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

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**A R T I C L E   I N F O**

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**A B S T R A C T**

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 (Σ2OHNAs) and interleukin (IL)-1β 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-1β, 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 (MPV/P) and OH-PAHs. In addition, ZOHNa was positively associated with IL-1β 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 can 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 (Pourafa 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 (Perrhoth and Castelo Branco, 2017).

Human anucleate discoid platelets average 0.5 μm in thickness and 1.5 to 3.0 μm in diameter. They normally circulate in peripheral blood at a concentration of 150–400 × 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)-1β, IL-6, IL-12, IL-15, TNF-α, IFN-γ, IL-10, and IL-17) and chemokines (MIP-1α, MIP-1β, RANTES, and IP-10) 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 regulatory 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 (Singbaril 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-K² 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-K² 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 °C until analysis for cytokins. Urine samples were preserved in −20 °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-hydroxyphenanthrene (3-OHPH), 4-hydroxyphenanthrene (4-OHPH), 9-hydroxyphenanthrene (9-OHPH), 2-hydroxypyrrole (2-OHPf), 9-hydroxypyrrole (9-OHPf) and 1-hydroxypyrrole (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 (R²) 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 μg/mmol Cr.

2.4. Hematologic parameter measurements

The parameters of routine blood indices, including leucocyte 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 hematometry 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-1β, and pro-angiogenic cytokines, including growth-related oncogene α (GROα) 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-1β, GROα and RANTES were incubated with child serum samples and analyzed according to the manufacturer’s...
instructions. A Lumines 200 device (Lumines, 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 ± 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 in-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 (ΣOHNa) and total hydroxylated PAHs (ΣOH-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 ΣOHNa exposure and IL-1β. 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, neutrophil-granulocyte ratio and monocyte ratio between the two groups \( (P > 0.05) \). Compared with those children from the reference area, chest circumference \( (50.3 ± 3.2 \text{ vs. } 49.5 ± 2.9, P < 0.05) \) and lymphocyte ratio \( (median: 49.4\% \text{ vs. } 48.5\%, P < 0.01) \) were decreased in the e-waste-exposed children.

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 ΣOHNa \( (median: 1.48 \mu g/mmol Cr \text{ vs. } 0.75 \mu g/mmol Cr) \), total hydroxyanthracene (ΣOHAPh) \( (median: 0.94 \mu g/mmol Cr \text{ vs. } 0.62 \mu g/mmol Cr) \), and ΣOH-PAHs \( (median: 3.05 \mu g/mmol Cr \text{ vs. } 1.76 \mu g/mmol Cr) \) compared to children from the reference group \( (all P < 0.001) \). We also observed that urinary levels of 2 + 9-OHFlu \( (median: 0.14 \mu g/mmol Cr \text{ vs. } 0.09 \mu g/mmol Cr) \) and 1-OHP \( (median: 0.19 \mu g/mmol Cr \text{ vs. } 0.14 \mu g/mmol Cr) \) 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-OHP, 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>
<thead>
<tr>
<th>Variables</th>
<th>Reference group (n = 121)</th>
<th>Exposed group (n = 118)</th>
<th>Statistics</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female, n, %)</td>
<td>76/45 (62.8, 37.2)</td>
<td>69/49 (58.5, 41.5)</td>
<td>χ² = 0.471</td>
<td>0.493</td>
</tr>
<tr>
<td>Age (year, mean ± SD)</td>
<td>4.3 ± 1.1</td>
<td>4.5 ± 0.9</td>
<td>t = 1.847</td>
<td>0.066</td>
</tr>
<tr>
<td>BMI (kg/m², mean ± SD)</td>
<td>15.1 ± 1.6</td>
<td>14.9 ± 1.4</td>
<td>t = 2.02</td>
<td>0.049</td>
</tr>
<tr>
<td>Head circumference (cm, mean ± SD)</td>
<td>49.9 ± 1.5</td>
<td>49.9 ± 1.7</td>
<td>r = 0.318</td>
<td>0.751</td>
</tr>
<tr>
<td>Chest circumference (cm, mean ± SD)</td>
<td>50.3 ± 3.2</td>
<td>49.5 ± 2.9</td>
<td>t = 2.093</td>
<td>0.037</td>
</tr>
<tr>
<td>Leukocyte count (×10¹²/L, median, IQR)</td>
<td>5.04 (7.28, 9.77)</td>
<td>8.17 (7.12, 9.65)</td>
<td>Z = -0.152</td>
<td>0.880</td>
</tr>
<tr>
<td>Neutrophil-granulocyte ratio (% median, IQR)</td>
<td>41.2 (36.4, 47.7)</td>
<td>41.5 (35.4, 50.2)</td>
<td>Z = -0.112</td>
<td>0.837</td>
</tr>
<tr>
<td>Lymphocyte ratio (% median, IQR)</td>
<td>49.4 (42.5, 54.7)</td>
<td>48.5 (39.8, 54.9)</td>
<td>Z = -2.630</td>
<td>0.009</td>
</tr>
<tr>
<td>Monocyte ratio (% median, IQR)</td>
<td>5.6 (4.8, 6.4)</td>
<td>6.0 (5.1, 7.2)</td>
<td>Z = -0.851</td>
<td>0.395</td>
</tr>
</tbody>
</table>

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

* Chi-square test was applied to compare categorical variables between the two groups.

† Independent t-test was used to compare the quantitative variables with normal distributions between the two groups.

‡ Mann-Whitney U test was conducted to compare variables in skewed distributions between the two groups.
1-OHNa, 2-OHNa, 2-OHPh, 3-OHPh, 4-OHPh, 2 + 9-OHFlu and 1-OHP, use of residence as a workplace and levels of 1-OHNa, 2-OHNa, 2-
OHPh, 4-OHPh and 2 + 9-OHFlu, ventilation of house and levels of 1-
OHNa and 2-OHPh, e-waste contamination within 50 m of residence
and levels of 1-OHNa, 2-OHNa, 1-OHPh, 2-OHPh and 4-OHPh. How-
ever, 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 × 10^9/L vs. 284 × 10^9/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-
flammatory 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 pla-
ettelet indices including MPV, PDW and P-LCR. Additionally, signi-
nificantly 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 relation-
ship (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.

1-OHNa, 1-hydroxynaphthalene; 2-OHNa, 2-hydroxynaphthalene; 1-OHPh, 1-hydroxyphenanthrene; 2-OHPh, 2-hydroxyphenanthrene; 3-OHPh, 3-hydro-
xynaphthalene; 4-OHPh, 4-hydroxyphenanthrene; 9-OHPh, 9-hydroxyphenanthrene; 2 + 9-OHFlu, 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; Cr, creatinine.

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

<table>
<thead>
<tr>
<th>Metabolite (μg/mmol Cr)</th>
<th>Reference group (n = 121)</th>
<th>Exposed group (n = 118)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Selected percentiles</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25th</td>
<td>50th</td>
<td>75th</td>
</tr>
<tr>
<td>1-OHNa</td>
<td>0.14</td>
<td>0.27</td>
<td>0.49</td>
</tr>
<tr>
<td>2-OHNa</td>
<td>0.22</td>
<td>0.45</td>
<td>0.84</td>
</tr>
<tr>
<td>1-OHPh</td>
<td>0.12</td>
<td>0.21</td>
<td>0.32</td>
</tr>
<tr>
<td>2-OHPh</td>
<td>0.06</td>
<td>0.10</td>
<td>0.16</td>
</tr>
<tr>
<td>3-OHPh</td>
<td>0.09</td>
<td>0.15</td>
<td>0.27</td>
</tr>
<tr>
<td>4-OHPh</td>
<td>0.05</td>
<td>0.07</td>
<td>0.13</td>
</tr>
<tr>
<td>9-OHPh</td>
<td>0.04</td>
<td>0.06</td>
<td>0.11</td>
</tr>
<tr>
<td>2 + 9-OHFlu</td>
<td>0.05</td>
<td>0.09</td>
<td>0.16</td>
</tr>
<tr>
<td>1-OHP</td>
<td>0.09</td>
<td>0.14</td>
<td>0.24</td>
</tr>
<tr>
<td>ΣOHNa</td>
<td>0.40</td>
<td>0.75</td>
<td>1.31</td>
</tr>
<tr>
<td>ΣOHPh</td>
<td>0.39</td>
<td>0.62</td>
<td>1.03</td>
</tr>
<tr>
<td>ΣOH-PAHs</td>
<td>1.08</td>
<td>1.76</td>
<td>2.82</td>
</tr>
</tbody>
</table>

1-OHNa, 1-hydroxynaphthalene; 2-OHNa, 2-hydroxynaphthalene; 1-OHPh, 1-hydroxyphenanthrene; 2-OHPh, 2-hydroxyphenanthrene; 3-OHPh, 3-hydro-
xynaphthalene; 4-OHPh, 4-hydroxyphenanthrene; 9-OHPh, 9-hydroxyphenanthrene; 2 + 9-OHFlu, 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; Cr, creatinine.
Six kinds of platelet indices (platelet count, MPV, PDW, P-LCR, PLR and MPVP) were associated with the 1-OHNa, 2-OHNa and ΣOHNa 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 Q2, 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 MPVP (all P < 0.05). The P value for the trends in the PCT quantile regression models was not significant. The results of unadjusted model are not shown.

### 3.7. Mediation of platelets in the relationship between ΣOHNa and IL-1β

Mediation analyses were used to understand the relationships between urinary ΣOHNa and IL-1β through seven platelet indices, as mediator variables (Table 5). After adjusting for age, gender and BMI, ΣOHNa was positively associated with IL-1β mediated through MPV (β = 0.024, 95% CI: 0.003, 0.064), PDW (β = 0.026, 95% CI: 0.004, 0.069), P-LCR (β = 0.020, 95% CI: 0.002, 0.057), and PLR (β = 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-1β) was increased in the e-waste-exposed children and was positively associated with urinary PAH
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 ace- naphthene in 3- to 5-year-old children, with their urinary 1-OHNa and 1-hydroxynaphthene (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 contact with e-waste, residence as a workplace, family member smoking, and ventilation of their house, indicate that 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). 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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

Table 3

| Table 3 | Comparison of platelet indices in preschool children. |
|-----------------|-----------------|-----------------|-----------------|
|                  | Reference group | Exposed group   | P-value         |
| Platelet count   | (x 10^11/L)     |                 |                 |
|                  | (n = 121)       | (n = 118)       |                 |
| 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         |
| MPV              | 0.37 (0.03, 0.04)| 0.03 (0.03, 0.04)| < 0.001         |
| MPV, mean platelet volume; PDW, platelet distribution width; PCT, thrombocytoct; 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

| Table 4 | Comparison of urinary cytokine concentrations (pg/mL) in preschool children. |
|-----------------|-----------------|-----------------|-----------------|
| Cytokine (pg/mL) | Reference group | Exposed group   | P-value         |
| Pro-inflammatory cytokines |                 |                 |                 |
| IL-1β           | 0.25 (0.25, 0.43)| 0.43 (0.25, 0.49)| < 0.001         |
| IP-10β          | 25.5 (22.9, 29.2)| 28.5 (24.5, 33.9)| 0.001           |
| Pro-angiogenic cytokines |                 |                 |                 |
| RANTES           | 63.7 (58.8, 72.9)| 65.2 (59.5, 72.7)| 0.639           |
| GROα            | 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. 

* Exposed group, n = 109; reference group, n = 111.  
* Exposed group, n = 108; reference group, n = 111.  
* 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.
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+ and CD4+ 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-1β. IL-1β 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-1β, or IL-6, in systemic circulation (Beneke et al., 2012). Furthermore, we observed that elevated urinary 1- and 2-OHNa and 4-OHPhe were related to increased IL-1β 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-OHPhe and 4-OHPhe concentrations were negatively associated with IL-1β, IL-10, and TNF-α 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

Fig. 4. Effect estimates and 95% confidence interval for quartiles of urinary OHNa and ΣOH-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; ΣOHNa, the sum of 1-OHNa and 2-OHNa; ΣOH-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.
marker in multiple chronic diseases, such as periodontitis, rheumatoid arthritis, and chronic obstructive pulmonary disease exacerbation (Kisacik et al., 2008; Ulasi 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 of these two values, MPVP, may be a more meaningful indicator (Kisacik et al., 2008; Ulasli et al., 2012; Wang et al., 2015). There is an arthritis, and chronic obstructive pulmonary disease exacerbation platelet volume to platelet count. PLR, ratio of platelet count to lymphocyte count; MPVP, ratio of mean platelet distribution width; PCT, thrombocytocrit; P-LCR, platelet-large cell ratio; IL: interleukin; CI: confidence interval; ΣOH-PAHs, the sum of 1-hydroxy-naphthalene and 2-hydroxy-naphthalene; MPV, mean platelet volume; PDPW, 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. α All models are adjusted for age, gender and BMI; 5000 bootstrap samples, n = 220. β Proportion of mediation = indirect effect/(direct effect + indirect effect) × 100. **Table 5: Mediation analysis of platelet indices as mediators between ΣOHNa and IL-1β.**

<table>
<thead>
<tr>
<th>Platelet count</th>
<th>ΣOHNa</th>
<th>β (95% CI)</th>
<th>Proportion of mediation (%)</th>
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<tr>
<td>Direct effect</td>
<td>0.130 (0.022, 0.239)</td>
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<td>0.005 (–0.015, 0.031)</td>
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<tr>
<td>MPV</td>
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<td>0.112 (0.006, 0.218)</td>
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</tr>
<tr>
<td>Indirect effect</td>
<td>0.024 (0.003, 0.064)</td>
<td>17.6</td>
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<tr>
<td>PDW</td>
<td>Direct effect</td>
<td>0.110 (0.004, 0.216)</td>
<td>–</td>
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<tr>
<td>Indirect effect</td>
<td>–0.006 (–0.033, 0.009)</td>
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<td>–</td>
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<tr>
<td>P-LCR</td>
<td>Direct effect</td>
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<td>PLR</td>
<td>Direct effect</td>
<td>0.119 (0.014, 0.225)</td>
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<tr>
<td>Indirect effect</td>
<td>0.017 (0.000, 0.052)</td>
<td>12.2</td>
<td>–</td>
</tr>
<tr>
<td>MPVP</td>
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<td>0.121 (0.012, 0.230)</td>
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<tr>
<td>Indirect effect</td>
<td>0.014 (–0.007, 0.049)</td>
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IL: interleukin; CI: confidence interval; ΣOH-PAHs, the sum of 1-hydroxy-naphthalene and 2-hydroxy-naphthalene; MPV, mean platelet volume; PDPW, 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. α All models are adjusted for age, gender and BMI; 5000 bootstrap samples, n = 220. β Proportion of mediation = indirect effect/(direct effect + indirect effect) × 100. **Table 5: Mediation analysis of platelet indices as mediators between ΣOHNa and IL-1β.**

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**Table 5: Mediation analysis of platelet indices as mediators between ΣOHNa and IL-1β.**

Our findings suggest that elevated urinary 1-OHNa and 2-OHNa concentrations are associated with increased IL-1β, platelet count, PCT and PLR, and decreased MPV, PDW, P-LCR and MPVP in preschool children. Moreover, ΣOHNa is positively associated with IL-1β 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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