



# Alterations in platelet indices link polycyclic aromatic hydrocarbons toxicity to low-grade inflammation in preschool children



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## ABSTRACT

**Background:** Environmental exposure to carcinogenic polycyclic aromatic hydrocarbons (PAHs) can disturb the immune response. However, the effect of PAHs on low-grade inflammation related to platelets in humans is unknown.

**Objectives:** We investigated the association of PAH exposure with low-grade inflammation and platelet parameters in healthy preschoolers.

**Methods:** The present study recruited 239 participants, aged 2–7 years, from an electronic-waste (e-waste)-exposed ( $n = 118$ ) and a reference ( $n = 121$ ) area. We measured ten urinary PAH metabolites, four types of immune cells and cytokines, and seven platelet parameters, and compared their differences between children from the two groups. Spearman correlation analysis was performed to explore the potential risk factors for PAH exposure and the associations between urinary monohydroxylated PAHs (OH-PAHs) and biological parameters. Associations between urinary PAH metabolites and platelet indices were analyzed using quantile regression models. Mediation analysis was used to understand the relationship between urinary total hydroxynaphthalene ( $\Sigma$ OHNa) and interleukin (IL)-1 $\beta$  through seven platelet indices, as mediator variables.

**Results:** We found higher urinary monohydroxylated PAH (OH-PAH) concentrations, especially 1-hydroxynaphthalene (1-OHNa) and 2-hydroxynaphthalene (2-OHNa), in children from the e-waste-exposed group than in the reference group. These were closely associated with child personal habits and family environment. A decreased lymphocyte ratio and increased pro-inflammatory cytokines, such as gamma interferon-inducible protein (IP)-10 and IL-1 $\beta$ , were found in the e-waste-exposed children. After adjustment for confounding factors, significantly negative correlations were found between levels of mean platelet volume (MPV), platelet distribution width (PDW), platelet-large cell ratio (P-LCR) and ratio of mean platelet volume to platelet count (MPVP) and OH-PAHs. In addition,  $\Sigma$ OHNa was positively associated with IL-1 $\beta$  mediated through MPV, PDW, P-LCR, and ratio of platelet count to lymphocyte count (PLR).

**Conclusions:** Platelet indices were significantly associated with the changes in urinary OH-PAH levels, which may be regarded as effective biomarkers of low-grade inflammation resulting from low PAH exposure in healthy children.

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants consisting of two or more fused aromatic rings with different structural arrangements. PAHs possess mutagenic and carcinogenic properties that have potential developmental and reproductive toxicity,

and neurotoxic, immunotoxic, and cytotoxic effects (Huo et al., 2019b; Luderer et al., 2017; Oliveira et al., 2019; Perera et al., 2018; Yao et al., 2019). Because of their volatility, PAHs can be diffused far from their original source and accumulate in a variety of environmental matrices, such as air, water, soil, dust, sediment and food (Gao et al., 2018). Human exposure to PAHs occurs through three key absorption

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pathways, namely ingestion, inhalation, and dermal contact (Ma and Harrad, 2015; Ruby et al., 2016). It is related to various adverse health effects, including poor fetal development (Huo et al., 2019b), oxidative stress (Lu et al., 2016), cardiovascular disease (Alshaarawy et al., 2016), inflammation (Ferguson et al., 2017), obesity (Poursafa et al., 2018) and diabetes (Yang et al., 2017). During early childhood, children experience very rapid growth and development in which complex processes, including the immature immune system, are specifically sensitive to environmental chemicals and easily disrupted by potential toxic exposure (Perloth and Castelo Branco, 2017).

Human nucleate discoid platelets average 0.5  $\mu\text{m}$  in thickness and 1.5 to 3.0  $\mu\text{m}$  in diameter. They normally circulate in peripheral blood at a concentration of  $150\text{--}400 \times 10^9$  platelets/L with a cycle time of 7 to 10 days, after which they are either used up in hemostasis or undergo programmed cell death (Mason et al., 2007; Vélez and García, 2015). Platelets play a dominant role in the regulation of thrombosis and hemostasis. There is also emerging evidence indicating that platelets intervene in tissue regeneration and angiogenesis, atherosclerosis, tumor metastasis, growth, and inflammation, and participate in innate and adaptive immune responses, as well as controlling lymphatic vessel development (Engelmann and Massberg, 2013; Franco et al., 2015; Gimbrone Jr. et al., 1969; Ho-Tin-Noe et al., 2011; Nurden, 2018; Rondina et al., 2013; Welsh et al., 2016; Xu et al., 2016). Platelets can express or release a range of immunomodulatory molecules (CD40L, TLRs, and P-selectin) and cytokines [interleukin (IL)-1 $\beta$  and transforming growth factor (TGF)- $\beta$ ], which can serve as immune effectors during the immune response (Herter et al., 2014; Xu et al., 2016). Also, it has been clinically observed that platelets play a regulative role in the acute inflammatory phase (Gawaz et al., 1995), and they have been recognized as crucial mediators of inflammation in various diseases, including myocardial infarction (Xu et al., 2006), vascular injury (Wang et al., 2005), dermal inflammation (Katoh, 2009) and acute kidney injury (Singbartl et al., 2001).

Two epidemiology studies reveal that there exists a dose-response association between PAH exposure and platelet indices, of which mean platelet volume (MPV) partially mediates the increased risk of atherosclerosis due to PAH exposure in adults (Hu et al., 2018; Yuan et al., 2019). In our previous studies, we noticed that electronic-waste (e-waste)-exposed children displayed altered innate and adaptive immune responses, including decreased natural killer (NK) cells, and increased erythrocyte adherence molecules and T cells (Dai et al., 2017; Huo et al., 2019a; Zhang et al., 2016; Zhang et al. 2017; Zheng et al., 2019). However, to our current knowledge, no human study has investigated the adverse effects of PAHs on platelets and low-grade inflammation in preschoolers, especially those from e-waste recycling area. Against this background, the current study aims to examine possible associations between urinary PAH metabolites, low-grade inflammation and alterations of platelet indices in preschoolers.

## 2. Materials and methods

### 2.1. Study population and sample collection

In the current study, a total of 239 participants, aged 2–7 years, were recruited between November and December 2015 from an e-waste-exposed area ( $n = 118$ ) and a reference ( $n = 121$ ) area. The e-waste exposed area, Guiyu, is an e-waste recycling town in Guangdong province located in south China, which has long history of e-waste dismantling and recycling (Huo et al., 2007). We chose Haojiang as the reference area because it is located approximately only 31.6 km from Guiyu, and is similar to Guiyu in terms of population, cultural background, residential lifestyle and socioeconomic status, but has no informal e-waste recycling sites. The children's parents or guardians completed a questionnaire to obtain detailed information regarding the child's demographic characteristics, behavior and dietary habits, parental socioeconomic status and education level, family medical and

health history, and dwelling environment. Written informed consent was obtained from the parents or guardians of each child before participation in the study. The study was approved by the Human Ethics Committee of Shantou University Medical College (SUMC2013XM-0076).

### 2.2. Sample collection

All participants took part in a basic physical examination, and their biological samples were collected on the same day. A 15 mL urine were collected into a polypropylene conical centrifuge tube from children after getting up in the morning. At an early fasting state between 8:00–9:00 am, a 4 mL venous blood of each child was drawn by professional nurse and collected into two vacuum blood tube either containing EDTA-K<sup>2</sup> as an anticoagulant or without anticoagulant. All blood and urine samples were placed on ice and transported to the laboratory. The blood samples in the EDTA-K<sup>2</sup> tube were used for routine blood examination. The blood sample without anticoagulant was centrifuged at 855g for 15 min to separate the serum, then stored at  $-80\text{ }^\circ\text{C}$  until analysis for cytokines. Urine samples were preserved in  $-20\text{ }^\circ\text{C}$  until PAH metabolites measurement.

### 2.3. Urinary PAH metabolite measurements

Urinary monohydroxylated PAH (OH-PAH) concentrations are considered as representative of internal PAH levels, and are commonly used for evaluating recent human exposure to PAHs (Li et al., 2008). Ten OH-PAHs were measured: 1-hydroxynaphthalene (1-OHNa), 2-hydroxynaphthalene (2-OHNa), 1-hydroxyphenanthrene (1-OHPh), 2-hydroxyphenanthrene (2-OHPh), 3-hydroxynaphthalene (3-OHPh), 4-hydroxyphenanthrene (4-OHPh), 9-hydroxyphenanthrene (9-OHPh), 2-hydroxyfluorene (2-OHFlu), 9-hydroxyfluorene (9-OHFlu) and 1-hydroxypyrene (1-OHP). The samples were analyzed by an Agilent 7890A gas chromatography and an Agilent 5975C mass spectrometer (Agilent Technologies Inc., USA). The detailed procedures used for analysis have been described in our previously published papers (Huo et al., 2019b; Zheng et al., 2019). All regression coefficients ( $R^2$ ) of standard curves were above 0.995. The percent relative standard deviation (%RSD) of quality control samples was 1.5–14.5%, and the recovery of all analytes was 80.0–125.0%. The concentration of urinary creatinine (Cr) was determined by the Cayman Chemical Creatinine Assay (Cayman Chemical Company, UK) based on Jaffe's reaction. Finally, OH-PAH concentrations were expressed as  $\mu\text{g}/\text{mmol Cr}$ .

### 2.4. Hematologic parameter measurements

The parameters of routine blood indices, including leukocyte count, neutrophilic-granulocyte ratio, lymphocyte ratio, monocyte ratio, MPV, platelet count, platelet distribution width (PDW), platelet-large cell ratio (P-LCR), and thrombocytocrit (PCT) were tested by a Sysmex XT-1800i automated hematology analyzer (Sysmex Corporation, Kobe, Japan). Each sample was measured within 8 h of blood collection, and calibration standards and quality controls were obtained from the manufacturer. The ratios of mean platelet volume to platelet count (MPVP) and platelet count to lymphocyte count (PLR) were calculated.

### 2.5. Serum cytokine measurements

Pro-inflammatory cytokines, such as gamma interferon-inducible protein (IP)-10 and IL-1 $\beta$ , and pro-angiogenic cytokines, including growth-related oncogene  $\alpha$  (GRO $\alpha$ ) and regulated upon activation, normal T cells expressed and secreted (RANTES), were measured using a ProcartaPlex Human Cytokine & Chemokine Panel 1A (eBioscience, USA) adopting the method by Zhang et al. (2016). Beads coated with anti-human IP-10, IL-1 $\beta$ , GRO $\alpha$  and RANTES were incubated with child serum samples and analyzed according to the manufacturer's

instructions. A Luminex 200 device (Luminex, USA) was used for data acquisition.

## 2.6. Statistical analysis

Normal and non-normal continuous variables were compared using Student's *t*-test and the Mann-Whitney *U* test, and were represented as mean  $\pm$  SD and median (interquartile range, IQR), respectively. Chi-square tests were performed to compare distribution differences of categorical variables. Spearman correlation tests were performed to explore the potential risk factors for PAH exposure and the relationships between urinary OH-PAHs and biological parameters, as well as presented as correlation coefficients ( $r_s$ ) with *P*-values. Variables with skewed distributions were ln-transformed prior to regression and mediation analysis. We divided the urinary OH-PAH levels into four dummy variables in accordance with quartiles, and chose the first quartile (Q1) as the reference variable to weigh the last three quartiles (Q2, Q3, Q4). Quantile regression models were used to evaluate the non-linear relationship between an interquartile range increase in urinary 1-OHNa, 2-OHNa, total hydroxynaphthalene ( $\Sigma$ OHNa) and total hydroxylated PAHs ( $\Sigma$ OH-PAH) levels and alterations of platelet parameters. Regression models were adjusted for confounders including gender, age, body mass index (BMI) and family member smoking, paternal and maternal education levels, and monthly household income. Mediation analysis was performed to investigate whether platelets mediate the association between  $\Sigma$ OHNa exposure and IL-1 $\beta$ . Differences were considered statistically significant at  $P < 0.05$ , using a two-tailed test. Statistical analysis was performed using SPSS version 24.0 (IBM Corp. Armonk, NY, USA), and figures were drawn by using GraphPad Prism software version 7.0 (GraphPad, San Diego, CA) and R project version 3.5.2 for windows (R Foundation for Statistical Computing, Vienna, Austria).

## 3. Results

### 3.1. General characteristics of the participants

Demographic characteristics of the 239 preschool children from the two groups are shown in Table 1. The average child age at enrollment was 4.5 and 4.3 years old in the e-waste-exposed group and reference group, respectively ( $P > 0.05$ ). Among the participants, males and females accounted for 58.5% and 41.5% in the e-waste-exposed group, and 62.8% and 37.2% in the reference group ( $P > 0.05$ ). No statistically significant differences were found when comparing BMI, head circumference, leukocyte count, neutrophilic-granulocyte ratio and monocyte ratio between the two groups (all  $P > 0.05$ ). Compared with those children from the reference area, chest circumference ( $50.3 \pm 3.2$  vs.  $49.5 \pm 2.9$ ,  $P < 0.05$ ) and lymphocyte ratio (median: 49.4% vs. 48.5%,  $P < 0.01$ ) were decreased in the e-waste-exposed

children.

### 3.2. Urinary concentrations of PAH metabolites

Table 2 and Fig. 1 show the distribution of urinary concentrations of OH-PAHs. The sum of 2 + 9-OHFlu was computed instead of the individual compounds during analysis, due to the concentrations of these individual metabolites to low and difficult to separate from other metabolite peaks. Children from the e-waste-exposed group had significantly higher median values of urinary  $\Sigma$ OHNa (median: 1.48  $\mu$ g/mmol Cr vs. 0.75  $\mu$ g/mmol Cr), total hydroxyphenanthrene ( $\Sigma$ OHPH) (median: 0.94  $\mu$ g/mmol Cr vs. 0.62  $\mu$ g/mmol Cr), and  $\Sigma$ OH-PAHs (median: 3.05  $\mu$ g/mmol Cr vs. 1.76  $\mu$ g/mmol Cr) compared to children from the reference group (all  $P < 0.001$ ). We also observed that urinary levels of 2 + 9-OHFlu (median: 0.14  $\mu$ g/mmol Cr vs. 0.09  $\mu$ g/mmol Cr) and 1-OHP (median: 0.19  $\mu$ g/mmol Cr vs. 0.14  $\mu$ g/mmol Cr) were significantly increased in the e-waste-exposed children, compared with the reference children (all  $P < 0.01$ ). However, there were no significant differences in 3-OHPH and 9-OHPH between the two groups (all  $P > 0.05$ ). As presented in Fig. 1, in the e-waste-exposed children, urinary 1-OHNa and 2-OHNa comprised the highest proportion among all OH-PAHs, accounting for 24.4% and 35.6% respectively, and urinary 9-OHPH and 2 + 9-OHFlu comprised the lowest proportion among all PAH metabolites, respectively accounting for 2.4% and 4.1%. A similar pattern was found in the reference children, with 1-OHNa, 2-OHNa, 9-OHPH, and 2 + 9-OHFlu accounting for 17.8%, 28.5%, 3.8%, and 5.5%, respectively.

Spearman rank correlation was employed to test the relationship of these PAH metabolites in all preschool children. All PAH metabolites were more closely and positively correlated with each other. Their correlation coefficients ranged from 0.375 (1-OHNa and 3-OHPH) to 0.794 (4-OHPH and 1-OHP, 4-OHPH and 9-OHPH) (all  $P < 0.001$ ) (Fig. 2).

### 3.3. Potential factors in relation to PAH exposure

In order to explore the potential factors affecting PAH exposure, the Spearman correlation coefficient matrix was calculated (Fig. 3). We found significantly negative correlations ( $P < 0.05$ ) between yearly milk product consumption and levels of 1-OHNa, 2-OHNa, and 4-OHPH, yearly iron-rich product consumption and levels of 1-OHNa and 2-OHNa, distance of residence from road and levels of 2-OHPH, 4-OHPH and 2 + 9-OHFlu, paternal education level and levels of 1-OHNa, 2-OHNa, 2-OHPH, 4-OHPH and 1-OHP, maternal education level and levels of 1-OHNa, 2-OHNa, 1-OHPH, 2-OHPH, 4-OHPH, 9-OHPH, 2 + 9-OHFlu, and 1-OHP. In addition, significantly positive correlations ( $P < 0.05$ ) were found between the frequency of child contact with e-waste and levels of 1-OHNa, 2-OHNa, 2-OHPH, 3-OHPH, 4-OHPH, 9-OHPH, 2 + 9-OHFlu and 1-OHP, family member smoking and levels of

**Table 1**  
Demographic characteristics of preschool children from reference and exposed groups.

Variables	Reference group (n = 121)	Exposed group (n = 118)	Statistics	P-value
Gender (male/female, n, %)	76/45 (62.8, 37.2)	69/49 (58.5, 41.5)	$\chi^2 = 0.471$	0.493 <sup>a</sup>
Age (year, mean $\pm$ SD)	4.3 $\pm$ 1.1	4.5 $\pm$ 0.9	$t = 1.847$	0.066 <sup>b</sup>
BMI (kg/m <sup>2</sup> , mean $\pm$ SD)	15.1 $\pm$ 1.6	14.9 $\pm$ 1.4	$t = -0.202$	0.287 <sup>b</sup>
Head circumference (cm, mean $\pm$ SD)	49.9 $\pm$ 1.5	49.9 $\pm$ 1.7	$t = 0.318$	0.751 <sup>b</sup>
Chest circumference (cm, mean $\pm$ SD)	50.3 $\pm$ 3.2	49.5 $\pm$ 2.9	$t = -2.093$	0.037 <sup>b</sup>
Leukocyte count ( $\times 10^9/L$ , median, IQR)	5.04 (7.28, 9.77)	8.17 (7.12, 9.65)	$Z = -0.152$	0.880 <sup>c</sup>
Neutrophil-granulocyte ratio (% , median, IQR)	41.2 (36.4, 47.7)	41.5 (35.4, 50.2)	$Z = -0.112$	0.837 <sup>c</sup>
Lymphocyte ratio (% , median, IQR)	49.4 (42.5, 54.7)	48.5 (39.8, 54.9)	$Z = -2.630$	0.009 <sup>c</sup>
Monocyte ratio (% , median, IQR)	5.6 (4.8, 6.4)	6.0 (5.1, 7.2)	$Z = -0.851$	0.395 <sup>c</sup>

SD, standard deviation; IQR, interquartile range; BMI, body mass index.

<sup>a</sup> Chi-square test was applied to compare categorical variables between the two groups.

<sup>b</sup> Independent *t*-test was used to compare the quantitative variables with normal distributions between the two groups.

<sup>c</sup> Mann-Whitney *U* test was conducted to compare variables in skewed distributions between the two groups.

**Table 2**  
Comparison of urinary OH-PAHs (µg/mmol Cr) in preschool children.

Metabolite (µg/mmol Cr)	Reference group (n = 121)			Exposed group (n = 118)			P-value
	Selected percentiles			Selected percentiles			
	25th	50th	75th	25th	50th	75th	
1-OHNa	0.14	0.27	0.49	0.29	0.55	1.09	< 0.001
2-OHNa	0.22	0.45	0.84	0.44	0.82	1.75	< 0.001
1-OHP	0.12	0.21	0.32	0.15	0.29	0.50	0.005
2-OHP	0.06	0.10	0.16	0.12	0.23	0.41	< 0.001
3-OHP	0.09	0.15	0.27	0.11	0.17	0.29	0.359
4-OHP	0.05	0.07	0.13	0.08	0.13	0.24	< 0.001
9-OHP	0.04	0.06	0.11	0.05	0.07	0.11	0.170
2 + 9-OHFlu	0.05	0.09	0.16	0.07	0.14	0.22	0.001
1-OHP	0.09	0.14	0.24	0.13	0.19	0.28	0.004
ΣOHNa	0.40	0.75	1.31	0.85	1.48	2.87	< 0.001
ΣOHP	0.39	0.62	1.03	0.60	0.94	1.58	< 0.001
ΣOH-PAHs	1.08	1.76	2.82	1.91	3.05	4.91	< 0.001

1-OHNa, 1-hydroxynaphthalene; 2-OHNa, 2-hydroxynaphthalene; 1-OHP, 1-hydroxyphenanthrene; 2-OHP, 2-hydroxyphenanthrene; 3-OHP, 3-hydroxynaphthalene; 4-OHP, 4-hydroxyphenanthrene; 9-OHP, 9-hydroxyphenanthrene; 2 + 9-OHFlu, 2 + 9-hydroxyfluorene; 1-OHP, 1-hydroxypyrene; ΣOHNa, the sum of 1-OHNa and 2-OHNa; ΣOHP, the sum of 1-OHP, 2-OHP, 3-OHP, 4-OHP and 9-OHP; ΣOH-PAHs, the sum of urinary monohydroxylated PAH metabolite concentrations; Cr, creatinine.



**Fig. 1.** The contribution of urinary PAH metabolites in preschool children from e-waste-exposed and reference groups (n = 239). 1-OHNa, 1-hydroxynaphthalene; 2-OHNa, 2-hydroxynaphthalene; 1-OHP, 1-hydroxyphenanthrene; 2-OHP, 2-hydroxyphenanthrene; 3-OHP, 3-hydroxynaphthalene; 4-OHP, 4-hydroxyphenanthrene; 9-OHP, 9-hydroxyphenanthrene; 2 + 9-OHFlu, 2 + 9-hydroxyfluorene; 1-OHP, 1-hydroxypyrene.

1-OHNa, 2-OHNa, 2-OHP, 3-OHP, 4-OHP, 2 + 9-OHFlu and 1-OHP, use of residence as a workplace and levels of 1-OHNa, 2-OHNa, 2-OHP, 4-OHP and 2 + 9-OHFlu, ventilation of house and levels of 1-OHNa and 2-OHP, e-waste contamination within 50 m of residence and levels of 1-OHNa, 2-OHNa, 1-OHP, 2-OHP and 4-OHP. However, no significant correlations (P > 0.05) were found between levels of PAH metabolites and yearly soy product consumption and monthly household income.

**3.4. Comparison of platelet parameters and serum cytokines**

Table 3 shows the distribution of platelet parameters in preschool children. E-waste-exposed children had higher median values of platelet count (330 × 10<sup>9</sup>/L vs. 284 × 10<sup>9</sup>/L), PCT (0.34 vs. 0.29), and PLR (85.7 vs. 71.9) than reference children (all P < 0.001). Preschoolers had a lower median value of MPVP in the e-waste-exposed group than those from the reference group (0.03 vs. 0.37, P < 0.001). The levels of MPV, PDW and P-LCR were not significantly different between the two groups (all P > 0.05).

Table 4 presents the median and interquartile range of serum pro-inflammatory and pro-angiogenic cytokine levels in preschool children. The median concentrations of IL-1β and IP-10 in the e-waste-exposed children were 0.43 pg/mL and 28.5 pg/mL, respectively, which were significantly higher than in the reference children (median: 0.25 pg/mL

and 25.5 pg/mL) (all P < 0.01). No significant differences were found in the pro-angiogenic cytokines, including RANTES and GROα, between the two groups (all P > 0.05).

**3.5. Relationship between platelet parameters and serum cytokines**

As shown in Fig. 2, we observed significantly negative relationships (P < 0.05) between concentrations of IL-1β and IP10 and three platelet parameters including MPV, PDW and P-LCR. Additionally, significantly positive relationships (P < 0.05) were observed between concentrations of GROα and another three platelet indices including platelet count, PCT and PLR. However, significantly negative relationship (P < 0.05) was found between GROα and MPVP. No significant relationships were observed between RANTES and platelet parameters (all P > 0.05).

**3.6. Relationship between urinary OH-PAHs and platelet parameters**

As presented in Fig. 2, we found significantly positive correlations (P < 0.05) between levels of platelet count and 1-OHNa, 2-OHNa, ΣOHNa and ΣOH-PAHs, and levels of PCT and 1-OHNa, 2-OHNa and ΣOHNa. In addition, significantly negative correlations (P < 0.05) were found between levels of MPV, PDW, P-LCR and MPVP and four OH-PAHs including 1-OHNa, 2-OHNa, ΣOHNa and ΣOH-PAHs.

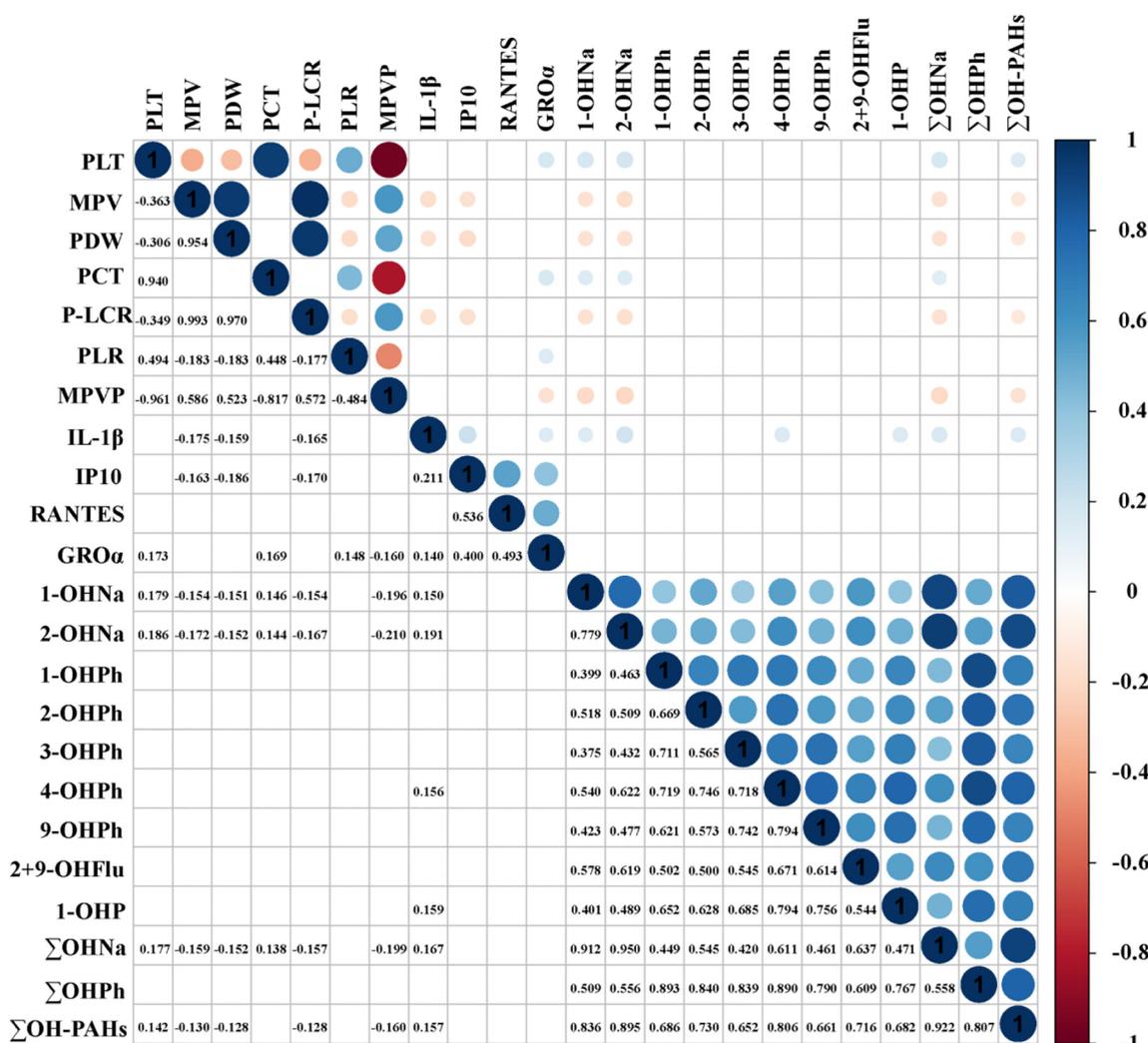


Fig. 2. Spearman correlation coefficient between urinary OH-PAHs and platelet indices and cytokines. 1-OHNa, 1-hydroxynaphthalene; 2-OHNa, 2-hydroxynaphthalene; 1-OHPh, 1-hydroxyphenanthrene; 2-OHPh, 2-hydroxyphenanthrene; 3-OHPh, 3-hydroxynaphthalene; 4-OHPh, 4-hydroxyphenanthrene; 9-OHPh, 9-hydroxyphenanthrene; 2 + 9-OHFlu, 2 + 9-hydroxyfluorene; 1-OHP, 1-hydroxypyrene;  $\Sigma$ OHNa, the sum of 1-OHNa and 2-OHNa;  $\Sigma$ OHPh, the sum of 1-OHPh, 2-OHPh, 3-OHPh, 4-OHPh and 9-OHPh;  $\Sigma$ OH-PAHs, the sum of urinary monohydroxylated PAH metabolite concentrations; MPV, mean platelet volume; PDW, platelet distribution width; PCT, thrombocytocrit; P-LCR, platelet-large cell ratio; PLR, ratio of platelet count to lymphocyte count; MPVP, ratio of mean platelet volume to platelet count; IL, interleukin; IP, gamma interferon-inducible protein; RANTES, regulated upon activation, normal T cells expressed and secreted; GRO, growth-related oncogene. Blue represents positive correlation; red represents negative correlation; a darker color indicates stronger correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Six kinds of platelet indices (platelet count, MPV, PDW, P-LCR, PLR and MPVP) were associated with the 1-OHNa, 2-OHNa and  $\Sigma$ OHNa when they were categorized in quartiles ( $P$  value for the trends were all  $< 0.05$ ). Compared with Q1, 1-OHNa in Q2 and Q4, and 2-OHNa in Q2, Q3 and Q4 were positively associated with platelet counts (all  $P < 0.05$ ), 1-OHNa and 2-OHNa in Q2 and Q3 were positively associated with PLR (all  $P < 0.05$ ). In addition, compared with Q1, 1-OHNa in Q2 and Q4, and 2-OHNa in Q3 and Q4 were inversely associated with MPV (all  $P < 0.05$ ), 1-OHNa in Q2 and Q4, and 2-OHNa in Q3 and Q4 were inversely associated with PDW (all  $P < 0.05$ ), 1-OHNa in Q4, and 2-OHNa in Q3 and Q4 were inversely associated with P-LCR (all  $P < 0.05$ ), 1-OHNa in Q2 and Q4, and 2-OHNa in Q2, Q3 and Q4 were inversely associated with MPVP (all  $P < 0.05$ ). The  $P$  value for the trends in the PCT quartile regression models was not significant. The results of unadjusted model are not shown.

### 3.7. Mediation of platelets in the relationship between $\Sigma$ OHNa and IL-1 $\beta$

Mediation analyses were used to understand the relationships

between urinary  $\Sigma$ OHNa and IL-1 $\beta$  through seven platelet indices, as mediator variables (Table 5). After adjusting for age, gender and BMI,  $\Sigma$ OHNa was positively associated with IL-1 $\beta$  mediated through MPV ( $\beta = 0.024$ , 95% CI: 0.003, 0.064), PDW ( $\beta = 0.026$ , 95% CI: 0.004, 0.069), P-LCR ( $\beta = 0.020$ , 95% CI: 0.002, 0.057), and PLR ( $\beta = 0.017$ , 95% CI: 0.000, 0.052), respectively. The estimated percentages mediated for the above mediators were 17.6%, 18.9%, 14.5%, and 12.2%, respectively.

## 4. Discussion

Previous studies have examined the effects of environmental PAH exposure on the immune response, cardiovascular diseases and platelet indices in animal models or adult populations (Chowdhury et al., 2017; Hu et al., 2018; Yuan et al., 2019). However, no studies are available on the relationship of PAH exposure and low-grade inflammation, as well as platelet alterations, in healthy preschoolers. In this study, we found pro-inflammatory cytokine (i.e. IL-1 $\beta$ ) was increased in the e-waste-exposed children and was positively associated with urinary PAH

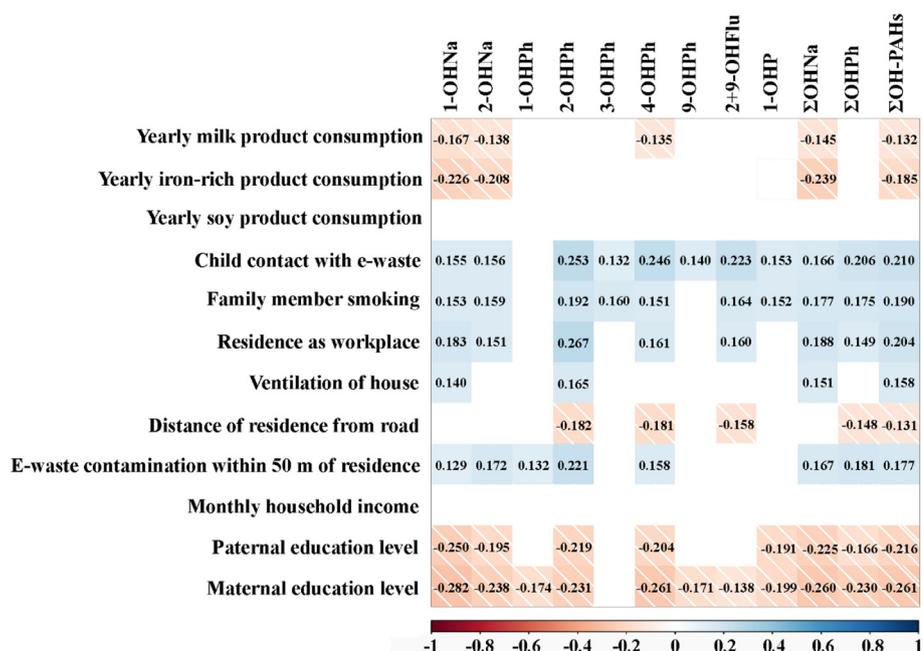


Fig. 3. Potential influencing factors related to child body burden of PAH exposure. 1-OHNa, 1-hydroxynaphthalene; 2-OHNa, 2-hydroxynaphthalene; 1-OHPH, 1-hydroxyphenanthrene; 2-OHPH, 2-hydroxyphenanthrene; 3-OHPH, 3-hydroxynaphthalene; 4-OHPH, 4-hydroxyphenanthrene; 9-OHPH, 9-hydroxyphenanthrene; 2 + 9-OHFu, 2 + 9-hydroxyfluorene; 1-OHP, 1-hydroxypyrene; ΣOHNa, the sum of 1-OHNa and 2-OHNa; ΣOHPH, the sum of 1-OHPH, 2-OHPH, 3-OHPH, 4-OHPH and 9-OHPH; ΣOH-PAHs, the sum of urinary monohydroxylated PAH metabolite concentrations. Blue represents positive correlation; red represents negative correlation; a darker color indicates stronger correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3  
Comparison of platelet indices in preschool children.

	Reference group (n = 121)	Exposed group (n = 118)	P-value
Platelet count ( $\times 10^9/L$ )	284 (249, 320)	330 (271, 379)	< 0.001
MPV (fl)	10.2 (9.80, 10.9)	10.2 (9.78, 10.7)	0.171
PDW (%)	11.9 (11.1, 13.0)	11.6 (10.9, 12.6)	0.076
PCT	0.29 (0.26, 0.33)	0.34 (0.29, 0.39)	< 0.001
P-LCR (%)	26.2 (22.8, 32.4)	25.8 (21.8, 30.4)	0.186
PLR	71.9 (60.9, 86.2)	85.7 (69.2, 108.9)	< 0.001
MPVP	0.37 (0.03, 0.04)	0.03 (0.03, 0.04)	< 0.001

MPV, mean platelet volume; PDW, platelet distribution width; PCT, thrombocytocrit; P-LCR, platelet-large cell ratio; PLR, ratio of platelet count to lymphocyte count; MPVP, ratio of mean platelet volume to platelet count. Data are presented as the median and interquartile range.

Table 4  
Comparison of serum cytokine concentrations (pg/mL) in preschool children.

Cytokine (pg/mL)	Reference group	Exposed group	P-value
Pro-inflammatory cytokines			
IL-1β <sup>a</sup>	0.25 (0.25, 0.43)	0.43 (0.25, 0.49)	< 0.001
IP-10 <sup>b</sup>	25.5 (22.9, 29.2)	28.5 (24.5, 33.9)	0.001
Pro-angiogenic cytokines			
RANTES <sup>b</sup>	63.7 (58.8, 72.9)	65.2 (59.5, 72.7)	0.639
GRO <sup>c</sup>	10.9 (8.42, 14.5)	12.0 (8.47, 16.2)	0.151

IL, interleukin; IP, gamma interferon-inducible protein; RANTES, regulated upon activation, normal T cells expressed and secreted; GRO, growth-related oncogene.

Data are presented by the median and interquartile range.

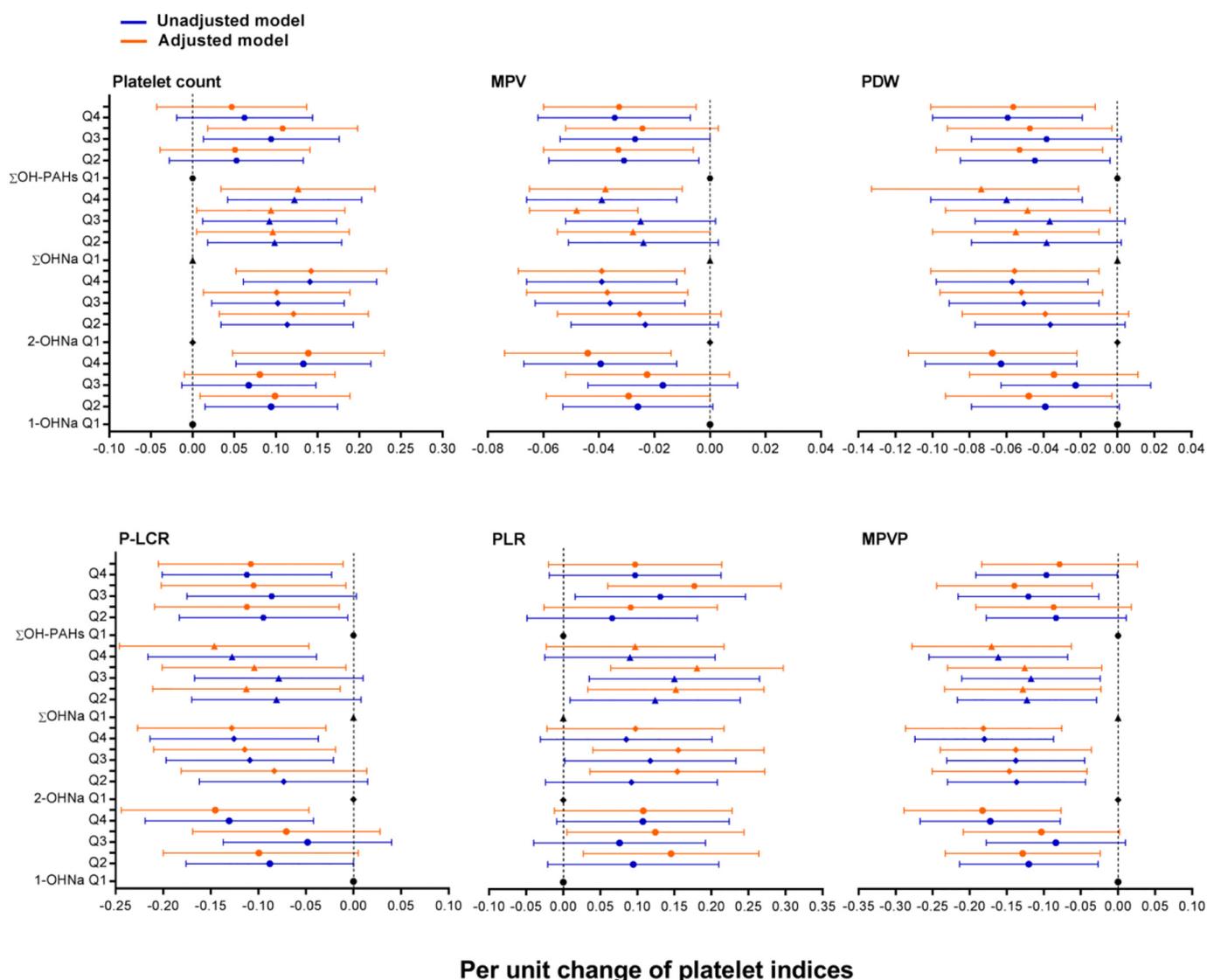
<sup>a</sup> Exposed group, n = 109; reference group, n = 111.

<sup>b</sup> Exposed group, n = 108; reference group, n = 111.

<sup>c</sup> Exposed group, n = 105; reference group, n = 110.

metabolites. We also observed an inverse association between urinary PAH metabolites (1-OHNa and 2-OHNa) and platelet indices (MPV, PDW, P-LCR and MPVP), but positive associations between PAH metabolites (1-OHNa and 2-OHNa) and platelet count and PCT. This study is the first of this kind and we have found that exposure to PAHs at low levels may impact on platelet parameters linked to the low-grade inflammation in preschoolers.

Intensive e-waste recycling activities are one of the most significant sources of PAHs. Studies have shown higher levels of PAHs in the plants, sediment, soil and ash from Guiyu, China due to its long history of unregulated e-waste recycling (Alabi et al., 2012; Gao et al., 2015). Accumulated PAHs in the environment can pose risks to the surrounding environment and humans. Our previous studies found total PAH concentrations of 108 ppb (Guo et al., 2012) in umbilical cord blood, and 68.5 μg/L in preschool child blood (Xu et al., 2015). Urinary ΣOH-PAH concentrations of 6.87 μg/g Cr (Huo et al., 2019b) and 6.32 μg/L (Zheng et al., 2019) have been measured in mothers and preschool children, respectively. We found that urinary PAH metabolite concentrations are higher in children from the e-waste-exposed group than the reference group, which is accordance with our previous research in Guiyu (Zheng et al., 2019). Additionally, results showed that 1-OHNa (0.55 μg/mmol Cr) and 2-OHNa (0.82 μg/mmol Cr) were the most abundant urinary PAH metabolites in preschool children in both groups, indicating this pattern of PAH exposure is comparable that of adults in other regions of China and 3-year-old children in Poland (Sochacka-Tatara et al., 2018; Yuan et al., 2019). Oliveira et al. recognized that indoor air is the major source of naphthalene and acenaphthene in 3- to 5-year-old children, with their urinary 1-OHNa and 1-hydroxyacenaphthene (1-OHAc) levels being the predominant PAH metabolites (Oliveira et al., 2017). For urinary 1-OHP in environmental and occupational PAH exposure assessments (Hansen et al., 2008), we suggest that biomonitoring PAH exposure should also consider 1-OHNa and 2-OHNa levels. Compared to adults, children have a larger lung surface area per kilogram of body weight and are more physically active, so their ventilation rates are usually higher, leading to a relatively greater exposure to environmental pollutants (Heacock et al., 2018). Considering a child's personal habits and family environment, the positive associations we observed between urinary OH-PAHs and e-waste contamination within 50 m of their residence, the frequency of child contact with e-waste, residence as a workplace, family member smoking, and ventilation of their house, indicate that e-waste and smoking are the main source of PAH exposure. Further, we found that elevated consumption of yearly milk and iron-rich products was related to decreased urinary 1-OHNa and 2-OHNa, which suggests consumption of dairy products and iron-rich foods may contribute to PAH metabolism in the body. In addition, parents having a high education level and residence far away from the road belong to the set of protective factors for PAH exposure in preschool children. As a result, staying



**Per unit change of platelet indices**

**Fig. 4.** Effect estimates and 95% confidence interval for quartiles of urinary OHNa and  $\Sigma$ OH-PAHs with platelet indices. Adjusted model adjusted for age, gender, BMI, family member smoking, paternal and maternal education levels, and monthly household income. 1-OHNa, 1-hydroxynaphthalene; 2-OHNa, 2-hydroxynaphthalene;  $\Sigma$ OHNa, the sum of 1-OHNa and 2-OHNa;  $\Sigma$ OH-PAHs, the sum of urinary monohydroxylated PAH metabolite concentrations; MPV, mean platelet volume; PDW, platelet distribution width; P-LCR, platelet-large cell ratio; PLR, ratio of platelet count to lymphocyte count; MPVP, ratio of mean platelet volume to platelet count.

away from e-waste and smoking exposure, increasing nutrition, improving the residential environment and increasing parental environmental awareness will be helpful in minimizing the effect of PAH exposure on preschoolers.

PAHs have the ability to compromise the human immune system. Low-molecular-weight PAHs at environmentally relevant concentrations (nM) can modulate inflammatory cytokine release, resulting in macrophage dysfunction (Wang et al., 2017). Our previous research revealed that e-waste-exposed children had a lower percentage of NK cells and higher counts of monocytes, neutrophils, eosinophils, basophils, CD8<sup>+</sup> and CD4<sup>+</sup> central memory T cells, and relevant inflammatory cytokines levels were also elevated. This suggests children's innate and adaptive immune responses are disrupted with their body tending toward a chronic inflammatory state (Cao et al., 2018; Zhang et al., 2016; Zhang et al., 2017). Our data showed that preschoolers from Guiyu have a decreased lymphocyte ratio and increased concentrations of pro-inflammatory cytokines, including IP10 and IL-1 $\beta$ . IL-1 $\beta$  is related to elevated endothelial permeability, hemostasis dysfunction and thrombosis (Hottz et al., 2013). Chronic low-grade

inflammation is characterized by raised concentrations of inflammatory markers, such as C-reactive protein, IL-1 $\beta$ , or IL-6, in systemic circulation (Beneke et al., 2012). Furthermore, we observed that elevated urinary 1- and 2-OHNa and 4-OHPh were related to increased IL-1 $\beta$  levels, implying that PAH exposure is closely related to child low-grade inflammation. However, another study conducted by Ferguson et al. (2017) observed that urinary 2- and 3-OHPh and 4-OHPh concentrations were negatively associated with IL-1 $\beta$ , IL-10, and TNF- $\alpha$  levels, which reflects an immunosuppressive effect in pregnant women. In our study, no correlations were found between urinary OH-PAHs and IP-10, which is in line with our previous results (Zheng et al., 2019). Moreover, Zheng et al. (2019) observed higher PAH exposure exacerbates vascular endothelial inflammation that may affect the development of the cardiovascular system in children.

Platelets are not only essential effector cells in hemostatic activity, but are also major inflammatory cells with pivotal roles in innate and adaptive immune responses (Semple et al., 2011; Vieira-de-Abreu et al., 2012). MPV, reflecting platelet production rate and stimulation, combined with platelet count, has been regarded as an inflammatory

**Table 5**  
Mediation analysis of platelet indices as mediators between  $\Sigma$ OHNa and IL-1 $\beta$ .

Model <sup>a</sup>	$\Sigma$ OHNa	
	$\beta$ (95% CI)	Proportion of mediation (%) <sup>b</sup>
Platelet count		
Direct effect	0.130 (0.022, 0.239)	–
Indirect effect	0.005 (–0.015, 0.031)	3.8
MPV		
Direct effect	0.112 (0.006, 0.218)	–
Indirect effect	0.024 (0.003, 0.064)	17.6
PDW		
Direct effect	0.110 (0.004, 0.216)	–
Indirect effect	0.026 (0.004, 0.069)	18.9
PCT		
Direct effect	0.141 (0.033, 0.249)	–
Indirect effect	–0.006 (–0.033, 0.009)	–4.1
P-LCR		
Direct effect	0.116 (0.010, 0.223)	–
Indirect effect	0.020 (0.002, 0.057)	14.5
PLR		
Direct effect	0.119 (0.014, 0.225)	–
Indirect effect	0.017 (0.000, 0.052)	12.2
MPVP		
Direct effect	0.121 (0.012, 0.230)	–
Indirect effect	0.014 (–0.007, 0.049)	10.5

IL: interleukin; CI: confidence interval;  $\Sigma$ OHNa, the sum of 1-hydroxynaphthalene and 2-hydroxynaphthalene; MPV, mean platelet volume; PDW, platelet distribution width; PCT, thrombocytocrit; P-LCR, platelet-large cell ratio; PLR, ratio of platelet count to lymphocyte count; MPVP, ratio of mean platelet volume to platelet count.

<sup>a</sup> All models are adjusted for age, gender and BMI; 5000 bootstraps samples,  $n = 220$ .

<sup>b</sup> Proportion of mediation = indirect effect/(direct effect + indirect effect)  $\times$  100.

marker in multiple chronic diseases, such as periodontitis, rheumatoid arthritis, and chronic obstructive pulmonary disease exacerbation (Kisacik et al., 2008; Ulasli et al., 2012; Wang et al., 2015). There is an inverse association between platelet count and MPV, and that the ratio of these two values, MPVP, may be a more meaningful indicator (Bessman et al., 1981; Lozano et al., 1998). Increased platelet count and decreased MPV are associated with low-grade inflammation, and can be considered as reliable inflammatory markers for assessment of disease activity in patients with severe periodontitis and osteoarthritis (Balta et al., 2014; Biricik et al., 2019; Wang et al., 2015; Zareifar et al., 2014). Our data revealed that e-waste-exposed preschoolers had higher platelet counts and lower MPVP than reference children, a result similar to that shown in male Pakistani cooks exposed to combustion emission (Kamal et al., 2016). We also observed that PCT was elevated in the e-waste-exposed children and was positively associated with platelet count in all preschoolers. PLR, which reflects the ratio of platelet count to lymphocyte count, has recently been recognized as a marker of a thrombotic and inflammatory state, and its elevation seems to be an addition to conventional risk factors of atherosclerosis, systemic inflammatory response syndrome and solid tumors (Templeton et al., 2014; Ucar et al., 2016; Yildiz et al., 2015). Usually platelet indices are biomarkers for potential diseases, such as cancer, colonic inflammation and thrombocytosis (Davis et al., 2014; Syed et al., 2007; Yan et al., 2013). Therefore, our finding of significant changes in platelet count, MPVP, PLR and PCT in the e-waste-exposed children indicates that they may be in a state of chronic low-grade inflammation.

Numerous studies have reported that PAH exposure may induce inflammatory responses involving changes in immune cells and relevant cytokines (Brito et al., 2010; Everett et al., 2010; Wang et al., 2017). Nevertheless, we did not find any significant associations between urinary PAH metabolites and levels of neutrophil-granulocyte, lymphocyte and monocyte (data not shown). At present, research on the effect of low PAH exposure on platelet-related inflammation risk in

preschoolers are lacking. In this study, significant associations were observed between increased OH-PAHs and elevated platelet count and PLR, and reduced MPV, PDW, P-LCR and MPVP. There were non-linear positive associations between 1-OHNa or 2-OHNa and platelet count and PLR, and non-linear negative associations between 1-OHNa or 2-OHNa and MPV, PDW, P-LCR and MPVP, suggesting increased thrombocytopenia, altered platelet morphology and elevated inflammation in preschoolers exposed to low levels of PAHs. Platelets have the capacity to synthesize and secrete pro-inflammatory cytokines, such as IL-1 $\beta$ , which can serve as a regulator of platelet number in healthy populations (Beaulieu et al., 2014; Hottz et al., 2013; Lindemann et al., 2001; Tunjungputri et al., 2018). Moreover, we found that  $\Sigma$ OHNa was positively associated with IL-1 $\beta$  mediated through MPV, PDW, P-LCR, and PLR in preschool children. Wang et al. (2017) reported that low-molecular-weight PAH exposure can lead to dysfunction of activated macrophages, such as altered phagocytosis and inhibition of LPS-triggered cytokines. We also found that low-molecular-weight PAH exposure, including 1-OHNa and 2-OHNa, affect the platelet count, activity and morphology, indicating the harm that low-molecular-weight PAHs cause to humans cannot be ignored. Yuan et al. (2019) noted some nonlinear relationships between  $\Sigma$ OHNa or  $\Sigma$ OHPh and MPV;  $\Sigma$ OHNa or  $\Sigma$ OH-PAHs and MPVP;  $\Sigma$ OHFlu, 1-OHP or  $\Sigma$ OH-PAHs and PLR; and  $\Sigma$ OHPh or 1-OHP and PDW in Wuhan adults. These participants' median value of urinary  $\Sigma$ OH-PAHs is 5.92  $\mu$ g/mmol Cr, much higher than in our sample of children. Therefore, even exposure to low levels of PAHs have a significant impact on platelets. In addition, platelets keep vascular integrity during inflammation through a mechanism that distinguishes classical platelet activation (Deppermann and Kubes, 2018). However, we did not find significant differences in levels of pro-angiogenic cytokines (RANTES and GRO $\alpha$ ) between the two groups, and no significant associations were found between PAH exposure and RANTES or GRO $\alpha$ . Given the immature immune system of preschool children, we speculate that even exposure to relatively low concentrations of 1-OHNa and 2-OHNa can markedly affect platelet-associated inflammation. Taking together the findings of this study, we show for the first time that alterations of platelet indices are more sensitive to PAH exposure in preschoolers than other inflammatory cells in peripheral blood, and may be better reflect low-grade inflammation in children. Although alterations of platelets generally do not generate prominent adverse responses in the body, long-term chronic low-grade inflammation and elevated thrombocytopenia may adversely affect the development of preschool children.

This study has the following limitations: First, because of the cross-sectional design of our study, we cannot identify a causal relationship between PAH exposure and platelet indices mediating inflammation. Second, we collected only urine samples at a single time point in the morning to assess one type of pollutant, PAHs, which are correlated with platelet parameters. In future research to confirm our findings, multiple measurements of PAH metabolites are needed to get a more accurate assessment of exposure. Next, there may have been other confounding factors with other pollutants (heavy metals and organic chemicals) and socioeconomic status, which also contributed to the observed changes in the inflammation process. In the regression model, we have adjusted some potential confounding factors as much as possible. Finally, the sample size relatively small, thus a long-term follow-up observation in large sample is necessary.

## 5. Conclusions

Our findings suggest that elevated urinary 1-OHNa and 2-OHNa concentrations are associated with increased IL-1 $\beta$ , platelet count, PCT and PLR, and decreased MPV, PDW, P-LCR and MPVP in preschool children. Moreover,  $\Sigma$ OHNa is positively associated with IL-1 $\beta$  mediated through MPV, PDW, P-LCR, and PLR. Platelet indices are sensitive to the PAH exposure, and may be effective biomarkers of low-grade inflammation due to PAH exposure in healthy children. Further

research is needed to validate the findings, clarify the potential biological mechanism and explore the possible disease risks in adulthood.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2019.105043>.

## Declaration of Competing Interest

The authors declare there have no conflict of interests.

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