of Table 3. Age and smoking were also positively significantly associated with plasma 8-OHdG. However, alcohol drinking, coffee drinking, and tea drinking were not associated with plasma 8-OHdG levels.

In contrast, none of the factors was found to be associated with urinary 8-OHdG levels measured by LC-MS/MS, either adjusted or non-adjusted for urinary creatinine. No association was found either inclusion or exclusion gender in the regression models (Table 4).

## Discussion

8-OHdG has been well studied as a marker of oxidized DNA damage. Due to noninvasiveness and easy collection, urinary 8-OHdG has been widely used as a biomarker of oxidative stress (Pilger and Rudiger 2006; Halliwell 2000). 8-OHdG is believed to be connected with environmental pollutants such as PAHs, heavy metals and even estrogenic chemicals and dose-effects relationships were observed between these chemical and urinary 8-OHdG levels (Li et al. 2015a; Li et al. 2015b; Zhang et al. 2016). However, the use of plasma 8-OHdG as a marker of oxidative damage is still limited (Chen et al. 2007; Liu et al. 2004; Alper et al. 2005). This study found that there was a statistically significant positive correlation between the log-transformed plasma 8-OHdG and the log-transformed urinary 8-OHdG. However, the plasma 8-OHdG was more sensitive than urinary 8-OHdG to detect increase of oxidative damage induced by risk factors.

The ideal in human studies has been suggested to measure steady state DNA damage as well as total damages measured by urinary DNA damage (Pilger and Rudiger 2006; Halliwell 2000). 8-OHdG concentration in lymphocytes isolated from peripheral blood is the best measure of steady state levels. 8-OHdG in lymphocytes is then excised by nucleotide excision repair enzymes and circulated in the plasma. Therefore, plasma 8-OHdG concentration in the peripheral blood may serve as a surrogate measure of steady state levels, although plasma 8-OHdG level is lower than urinary 8-OHdG levels. The amount of modified nucleosides excreted into urine is considered to represent the whole body oxidative DNA damage (Pilger and Rudiger 2006; Halliwell 2000). Both plasma and urinary 8-OHdG are combined measurements of rates of damage and rates of repair. Therefore, the plasma DNA damage may be correlated with urinary total DNA damage. This study found that there was a statistically significant correlation between plasma and urinary 8-OHdG. However, the levels of oxidized bases can change not only because of changes in the rate of oxidative DNA damage, but also because of alterations in the rate of repair (Pilger and Rudiger 2006; Halliwell 2000). Arguments have been raised whether greater "throughput" of DNA base oxidation is deleterious even if it does not result in significant

**Table 4** Multiple linear regression of urinary log (8-OHdG) measured by LC-MS/MS on risk factors

| Variable | Model 1[a] Coefficient (β) | SE (β) | *P* value | Model 2[a] Coefficient (β) | SE (β) | *P* value |
|---|---|---|---|---|---|---|
| Age (years) | 0.005 | 0.004 | 0.28 | 0.005 | 0.004 | 0.28 |
| Gender (male/female) | −0.05 | 0.09 | 0.60 | – | – | – |
| Smoking (yes/no) | −0.05 | 0.10 | 0.58 | −0.07 | 0.09 | 0.42 |
| Alcohol drinking (yes/no) | 0.13 | 0.10 | 0.20 | 0.13 | 0.10 | 0.21 |
| Coffee (yes/no) | 0.13 | 0.10 | 0.18 | 0.14 | 0.09 | 0.15 |
| Tea (yes/no) | −0.06 | 0.07 | 0.43 | −0.05 | 0.07 | 0.48 |

[a] Model 1 includes males and females; Model 2 only includes males since no smokers in females

rises in the steady state levels of DNA base damage products (Pilger and Rudiger 2006; Halliwell 2000).

If only one set of measurements can be made, the steady state DNA damage measurement might be preferable because miscoding induced by oxidized bases is presumably what determines the risk of mutation and in turn the risk of cancer development (Pilger and Rudiger 2006; Halliwell 2000). Our study showed plasma 8-OHdG is more sensitive than urinary 8-OHdG to detect risk factors induced oxidative damages, which is consistent with this suggestion. Plasma 8-OHdG levels have been found previously to be associated with the brain contents of 8-OHdG and suggested that 8-OHdG as a peripheral biomarker may be an indicator of oxidative brain damage in acute cerebral infarction (Liu et al. 2004).

Susceptibility to DNA oxidation damages shows an inter-individual variation, possibly linked to differences in metabolic capacity, repair activity, or antioxidant defense (Pilger and Rudiger 2006; Halliwell 2000). Since we collected the blood and urine samples at the same time from the individual subject, this concern will not be the case in this study. Many previous studies have identified age, gender and smoking as determinants of plasma 8-OHdG (Black et al. 2016; Suzuki et al. 2003), but no complete agreement with urinary 8-OHdG (Besaratinia et al. 2001; Kimura et al. 2006; Kasai et al. 2001; Loft et al. 1992; Prieme et al. 1998; Pilger et al. 2001; Proteggente et al. 2002; Wang et al. 2011b). In our study, we also found the influence of age and smoking on the levels of plasma 8-OHdG but not on urinary 8-OHdG. Other risk factors such as alcohol drinking, coffee drinking and tea drinking were not associated with plasma and urinary 8-OHdG in this study. However, some but not all experimental studies suggest that alcohol consumption could increase 8-OHdG (Wieland and Lauterburg 1995; van Zeeland et al. 1999). The coffee and tea drinking has been linked to reduction of oxidative DNA damage, but studies found that urinary 8-OHdG concentrations tended to decrease with coffee and tea consumption only in healthy women (Hori et al. 2014) and smokers (Hakim et al. 2003). The reasons for these discrepancies are unknown, though they may be due to variations in laboratory techniques or participants' race, age, gender, and type of alcohol, coffee, and tea consumption. Base on our study, the plasma 8-OHdG is more sensitive than urinary 8-OHdG to detect increased oxidative damages induced by risk factors.

Analysis of 8-OHdG levels has been performed by methods such as high performance liquid chromatography with electrochemical detection (HPLC-ECD), gas chromatography with mass spectrometry (GC/MS), enzyme-linked immunosorbent assay (ELISA), and liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Pilger and Rudiger 2006; Halliwell 2000). To our knowledge, the sensitivity and specificity of LC-MS/MS method are significantly better than those of ELISA or HPLC-ECD. Hence a statistically significant difference in urinary levels of 8-OHdG between exposed and control workers was detected by the LC-MS/MS method, but not by the ELISA method (Hu et al. 2004). Moreover, a dose-effect relationship was observed in urinary levels of 8-OHdG in children exposed to PAHs and benzene when using by LC-MS/MS method (Li et al. 2015a, b), but not by HPLC-ECD (Fan et al. 2012). LC-MS/MS method provides high selectivity, sensitivity, and no derivatization step is required in the analysis. In addition, the targeted analyze can be quantitated reliably and accurately with the application of the isotope-dilution method (Hu et al. 2004). Therefore, it has been suggested to continue aggressively the development of MS-based methods (especially LC-MS) for the measurement of DNA base oxidation products (Halliwell 2000).

## Conclusion

Our results showed there is a positive relation between the biomarkers of plasma (steady state DNA damage) and urinary 8-OHdG (total DNA damage). The plasma 8-OHdG is more sensitive than urinary 8-OHdG to detect elevation of oxidative damages induced by risk factors such as age, gender and smoking. However, there are still critical aspects related to the analytical challenge, inter- and intra-individual variation, and inter-laboratory differences of plasma 8-OHdG measurement. Further work is needed to reach a consensus on the use of plasma level of 8-OHdG.

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