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RESEARCH ARTICLE

Urinary metabolites of polycyclic aromatic hydrocarbons in pregnant women and their association with a biomarker of oxidative stress

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Abstract

Exposure to polycyclic aromatic hydrocarbons (PAHs) during pregnancy may pose adverse health risk to both the mothers and babies. In the present study, 188 pregnant women of different trimesters were recruited in Guangzhou, south China, and nine hydroxyl PAHs (OH-PAHs) and a biomarker of DNA oxidative damage, 8-hydroxy-2′-deoxyguanosine (8-OHdG), were determined in their urine samples. All OH-PAHs except for 4-hydroxyphenanthrene and 6-hydroxychrysene were found in > 90% samples, with total concentration in the range of 0.52 to 42.9 μg/g creatinine. In general, concentration levels of OH-PAHs in pregnant women were lower than those in general population in the same research area but with higher levels in working women than in housewives. The mean daily intakes of PAHs from dietary estimated by urinary OH-PAHs were 0.021, 0.004, 0.047, and 0.030 μg/kg_bw/day for naphthalene, fluorene, phenanthrene, and pyrene, respectively, which were much lower than the reference doses (20, 30, and 40 μg/kg_bw/day for naphthalene, pyrene, and fluorene, respectively) derived from chronic oral exposure data by the United States Environmental Protection Agency. The low exposure levels of PAHs may be attributed to the traditional dietary taboo of Chinese pregnant women, which is to minimize the consumption of "toxic" food. The concentrations of 8-OHdG (4.67–49.4 μg/g creatinine) were significantly positively correlated with concentrations of several OH-PAHs, such as metabolites of naphthalene, fluorene, and phenanthrene ($r = 0.3–0.6$). In addition, the concentrations of 8-OHdG were higher in working women than in housewives when exposed to the same levels of PAHs, partly indicating the possible relation between work-related pressure for working women and the oxidative stress.

Keywords OH-PAHs · Pregnant women · Oxidative stress · Working pressure · Exposure assessment · 8-OHdG

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic pollutants derived from pyrogenic, petrogenic, and biological sources (Abdel-Shafy and Mansour 2016). Many PAHs are well-known carcinogens, such as benzo[a]pyrene (Abdel-Shafy and Mansour 2016). Human exposure to PAHs mainly occurs through dietary intake, air inhalation, and dermal contact (Domingo and Nadal 2015; Fernando et al. 2016; Xia et al. 2010; Zhang et al. 2014). After entering the human body, PAHs are quickly metabolized to hydroxyl PAHs (OH-PAHs), perhaps in several hours, and some OH-PAHs are useful biomarkers of human exposure to PAHs (Li et al. 2012).

A lot of previous studies have estimated human exposure doses of PAHs based on their concentrations in potential environmental sources, such as food, airborne, and indoor dust, which however may not account for exposure doses from all potential sources. Recent biomonitoring efforts attempted to gain more information in this regard. Assessment of PAH exposure for pregnant women is important, because their health strongly impacts the babies they gave birth to. The first biomonitoring study of PAHs in pregnant women measured 1-hydroxypyrene (1-OH-Pyr) in urine samples from 204

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pregnant women in Spain (Llop et al. 2008). Subsequent studies were carried out in Japan, Korea, Poland, the United States (U.S.), Canada, Peru, Saudi Arabia, Brazil, and China (Adetona et al. 2013; Al-Saleh et al. 2013; Kim et al. 2011; Li et al. 2015a; Machado et al. 2015; Nethery et al. 2012; Polanska et al. 2014; Polańska et al. 2011; Suzuki et al. 2010; Woodruff et al. 2011), but only limited studies involved multiple OH-PAHs, which reported extremely high concentrations of OH-PAHs in Chinese pregnant women (Li et al. 2015a). It should be noted that the pregnant women in Li et al.'s study were recruited in Taiyuan, Shanxi Province, which is known for severe PAH pollution in both air and food (Xia et al. 2010, 2013). Clearly, the results obtained thus far may not be generalized to Chinese pregnant women. In addition, studies have also been conducted on the variability in the concentrations of PAH metabolites during the entire period of pregnancy (Adetona et al. 2013).

As carcinogens, PAHs can be an exogenous source of reactive oxygen species, causing oxidative DNA damage (Valavanidis et al. 2009). For example, increased sperm DNA damage was associated to PAH exposure for males (Han et al. 2011). 8-Hydroxy-2′-deoxyguanosine (8-OHdG) is one of the by-products derived from DNA damage, a sensitive marker of oxidative stress after exposure to various environmental pollutants (Valavanidis et al. 2009). Increased concentrations of 8-OHdG were found to associate with the increased exposure levels of metals, phthalates, bisphenols, and PAHs (Guo et al. 2014; Kuang et al. 2013; Wong et al. 2005; Zhang et al. 2016). By far, one study in Saudi Arabia examined the correlation between the concentrations of OH-PAHs and 8-OHdG in urine of pregnant women, but a single biomarker (1-OH-Pyr) was used (Al-Saleh et al. 2013). Iman et al. suggested that 1-OH-Pyr may not be an applicable biomarker of PAH exposure, because no significant correlation was found between the concentrations of PAHs and 1-OH-Pyr (Al-Saleh et al. 2013). Other researchers also suggested that sole use of 1-OH-Pyr may not reflect the entire PAH exposure (Fan et al. 2012). Therefore, additional efforts should be directed toward identifying more OH-PAHs to appropriately examine the correlation between PAH exposure and its oxidative outcome.

In the present study, we recruited 188 pregnant women at different trimesters from Guangzhou, south China. OH-PAHs and 8-OHdG were determined in their urine samples. We aimed to estimate the internal exposure of PAHs for pregnant women during pregnancy by analyzing their urinary metabolites and to check the relationship between concentrations of OH-PAHs or daily exposure doses of PAHs and oxidative stress (8-OHdG).

Materials and methods

Samples and standards

The present study was a part of our ongoing efforts to assess pregnant women's exposure to endocrine disruptors during different gestational periods, which was approved by the ethics committee of Jinan University. From January to March of 2015, pregnant women were randomly recruited during their regular pregnancy checks. The detailed objectives and design of the study were explained to the recruited pregnant women before spot urine samples were collected. The urine samples were transferred to the laboratory within 4 h and maintained at − 20 °C until analysis. A previous study confirmed that urinary OH-PAHs were stable for several years, even going through several freeze and thaw cycles when stored at − 20 °C (Gaudreau et al. 2016). Personal data were also obtained during sample collection (Table 1).

Nine target analytes, including 1-hydroxynaphthalene (1-OH-Nap), 2-hydroxynaphthalene (2-OH-Nap), 1-hydroxyphenanthrene (1-OH-Phen), 2-hydroxyphenanthrene (2-OH-Phen), 3-hydroxyphenanthrene (3-OH-Phen), 4-hydroxyphenanthrene (4-OH-Phen), 2-hydroxyfluorene (2-OH-Fluo), 6-hydroxychrysene (6-OH-Chry), and 1-OH-Pyr, were purchased from AccuStandard (New Haven, CT) or Cambridge Isotope Laboratories (Andover, MA). Isotope-labeled standards, including 2-OH-Nap-d_7, 1-OH-Nap-d_7, 1-OH-Pyr-d_9, $^{13}C_4$-4-OH-Phen, $^{13}C_6$-3-OH-Phen, $^{13}C_6$-2-OH-Fluo, creatinine-d_5, and $^{15}N_5$-8-OHdG, were acquired from C/D/N Isotopes (Quebec, Canada) or Cambridge Isotope Laboratories. 8-OHdG and creatinine were obtained from Sigma-Aldrich (St Louis, MO). All reagents are of HPLC grade or better.

Sample preparation and instrumental analysis

Concentrations of OH-PAHs in urine samples were determined with an isotope dilution method described previously (Guo et al. 2013). Briefly, upon addition of the internal standards, 2 mL of urine was enzymatically deconjugated overnight at 37 °C with β-glucuronides and liquid-liquid extracted with organic solvent twice (80% pentane/20% toluene in volume). In each cycle, the mixture was shaken for 30 min and centrifuged for 20 min, and the supernatant was transferred to another clean glass tube. Subsequently, two extracts were combined and washed with 1 mL of 1 M $AgNO_3$ solution. The combined extract was concentrated to near dryness under a gentle nitrogen stream and dissolved with methanol to 0.4 mL. For 8-OHdG and creatinine, the urine samples were diluted 10 and 10,500 times, respectively, with Milli-Q water without further purification for instrumental analysis (Guo et al. 2014; Zhang et al. 2016).

Table 1 General information of pregnant women from Guangzhou, south China

Pregnant women ($n = 188$)	
Age (year)	29.2 ± 3.8 (mean)
Height (cm)	159.6 ± 4.3
Weight (kg)	60.0 ± 8.5
Job status	Working women, $n = 138$; housewife, $n = 24$; unknown, $n = 26$
Occupation	Office stuff, $n = 92$; teacher, $n = 16$; saleswomen, $n = 9$; doctor, $n = 5$; programmer, $n = 5$; other jobs, $n = 11$
Fasting in the morning	Yes, $n = 48$; no, $n = 140$
Week of pregnant (week)	23.8 ± 9.7
Time living in Guangzhou (year)	7.45 ± 6.92

A high-performance liquid chromatograph equipped with a tandem mass spectrometry system (HPLC-MS-MS; Shimadzu LC-30A LC system plus an ABSciex 5500 triple quadrupole MS) was used to quantify OH-PAHs, creatinine, and 8-OHdG. Specific gravity of urine was measured using a handheld pen refractometer (ATAGO, Tokyo). The refractometer was calibrated with HPLC water to set zero value and then used to measure specific gravity. The details of urine sample preparation and instrumental analysis are described in the Supporting Information (SI).

Quality assurance/quality controls

For each batch of 26 urine samples, two method blanks, two matrix samples, and either two blank spiked or two matrix spiked samples were processed. Method blank samples and matrix samples were Milli-Q water and mixture of urine from non-experimental subjects, respectively. The quality assurance/quality control (QA/QC) results are shown in SI Table S1. Almost no OH-PAHs were detected in method blanks, and the mean recoveries of the internal standards in all matrix spiked samples/duplicates were in the range of 43–91% (isotope dilution method, so acceptable). For 8-OHdG, it was not detected in 3 blanks in each diluted batch. All data were recovery adjusted. The reporting limit, defined as the lowest calibration concentration divided by the actual sample weight, was 0.10 ng mL^{-1} for 1-OH-Nap and 2-OH-Nap, 0.02 ng mL^{-1} for 2-OH-Fluo, 2-OH-Phen, and 3-OH-Phen, and 0.05 ng mL^{-1} for all other target analytes.

Data analysis

Creatinine-adjusted concentrations of OH-PAHs and 8-OHdG were reported in the present study unless specified otherwise. Concentrations below the reporting limits were set as the reporting limit divided by the square root of 2 (Succop et al. 2004). The sums of 1- and 2-OH-Nap, 1-, 2-, and 3-Phen, and all nine analytes excluding 6-OH-Chry and 4-OH-Phen (hardly detected in samples) were defined as $\sum_2$OH-Nap, $\sum_3$OH-Phen, and $\sum_7$OH-PAH, respectively. The raw data acquired in the present study were not normally distributed as suggested by the Kolmogorov-Smirnov test. Thus, non-parametric tests (Mann-Whitney H or Kruskal-Wallis U) were used to analyze differences between and among groups, and Spearman's rank correlation coefficient was used for analysis of the relationship, if any, between two sets of data. Statistical significance for all analyses was set as $p < 0.05$. All statistical analyses were performed using SPSS version 22.0.

Results and discussion

Occurrence of OH-PAHs in urine

Nine hydroxyl PAH metabolites in 188 urine samples from pregnant women were determined. It should be noted that 2-OH-Fluo reported in the present study is the sum of three isomers of hydroxyfluorene (i.e., 2-, 3-, and 9-OH-Fluo) as they were not separable with the instrument used. Among the nine analytes, the detection frequencies of 4-OH-Phen (11%) and 6-OH-Chry (2%) were low, whereas the other seven target compounds were almost found in all urine samples (> 90%). The profile of detection frequencies was consistent with previous urine studies (Guo et al. 2013; Li et al. 2008; Nethery et al. 2012; Yang et al. 2015). Generally, water-soluble metabolites, mostly as glucuronic acid conjugates, with molecular mass < 475 Da, are preferentially excreted in urine, and those with higher molecular masses appear in feces (Yang et al. 2009). High molecular weight metabolites of PAH, e.g., metabolites of chrysene, were not frequently detected in urine (Li et al. 2008; Ramesh et al. 2004).

Concentrations of $\sum_7$OH-PAH ranged from 0.52 to 42.9 μg/g (Fig. 1, with detailed data presented in SI Table S2), with 1- and 2-OH-Nap as the predominant congeners (69%), followed by metabolites of phenanthrene (17%), 2-OH-Fluo (7%), 1-OH-Pyr (7%), and 6-Chry (1%). In addition, concentrations of 2-OH-Nap were much greater than those of 1-OH-Nap in all samples (2 to 105 times). Among

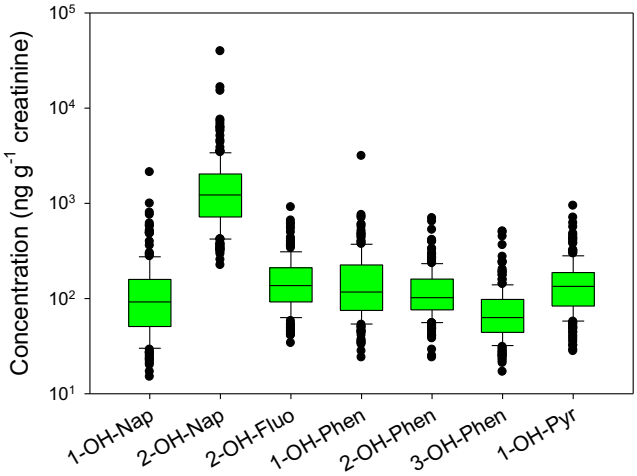

Fig. 1 Concentrations of OH-PAHs in urine samples of pregnant women, south China (ng/g creatinine). The horizontal lines represent the 10th, 50th, and 90th percentiles, and the boxes represent the 25th and 75th percentiles. Outliers are shown as individual points

four OH-Phen isomers, 1-OH-Phen was the most abundant one, accounting for 41% of the total concentration, followed by 2-OH-Phen (34%), 3-OH-Phen (21%), and 4-OH-Phen (4%).

The concentrations of OH-PAHs in pregnant women in the present study were generally comparable to other similar studies in other countries (SI Table S3). For example, the geometric mean concentration of 1-OH-Pyr, which was used frequently as a biomarker of human exposure to PAHs, was 0.13 μg/g in the present study and 0.063, 0.136, and 0.324 μg/g in pregnant women in the U.S., Canada, and Peru, respectively. However, the levels of PAH exposure in pregnant women in our study were orders of magnitude lower than general population, including children in kindergarten and non-smoking college students (Fig. 2), in the same study area reported by other studies in south China (Fan et al. 2012; Li et al. 2015a, b, c). The main routes for human exposure to PAHs in China were air/particle inhalation or diet consumption, with the later one as predominant generally (> 70%) (Zhang et al. 2014). So the low levels of OH-PAHs in pregnant women here may be attributed to concentration variation of PAHs in air or their food. Air studies in this area reported that concentrations of PAHs in air were generally higher in winter than those in summer recent years, with a concentration ratio range of 1.29–20.5 (mean 5.97), and were gradually decreased from 2004 to 2016 (Yan et al. 2019). The three PAH biomonitoring studies in Guangzhou were carried out at different years: June 2013 for children, November 2011 for college students, and January to March 2015 for our study, so the low levels of OH-PAHs in pregnant women may be partly explained by the low concentrations of PAHs in the air. In addition, certain unique life styles adopted by Chinese pregnant women may help to lessen their exposure to PAHs. Several studies from Hong Kong and Macau (south China) reported that antenatal taboos are still commonly practiced by contemporary Chinese women, including behavior and diet, in order to mitigate adverse effects associated with pregnancy and birth (Lau 2012; Lee et al. 2009). For example, some metaphysically "toxic" food, such as fish, beef, goose, mutton, and rabbit meat, which may contain PAHs especially after barbecue, are avoided, as traditionally toxins present in these food are believed to affect fetal health (Lau 2012; Lee et al. 2009). In any case, pregnant women in south China were generally subject to PAH exposure but not at high level.

Distribution patterns of OH-PAHs by demographics

Non-parametric tests were used to analyze the distribution patterns of OH-PAHs in pregnant women by different groups (Table 2). It should be noted that data analysis adjusted by creatinine and urine gravity gave almost similar results. The results indicated that no significant difference in concentration

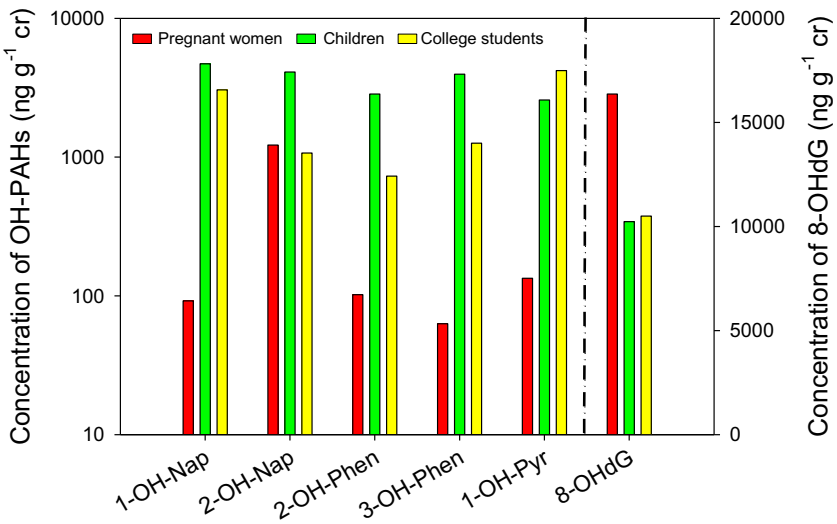

Fig. 2 Concentrations of OH-PAHs and 8-OHdG among different population in Guangzhou, south China. Data of pregnant women were acquired from the present study, and data of children and students were obtained from two previous studies (Li et al. 2015b, c)

Table 2 Concentrations of OH-PAHs and 8-OHdG in pregnant women in Guangzhou, south China (geometric mean; μg/g creatinine)

	n	1-OH-Nap	2-OH-Nap	∑OH-Nap	2-OH-Fluo	1-OH-Phen	2-OH-Phen	3-OH-Phen	∑OH-Phen	1-OH-Pyr	∑OH-PAH	8-OHdG
Age (year)												
19–30	127	0.10	1.32	1.45	0.14	0.13	0.11	0.07	0.33	0.13	2.17	16.5
31–42	61	0.09	1.12	1.23	0.14	0.13	0.11	0.07	0.34	0.13	1.91	15.2
Height (cm)												
150–160	121	0.10	1.37	1.48	0.15	0.13	0.11	0.07	0.33	0.13	2.21	16.3
161–170	67	0.09	1.08	1.20	0.14	0.13	0.11	0.06	0.33	0.12	1.87	15.7
Weight (kg)												
40.0–50.0	26	0.08	1.19	1.23	0.12	0.11	0.08**[a]	0.06*	0.27	0.11	1.90	14.3
50.1–60.0	70	0.11	1.43	1.56	0.15	0.13	0.11	0.07	0.33	0.13	2.28	17.6
60.1–70.0	77	0.10	1.23	1.35	0.15	0.15	0.13	0.07	0.38	0.15	2.13	15.4
> 70.0	15	0.07	0.86	0.93	0.10	0.10	0.11	0.04	0.26	0.09	1.43	15.9
Trimester												
First	29	0.10	1.91*	2.03*	0.16	0.11	0.09**	0.08	0.30	0.12	2.76	13.8
Second	90	0.10	1.19	1.31	0.15	0.14	0.11	0.07	0.33	0.13	1.99	16.7
Third	69	0.09	1.15	1.25	0.13	0.13	0.13	0.06	0.35	0.14	1.96	16.4
Times of pregnancy												
1	129	0.95	1.23	1.35	0.14	0.13	0.11	0.06	0.33	0.13	2.05	16.0
> 1	57	0.94	1.30	1.41	0.14	0.13	0.11	0.07	0.34	0.14	2.12	16.4
Fasting in the morning												
Yes	47	0.10	1.63*	1.78*	0.16	0.14	0.11	0.08	0.35	0.15	2.61*	15.0
No	140	0.09	1.15	1.26	0.14	0.13	0.11	0.06	0.33	0.12	1.93	16.5
Job status												
Have a job	138	0.10	1.25	1.37	0.14*	0.13	0.11*	0.07**	0.34*	0.13*	2.09	16.9**
No job	24	0.08	1.10	1.19	0.11	0.09	0.09	0.05	0.25	0.10	1.74	12.8

[a] Non-parametric test

* $p < 0.05$

** $p < 0.01$

One sample information in the group of whether fasting in the morning and 26 sample information in the group of whether having a job were missing

of OH-PAHs was found among different groups of age, height, and multiparous, while significantly varied levels were observed among different groups of weight, trimester, status of fasting in the morning, and job situation. For example, significantly higher levels of 2-OH-Nap, $\sum_2$OH-Nap, and $\sum_7$OH-PAH were found in pregnant women fasting in the morning. Working women contained higher levels of all OH-PAHs, except for metabolites of naphthalene, than housewives. Women undergoing the first trimester also contained significantly higher concentrations of 2-OH-Nap, $\sum_2$OH-Nap, 2-Fluo, 2-OH-Phen, and $\sum_7$OH-PAH than those undergoing the second and third trimesters if the data were not adjusted by creatinine or urine gravity.

It should be noted that the urine samples representing different trimesters of pregnancy were collected from different women, which may have introduced uncertainties when individual samples are compared. Another follow-through study in Trujillo, Peru, however, also found a significant increase in the concentrations of OH-Phen across the trimesters (Adetona et al. 2013), similar to our study. The variability in the levels of OH-PAHs in pregnant women may have resulted from the different metabolism abilities across trimesters. For example, concentrations of dibenzo[def,p]chrysene (DBC) and its hydroxylated metabolites in blood and tissues were significantly higher in pregnant mice than in non-pregnant mice when exposed to 15 mg/kg DBC by oral gavage, which may be explained by reduced enzyme activity of over 30 types of P450s in the pregnant mice (Crowell et al. 2013). Furthermore, the high levels of OH-PAHs in working women (office stuff, teacher, doctor, saleswomen, and programmer) were apparently not derived from occupational exposure, as indicated in Table 1 that none of these women were affiliated with any well-known occupations with intensive exposure to PAHs, such as coke workers, chefs, or firefighters. The high exposure

Table 3 Estimated daily intake of PAHs for pregnant women in south China (geometric mean, μg/kg_bw/day)

	Naphthalene	Fluorene	Phenanthrene	Pyrene	Total PAHs[a]
Total	0.021	0.004	0.047	0.030	0.105
Trimesters					
First	0.033**[b]	0.005*	0.046	0.030	0.120
Second	0.020	0.004	0.048	0.030	0.106
Third	0.017	0.003	0.045	0.029	0.099
Job status					
Have a job	0.020	0.004	0.048*	0.030*	0.106*
No job	0.018	0.003	0.035	0.022	0.082

[a] Total PAHs refers to the sum of four PAHs

[b] Non-parametric test

* $p < 0.05$

** $p < 0.01$

levels of OH-PAHs for working women may be partly attributed to environmental tobacco smoke or traffic-related PAHs (Aquilina et al. 2010; Dunbar et al. 2001; WHO guidelines for indoor air quality: selected pollutants 2010). For example, a national report on urbanizing China in 2010 showed that the average time for working people to commute to workplaces was approximately 46 min per day in Guangdong Province, which ranked no. 5 in China (Zhu 2009). As a result, working women were subject to higher traffic-related PAHs in heavy traffic time, which housewives try to avoid generally. Besides, although smoking is prohibited in most workplaces, a survey on passive smoking in public working places in Shanghai, another megacity in China, indicated that more than half (55%) of the employees surveyed were passively exposed to tobacco smokes in working environments, such as restaurants, hotels, shopping malls, hospitals, university campus, and daycare centers (Li 2010). Both environmental tobacco smoke and traffic-related PAHs are important sources of PAH exposure (Aquilina et al. 2010; Dunbar et al. 2001; WHO guidelines for indoor air quality: selected pollutants 2010). It was reported that motor vehicle emissions contributed 46% of the masses of individual PAHs in ambient airborne particles in the U.S. and environmental tobacco smoke was a significant source of inhalation exposure to high molecular weight PAHs (Aquilina et al. 2010; Dunbar et al. 2001; WHO guidelines for indoor air quality: selected pollutants 2010).

Exposure to PAHs during pregnancy

With the urinary concentration data of OH-PAHs, the daily intake (DI) of PAHs (μg/kg_bw/day) through diet can be estimated by the following equation (Guo et al. 2013, 2014; Li et al. 2012),

$$\mathrm{DI} = \frac{C \times F\mathrm{cre}}{BW \times f \times 1000} \times \frac{MW\mathrm{p}}{MW\mathrm{m}}$$

where C is the urinary concentration of OH-PAHs (ng/g creatinine), F_{cre} is the creatinine excretion rate (g/day), BW is the body weight (kg), f is the ratio of OH-PAHs excreted in urine relative to the total exposure dose, and MW_p and MW_m are the molecular weights of parent PAHs and their metabolites (g/mol), respectively. The value of F_{cre} was set as 1.0 g/day and f was assigned to 100%, 6.8%, 60%, and 11% for naphthalene, pyrene, fluorene, and phenanthrene, respectively (Li et al. 2012).

The estimated DI of phenanthrene was the highest among all target analytes, with a geometric mean of 0.047 μg/kg_bw/day, followed by those of pyrene (0.030 μg/kg_bw/day), naphthalene (0.021 μg/kg_bw/day), and fluorene (0.004 μg/kg_bw/day) (Table 3). Generally, exposure to PAHs declined from the first trimester to the third trimester, and working women were subject to higher exposure than housewives. Specifically, pregnant women in the earlier trimesters were exposed to significantly higher doses of naphthalene and fluorene than those in later trimesters. Working pregnant women were also exposed to significantly higher doses of phenanthrene, pyrene, and total PAHs than pregnant housewives. These results were consistent with the concentrations of PAH metabolites in urine samples as discussed above.

The reference doses of naphthalene, pyrene, and fluorene derived from chronic oral exposure data by the U.S. Environmental Protection Agency were 20, 30, and 40 μg/kg_bw/day, respectively. Apparently, all exposure doses for pregnant women in the present study were far lower than these values, indicating low health risk from dietary exposure to PAHs.

Association of 8-OHdG with PAH exposure

Urinary concentrations of 8-OHdG in pregnant women in the present study ranged from 4.67 to 49.4 μg/g, significantly higher than those in children and college students from the same study area (Fig. 2) (Fan et al. 2012; Li et al. 2015a, b,

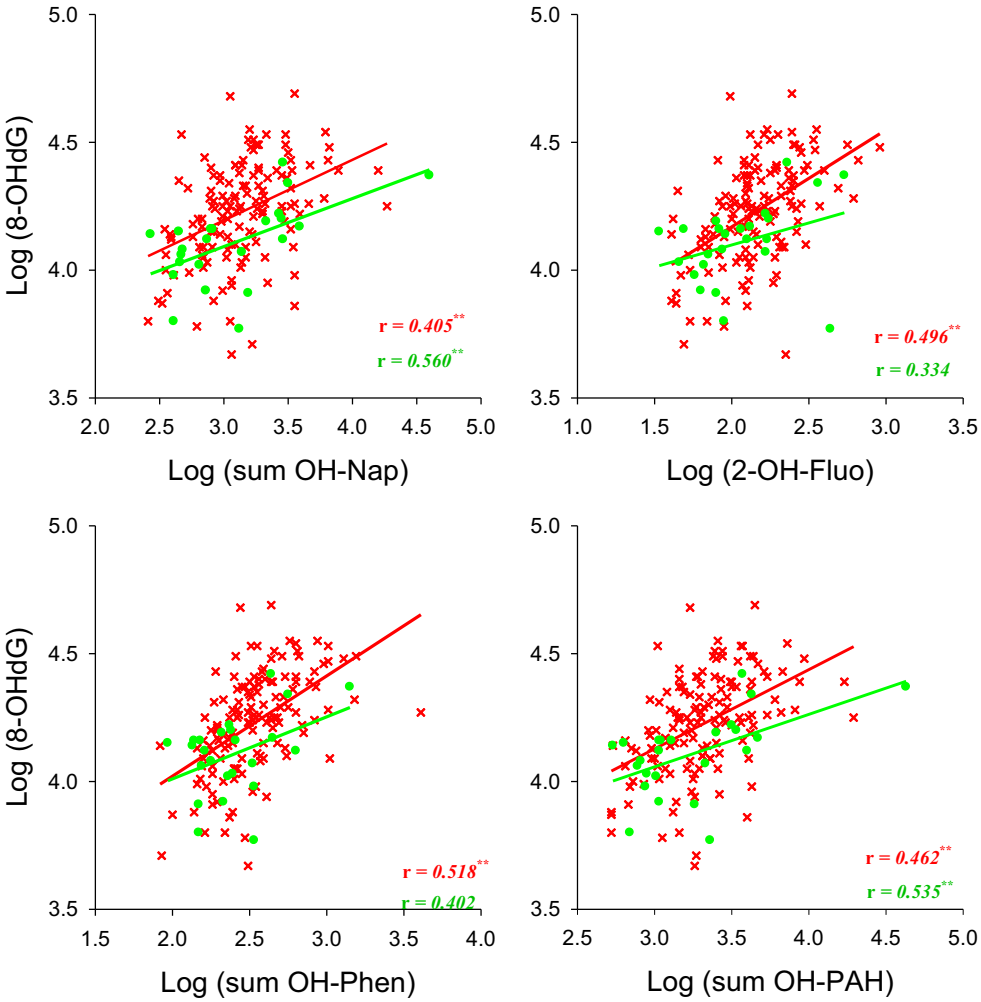

Fig. 3 Pearson correlations of urinary ∑OH-PAHs concentrations with urinary 8-OHdG concentrations in pregnant women. Samples are divided into working women and housewives. Log-transformed (common) concentrations were used for analysis. Red color represents working women while green represents housewives ("*r*" equals to correlation coefficient)

c). This was consistent with previously reported results that increased oxidative stress during pregnancy may associate with placental DNA (Hung et al. 2010; Myatt and Cui 2004). Maternal oxidative damage in pregnancy was suggested to associate with small baby birth size (Min et al. 2009), and Hsieh et al. (2012) set the threshold value as 74.5 μg/g for 8-OHdG. All concentrations of 8-OHdG in pregnant women did not exceed this value in the present study.

The concentrations of 8-OHdG were not significantly different among groups of different ages, heights, weights, trimesters, and times of pregnancy, except for groups between working women and housewives (Table 1). Similarly, Chiba et al. (2010) also reported that levels of 8-OHdG in umbilical cord blood were not influenced by maternal factors, such as maternal age, number of pregnancies and live births, and weight increase. As concentrations of 8-OHdG were heavily affected by job status in the present study, pregnant women were divided into working women and housewives to further explore the relationship between oxidative stress and PAH exposures (Fig. 3). Since PAH exposure may trigger oxidative stress, it was reasonable to obtain positive relationships (r = 0.3–0.6) between levels of 8-OHdG and those of $\sum_2$OH-Nap, 2-OH-Flou, $\sum_3$OH-Phen, and $\sum_7$OH-PAH for both groups (Fig. 3). In fact, the concentrations of 8-OHdG and several individual PAH metabolite, such as 1-OH-Nap, 2-OH-Nap, 1-OH-Phen, 2-OH-Phen, 3-OH-Phen, and 1-OH-Pyr, were also positively correlated (SI Fig. S1).

For working women, 8-OHdG was significantly ($p < 0.01$) correlated with each individual PAH metabolite and $\sum_2$OH-Nap, $\sum_3$OH-Phen, and $\sum_7$OH-PAH. On the other hand, 8-OHdG was not significantly ($p > 0.05$) associated with 2-OH-Flou, 2-OH-Phen, 3-OH-Phen, and 1-OH-Pyr for housewives. These results indicated that PAH exposure was associated with oxidative damage in pregnant women, particularly if pregnant women had jobs. In addition, the concentrations of 8-OHdG were higher in working women than in housewives when exposed to the same level of PAHs (Fig. 3), indicating more oxidative damage suffered by working women. One of the potential factors may be work-related stress. Two recent studies on manufactory workers in Japan found that work-

related stress was significantly and positively related to urinary concentrations of 8-OHdG, particularly in female workers (Inoue et al. 2009; Irie et al. 2001).

Previous studies indicated that exposure to PAHs during pregnancy may pose potential adverse health effects on both pregnant women and their babies (Choi et al. 2006, 2008; Dejmek et al. 2000; Suzuki et al. 2010) and would also increase oxidative stress (Han et al. 2011; Kim et al. 2011; Nethery et al. 2012). Chen et al. (2000) analyzed chemical exposure for 792 pregnant workers in a petrochemical industry in Beijing and discovered that the combination of low-level benzene exposure and occupational stress was associated with low birth weights. However, potential health risk due to chemical exposure and work stress combined has remained largely unknown (Grajewski et al. 2016). Our study in some degree suggested increased oxidative damage when work stress was combined with PAH exposure. The results from the present study also suggested the urgency to evaluate the health risk of pregnant women subjected to potential chemical exposure.

Conclusion

Concentrations of urinary PAH metabolites in pregnant women were lower than general population in the same research area, which might be attributed to relative low dietary or inhalation exposure. Several levels of OH-PAHs changed during pregnancy, which may partly result from the different metabolism abilities across trimesters. Significantly positive relationships were observed between levels of OH-PAHs and 8-OHdG, especially for working women. In addition to PAH exposure, working pressure may also have relation with oxidative stress. Our results from this pilot biomonitoring study indicated that a further research on lifestyles and PAHs in special dietary for pregnant women during pregnancy is needed.

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Compliance with ethical standards

The present study was approved by the ethics committee of Jinan University.

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