Ambient fine particulate matter inhibits innate airway antimicrobial activity in preschool children in e-waste areas

Shaocheng Zhang, Xia Huo, Yu Zhang, Yu Huang, Xiangbin Zheng, Xijin Xu

1. Introduction

Fine particulate matter (PM_{2.5}, which denotes particulate matter with an aerodynamic diameter less than or equal to 2.5 \mu m) air pollution is a prominent worldwide environmental problem and a critical global public health risk factor (Apte et al., 2015; Cohen et al., 2017; Fu et al., 2007; Hooper et al., 2018; Strickland et al., 2016; Zhang et al., 2018). Long-term exposure to PM_{2.5} is associated with increased risk of all natural, cardiovascular, and respiratory mortality (Hsu et al., 2017; Shen et al., 2018; Shiraiwa et al., 2017; Wang et al., 2018; Wong et al., 2015; Yin et al., 2017). The Global Burden of Diseases Study 2015 ranked ambient PM_{2.5} as the fifth highest mortality risk factor, contributing to approximately 4 million deaths and 103 million disability-adjusted life years, with the highest mortality being in east and south Asia (Cohen et al., 2017; Silva et al., 2016). Although PM_{2.5} can cause a series of adverse health effects, it is thought to primarily attack the airway and cause respiratory diseases, of which lower respiratory infection is the crucial cause of death in children younger than 5 years old (Cohen et al., 2017; Cong et al., 2018; Hooper et al., 2018; Mazidi and Speakman, 2017; Naghavi et al., 2015; Strickland et al., 2016; WHO, 2016; Zhang et al., 2018).

Epidemiological studies have indicated a positive association between PM_{2.5} exposure and innate airway pathogen infection (Neupane et al., 2010; Strickland et al., 2016). PM_{2.5} exposure may down-regulate airway antimicrobial proteins and peptides (AMPs), thereby accelerating airway pathogen infection. However, epidemiological research is scarce. Hence, we estimated the associations between individual PM_{2.5} chronic daily intake (CDI) and the levels of the airway AMP salivary agglutinin (SAG), as well as peripheral leukocyte counts and pro-inflammatory cytokines, of preschool children in Guiyu (an e-waste area) and Haojiang (a reference area located 31.6 km to the east of Guiyu). We recruited 581 preschool children from Guiyu and Haojiang, of which 222 were included in this study for a matching design (Guiyu: n = 110 vs. Haojiang: n = 112). Air PM_{2.5} pollution data was collected to calculate individual PM_{2.5} CDI. The mean concentration of PM_{2.5} in Guiyu was higher than in Haojiang, resulting in a higher individual PM_{2.5} CDI. Concomitantly, saliva SAG levels were lower in Guiyu children (5.05 ng/mL) than in Haojiang children (8.68 ng/mL), and were negatively correlated with CDI. Additionally, peripheral counts of white blood cells, and the concentrations of interleukin-8 and tumor necrosis factor-alpha, in Guiyu children were greater than in Haojiang children, and were positively correlated with CDI. Similar results were found for neutrophils and monocytes.

Abbreviations: PM_{2.5}, fine particulate matter; SAG, salivary agglutinin; SPD, surfactant protein D; BMI, body mass index; CDI, chronic daily intake; AMP, antimicrobial protein and peptide; WBC, white blood cell; IL, interleukin; TNF, tumor necrosis factor; E-waste, electronic waste; CI, confidence interval

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can stimulate oxidative stress and platelet-activating factor, subsequently inducing cell senescence, which reduces the expression of airway antimicrobial proteins and peptides (AMPs), thereby enabling pathogens, such as Streptococcus pneumoniae, Pseudomonas aeruginosa and Mycobacterium tuberculosis, to adhere to airway epithelial cells, and consequently increase airway infection (Chen et al., 2018; Mushitaq et al., 2011; Rivas-Santiago et al., 2015). Moreover, PM$_{2.5}$ can adsorb AMPs to decrease the amount of functional AMPs, and therefore suppress AMP antimicrobial capability and elevate vulnerability to airway pathogen infection (Vargas Buonfiglio et al., 2017). Collectively, PM$_{2.5}$ could down-regulate AMPs to weaken airway innate antimicrobial defense.

Salivary agglutinin (SAG), also known as lung scavenger receptor glycoprotein, has been identified to play a critical role in innate airway immune antimicrobial defense (Fabian et al., 2012; Prakobphol et al., 2000; Reichhardt et al., 2017; Reichhardt and Meri, 2016). SAG, one of the major AMPs, was originally found in saliva and is present in bronchoalveolar lavage and other mucosal fluids, but not in blood (Ericson and Rundegren, 1983; Gunput et al., 2016; Holmskov et al., 1997; Reichhardt et al., 2014; Reichhardt et al., 2016; Sonesson et al., 2011). Previously, it was determined that SAG induces microbial adhesion and aggregation via pathogen-associated molecular patterns of microbes, subsequently promoting their clearance (Chu et al., 2013; Li et al., 2017b; Madsen et al., 2016; Reichhardt et al., 2017; Reichhardt and Meri, 2016). SAG also can competitively inhibit microbial colonization through adhering directly to host cells to facilitate pathogen clearance (Boks et al., 2016). On the other hand, SAG binds to endogenous molecules in a calcium-dependent manner, and there exists a cooperative antiviral effect of SAG and surfactant protein D (SPD) in the respiratory innate immune system (Holmskov et al., 1997; Ligenberg et al., 2001; Madsen et al., 2010; Reichhardt et al., 2017; Reichhardt and Meri, 2016; Hartshorn et al., 2006; White et al., 2005). SPD, secreted by alveolar epithelial type II cells and Clara cells, is mainly distributed in the lung and can be transported into the blood. The primary function of SPD is the agglutination and removal of microbes, which protects against pathogen invasion and infection (Du et al., 2016; Hillaire et al., 2013; Sorensen, 2018; Takahashi et al., 2006; Wong et al., 2018).

To date, studies on PM$_{2.5}$ and innate airway antimicrobial activity are few, and are mainly in vitro experiments. Our previous investigations indicated that PM$_{2.5}$ concentrations and PM$_{2.5}$ heavy metal concentrations are higher in Guiyu, one of the biggest electronic waste (e-waste) regions. AMPs could down-regulate AMPs to weaken airway innate antimicrobial defense. To address this relationship, the present investigation estimates individual PM$_{2.5}$ chronic daily intake (CDI), levels of SAG and SPD, peripheral leukocyte counts, and pro-inflammatory cytokines in preschool children. Additionally, we also determine the correlations among CDI, SAG level, peripheral leukocyte count, and pro-inflammatory cytokines.

2. Materials and methods

2.1. Study population

A total of 581 preschool children (2–7 years old) were recruited from two kindergartens in Guiyu and Haojiang during November to December 2017. To account for the impact of age and gender, we used a matching design. Ultimately, 222 preschool children (approximately 5 years old) were included in this study (Guiyu: n = 110 vs. Haojiang: n = 112). Their guardians supplied signed informed consent prior to recruitment. Both Guiyu (the e-waste exposed area) and Haojiang (the reference area, located 31.6 km to the east of Guiyu) are small towns in Shantou, China. Except for the lack of e-waste pollution in Haojiang, the two areas are similar in ethnicity, cultural background and socio-economic status (Zeng et al., 2016; Zhang et al., 2017). All children had lived in their present address for more than one year. All children were free of infectious diseases, respiratory diseases or any known medical conditions. A questionnaire on general characteristics, dwelling environment, living habits of children, family history of disease, monthly household income and parental educational level was completed by the guardians. All protocols in this investigation were approved by the Human Ethics Committee of Shantou University Medical College, China.

2.2. Air PM$_{2.5}$ pollution and individual CDI

Several investigations have shown that air pollution data from monitoring stations can be used to estimate and calculate individual daily exposure (e.g. radius less than or equal to 15 km) (Cong et al., 2018; Darrow et al., 2011; Ivy et al., 2008; Li et al., 2017a; Yorifuji et al., 2017). Daily PM$_{2.5}$ data from Chaoan and Haojiang air quality monitoring stations was obtained from the Ministry of National Environmental Protection (http://106.37.208.233:20035/), from October to December 2017. From participant residential addresses, kindergarten locations and the geographical location of the corresponding air quality monitoring station, all participants lived within an 8 km radius of the corresponding monitoring station (Cong et al., 2018). We calculated individual CDI of PM$_{2.5}$ using a method described previously (Betha and Balasubramanian, 2011; Betha et al., 2013; Zheng et al., 2016). Briefly, CDI (ng·kg$^{-1}$·day$^{-1}$) = total dose (TD, ng·m$^{-3}$) × inhalation rate (IR, m$^3$·day$^{-1}$)/body weight (kg), and TD = C × E, where C is the mean concentration of PM$_{2.5}$ (including before and during the sampling) and E represents the deposition fraction of PM$_{2.5}$, all calculated using parameters of 5-year-old child, as described in prior investigations (Table A. 1) (ICRP, 1994; Zheng et al., 2016). The hours of outdoor exposure (0.5 h, 1 h, 2 h, 3 h, and 4.8 h) were used to determine the corresponding IR of children based on time spent outdoors (less than or equal to 0.5 h, 0.5–1 h, 1–2 h, 2–3 h, and > 3 h) (Zheng et al., 2016).

2.3. General physical test and biological measurements

As described previously, a general physical test, including height, weight and chest circumference, was executed by a trained physician, and fasting venous blood was obtained by a nurse (Lin et al., 2017; Zeng et al., 2017). Whole blood was used for peripheral leukocyte counts with a Sysmex XT-1800i automatic hematological analyzer, as in previous studies (Fessler et al., 2017; Zeng et al., 2017). Because the EDTA anticoagulant could underestimate the value of SPD (Bratcher and Gaggar, 2014), anticoagulant-free tubes were used to collect and clot blood at room temperature for 30 min, then serum was separated by centrifugation at 1000g for 15 min, and serum SPD concentration was determined with a Quantikine® ELISA kit according to the manufacturer’s instructions (R&D Systems Inc., USA). Sensitivity was 0.11 ng/mL (0.02–0.37 ng/mL), accuracy of intra- and inter-assays was within 6.2%–8.2% and 8.7%–9.3%, respectively. In addition, serum pro-inflammatory cytokines were measured with a ProcartaPlex Multiplex Immunoassay for Simplex Kits and Combinable Panels according to the manufacturers’ instructions (Thermo Fisher Scientific Inc., Austria), and the assayed results were analyzed with a Luminex® 200™ (Luminex Inc., USA). Lastly, the remaining whole blood and sera were aliquoted and stored at −80 °C until analysis.

Saliva was collected without stimulation from all participants as previously described (Gunput et al., 2016), and was centrifuged at 1000g for 20 min (4 °C) to remove cellular debris and collect supernatant, which was used for SAG detection. Saliva SAG level was quantified with a quantitative sandwich ELISA kit following the
manufacturer's technical manual (ELISAGenie Inc., UK). Measuring range was 0.312–20 ng/mL, sensitivity was 0.188 ng/mL, intra- and inter-assay accuracies were within 8% and 10%, respectively. The remainder of saliva supernatant was aliquoted and stored at −80 °C until analysis.

2.4. Statistical analysis

The independent-sample t-test, the Mann-Whitney U test, and the Pearson chi-square test were used to compare the differences between the two study groups, as appropriate. Data was depicted with mean and standard deviation, or median and interquartile range, according to distribution characteristics. A natural logarithm transformation was used to construct approximate normal distributions. Pearson correlation analyses were applied to define the associations of confounders and CDI. Simultaneously, the associations of CDI to SAG, peripheral leukocyte counts and pro-inflammatory cytokines were assessed using a multivariable adjusted linear regression model. As described in previous literature, confounders consisted of gender, age, body mass index (BMI), chest circumference, outdoor time, pencil biting, contact with e-waste, distance between residence and road, residence within 50 m of an e-waste site, family history of asthma, family member daily cigarette consumption, parent educational level, and monthly household income (Cong et al., 2018; Zeng et al., 2016; Zheng et al., 2016). All analyses were performed with SPSS 19.0 (IBM Corporation, USA) and GraphPad Prism 7.0 (GraphPad, CA). A P < 0.05 was considered as statistically significant in a two-tailed test.

3. Results and discussion

3.1. General characteristics of the study population

There were 222 preschool children enrolled in the study (Table 1). The mean age of the Haojiang children (n = 112) was 4.75 ± 0.82 years, and 4.71 ± 0.82 years for the Guiyu children (n = 110) (P > 0.05). There was no significant difference for gender between the two groups (P > 0.05). Even though chest circumference, height and weight of Guiyu children were smaller than Haojiang children (all P > 0.05), the body mass index (BMI) and family history of asthma were similar in both groups (all P > 0.05). Compared with Haojiang children, Guiyu children spent less time outdoors and had unhealthy living habits, including pencil biting, contacting e-waste, poor residential environment (such as daily smoking of a family member, distance between residence and road, and distance of residence within 50 m from an e-waste site) (all P < 0.05). Moreover, the parental educational level was lower, and parents had a lower monthly household income in Guiyu (all P < 0.05).

3.2. Ambient PM<sub>2.5</sub> pollution and factors influencing individual CDI

The mean concentration of PM<sub>2.5</sub> in Guiyu was significantly higher than Haojiang (39.06 μg/m³ vs. 26.68 μg/m³, P < 0.001) (Fig. 1A), which is consistent with our previous findings and governmental monitoring (Cong et al., 2018; Zheng et al., 2016). Remarkably, although the average PM<sub>2.5</sub> concentrations in both areas exceed the current guidelines of the World Health Organization (WHO) for ambient PM<sub>2.5</sub> (10 μg/m³ annual mean and 25 μg/m³ 24-hour mean), as for the national ambient air quality standards of China, the average PM<sub>2.5</sub> level in Guiyu surpasses annual mean levels (level I and II, 15 μg/m³ and 35 μg/m³ respectively) and the 24-hour mean level I (35 μg/m³), whereas in Haojiang, only the annual mean level I was exceeded (MEEPRC, 2012; WHO, 2018). These high levels in Guiyu might be attributed to primitive and irregular operations in e-waste recycling, such as open-air burning, roasting and dumping residue and ash, which accelerate particle and droplet emission into the ambient atmosphere (Cong et al., 2018; Zhang et al., 2016; Zheng et al., 2016).

### Table 1

<table>
<thead>
<tr>
<th>General characteristics of the study population.</th>
<th>Haojiang (n = 112)</th>
<th>Guiyu (n = 110)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (boys/girls)</td>
<td>58/54</td>
<td>57/53</td>
<td>0.996&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age (mean ± SD, years)</td>
<td>4.75 ± 0.82</td>
<td>4.71 ± 0.82</td>
<td>0.676&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Height (mean ± SD, cm)</td>
<td>108.44 ± 6.75</td>
<td>105.10 ± 6.35</td>
<td>0.000&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight (mean ± SD, kg)</td>
<td>18.19 ± 2.95</td>
<td>16.76 ± 2.29</td>
<td>0.000&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI (mean ± SD, kg/m²)</td>
<td>15.40 ± 1.39</td>
<td>15.13 ± 1.17</td>
<td>0.115&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chest circumference (mean ± SD, cm)</td>
<td>52.58 ± 5.53</td>
<td>50.97 ± 2.48</td>
<td>0.006&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Child outdoor time [n (%), hour]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.5</td>
<td>3 (2.7)</td>
<td>14 (13.0)</td>
<td>0.000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5–1</td>
<td>25 (22.3)</td>
<td>40 (37.0)</td>
<td></td>
</tr>
<tr>
<td>&gt; 1</td>
<td>48 (42.8)</td>
<td>28 (25.9)</td>
<td></td>
</tr>
<tr>
<td>&lt; 3</td>
<td>19 (17.0)</td>
<td>22 (20.4)</td>
<td></td>
</tr>
<tr>
<td>&gt; 3</td>
<td>17 (15.2)</td>
<td>4 (3.7)</td>
<td></td>
</tr>
<tr>
<td>Child pencil biting (yes/no)</td>
<td>11/101</td>
<td>30/80</td>
<td>0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Family history of asthma [n (%)]</td>
<td>2 (1.8)</td>
<td>2 (1.8)</td>
<td>0.985&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Family member daily cigarette consumption [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoking</td>
<td>56 (50.0)</td>
<td>31 (28.7)</td>
<td></td>
</tr>
<tr>
<td>&lt; 2 cigarettes</td>
<td>16 (14.3)</td>
<td>8 (7.4)</td>
<td></td>
</tr>
<tr>
<td>&lt; 10 cigarettes</td>
<td>18 (16.1)</td>
<td>27 (25.0)</td>
<td></td>
</tr>
<tr>
<td>&lt; 20 cigarettes</td>
<td>16 (14.3)</td>
<td>28 (25.9)</td>
<td></td>
</tr>
<tr>
<td>&gt; 20 cigarettes</td>
<td>6 (5.3)</td>
<td>14 (13.0)</td>
<td></td>
</tr>
<tr>
<td>Distance between residence and road [n (%), m]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 10</td>
<td>3 (2.7)</td>
<td>45 (49.4)</td>
<td></td>
</tr>
<tr>
<td>&lt; 50</td>
<td>22 (19.6)</td>
<td>26 (24.1)</td>
<td></td>
</tr>
<tr>
<td>&gt; 100</td>
<td>60 (53.6)</td>
<td>11 (10.2)</td>
<td></td>
</tr>
<tr>
<td>Father’s educational level [n (%)]</td>
<td></td>
<td></td>
<td>0.000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Middle school or lower</td>
<td>24 (21.4)</td>
<td>80 (73.4)</td>
<td></td>
</tr>
<tr>
<td>Secondary school</td>
<td>21 (18.8)</td>
<td>10 (9.2)</td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>16 (14.3)</td>
<td>11 (10.1)</td>
<td></td>
</tr>
<tr>
<td>College/university</td>
<td>51 (45.5)</td>
<td>8 (7.3)</td>
<td></td>
</tr>
<tr>
<td>Mother’s educational level [n (%)]</td>
<td></td>
<td></td>
<td>0.000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Middle school or lower</td>
<td>34 (30.3)</td>
<td>79 (72.5)</td>
<td></td>
</tr>
<tr>
<td>Secondary school</td>
<td>16 (14.3)</td>
<td>12 (11.0)</td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>18 (16.1)</td>
<td>5 (4.6)</td>
<td></td>
</tr>
<tr>
<td>College/university</td>
<td>44 (39.3)</td>
<td>13 (11.9)</td>
<td></td>
</tr>
<tr>
<td>Monthly household income [n (%), yuan]</td>
<td></td>
<td></td>
<td>0.000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt; 3000</td>
<td>12 (10.7)</td>
<td>21 (21.0)</td>
<td></td>
</tr>
<tr>
<td>&gt; 4500</td>
<td>18 (16.1)</td>
<td>23 (23.0)</td>
<td></td>
</tr>
<tr>
<td>&gt; 6000</td>
<td>19 (17.0)</td>
<td>30 (30.0)</td>
<td></td>
</tr>
<tr>
<td>&gt; 6000</td>
<td>63 (56.2)</td>
<td>26 (26.0)</td>
<td></td>
</tr>
</tbody>
</table>

BMI, body mass index. SD, standard deviation. Statistical significance, P < 0.05.  
<sup>a</sup> Analysis by Pearson chi-square test.  
<sup>b</sup> Analysis by independent-sample t-test.

Similarly, the median individual PM<sub>2.5</sub> CDI in Guiyu children was higher than Haojiang children (1.40 ng/kg·day<sup>−1</sup> vs. 0.88 ng/kg·day<sup>−1</sup>, P < 0.001) (Fig. 1B). In addition, Pearson correlation analysis, applied to explore if there were certain factors related to individual PM<sub>2.5</sub> CDI, suggested that individual PM<sub>2.5</sub> CDI was positively correlated with unhealthy living habits (including pencil biting and contact with e-waste), daily smoking of a family member, and residence within 50 m from an e-waste site (r<sub>x</sub> = 0.225, r<sub>y</sub> = 0.302, r<sub>z</sub> = 0.233, and r<sub>w</sub> = 0.344, respectively, all P < 0.01), whereas it was negatively associated with age, BMI, chest circumference, time spent outdoors, distance between residence and road, and parental educational level (father/mother), as well as monthly household income (r<sub>x</sub> = −0.327, r<sub>y</sub> = −0.358, r<sub>z</sub> = −0.413, r<sub>w</sub> = −0.134, r<sub>u</sub> = −0.472, r<sub>v</sub> = −0.419, r<sub>v</sub> = −0.350, and r<sub>v</sub> = −0.155, respectively, all P < 0.05) (Table A.2). Collectively, individual CDI might be attributable to unhealthy living habits, poor residential environment, low parental educational level, and poor household income. This is consistent with prior descriptions, indicative of e-waste air pollution and child habits promoting pollution exposure (Lu et al., 2018; Zahran...
et al., 2013).

3.3. AMP concentrations and the associations with individual PM2.5 CDI

As shown in Fig. 2, the level of saliva SAG in Guiyu children was lower than Haojiang children (5.05 ng/mL vs. 8.68 ng/mL, \( P < 0.001 \)). Previous studies have suggested that PM2.5 exposure could weaken airway AMP defenses through downregulating expression of and adhesion to AMPs, thereby accelerating airway susceptibility to \( \text{Staphylococcus aureus} \), \( \text{Streptococcus pneumoniae} \), \( \text{Pseudomonas aeruginosa} \) and \( \text{Mycobacterium tuberculosis} \) (Chen et al., 2018; Mushtaq et al., 2011; Rivas-Santiago et al., 2015; Vargas Buonfiglio et al., 2017). To determine the correlation between individual PM2.5 CDIs and saliva SAG levels, a multivariable adjusted linear regression model was used (Table 2). In unadjusted regression analysis, CDI was negatively correlated with natural logarithm-transformed saliva SAG level (Ln-SAG) \( \beta \text{(95% CI)} = -0.796 (-1.369, -0.222), P < 0.01 \). The correlation remained significant after further adjustment for gender, age, height, chest circumference, pencil biting, contact with e-waste, distance between residence and road, residence within 50 m of an e-waste site, family history of asthma, family member daily cigarette consumption, parental educational level, and monthly household income \( \beta \text{(95% CI)} = -0.222 (-0.113, -0.331), P < 0.001 \). However, unexpectedly, the serum SPD concentration in Guiyu children was similar to Haojiang children (8.70 × 10^9/L vs. 7.36 × 10^9/L, \( P > 0.05 \)) (Fig. 2). Impaired air-blood barrier integrity plays an elemental role in secreted lung protein translocation into the bloodstream (Hastings et al., 1992). PM2.5 exposure could cause acute and chronic inflammatory lung injury, which facilitates SPD leakage from the airway into the bloodstream (Fujita et al., 2005; Gaunsbaek et al., 2013; Wang et al., 2017). A prior study has indicated that cigarette smoke exposure could decrease SPD levels in bronchoalveolar lavage fluid while simultaneously enriching SPD in serum (Moazed et al., 2016). In short, the present study shows that the greater the individual CDI, the lower the saliva SAG level, and there might be impaired antimicrobial activity in the airway.

3.4. Peripheral leukocyte count and pro-inflammatory cytokines

Our results showed that the absolute counts of white blood cells (WBCs), neutrophils and monocytes in Guiyu children were higher than in Haojiang children (\( 8.70 \times 10^9/L \text{ vs. } 7.36 \times 10^9/L, 4.22 \times 10^9/L \text{ vs. } 3.25 \times 10^9/L \), and \( 0.57 \times 10^9/L \text{ vs. } 0.37 \times 10^9/L \), respectively, all \( P < 0.001 \)) (Fig. 3A). In addition, the concentrations of interleukin (IL)-8 and tumor necrosis factor (TNF)-\( \alpha \) in Guiyu children were higher than Haojiang children (2.685 pg/mL vs. 1.847 pg/mL, and 5.206 pg/mL vs. 3.357 pg/mL, respectively, all \( P < 0.001 \)).

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**Table 2** Associations between CDI and Ln-SAG level in preschool children.

<table>
<thead>
<tr>
<th>CDI</th>
<th>Ln-SAG</th>
<th>( \beta ) (95% CI)</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>(-0.796 (-1.369, -0.222))</td>
<td>-0.193</td>
<td>0.007</td>
</tr>
<tr>
<td>Model 2</td>
<td>(-0.941 (-1.769, -0.113))</td>
<td>-0.228</td>
<td>0.026</td>
</tr>
<tr>
<td>Model 3</td>
<td>(-0.982 (-1.864, -0.100))</td>
<td>-0.238</td>
<td>0.029</td>
</tr>
<tr>
<td>Model 4</td>
<td>(-1.215 (-2.293, -0.137))</td>
<td>-0.294</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Model 1: unadjusted.
Model 2: adjusted for gender, age, height, and chest circumference.
Model 3: adjusted for gender, age, height, chest circumference, pencil biting, and contact with e-waste.
Model 4: adjusted for gender, age, height, chest circumference, pencil biting, contact with e-waste, distance between residence and road, residence within 50 m of an e-waste site, family history of asthma, family member daily cigarette consumption, parental educational level, and monthly household income.

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**Fig. 2.** Levels of airway antimicrobial proteins and peptides in preschool children.

SAG, salivary agglutinin. SPD, surfactant protein D.

Data are presented as median (interquartile range), as analyzed by the Mann-Whitney \( U \)-test, \( *** P < 0.001 \).
Peripheral leukocytes are biomarkers of pathogen infection as well as inflammation, and the peripheral WBC count is a potential predictor of human mortality from all causes (Chabot-Richards and George, 2014; Fessler et al., 2017; de Labry et al., 1990). In addition, neutrophils play a crucial role in host immune defense against pathogen infection, and activated neutrophils can induce monocyte/macrophage recruitment and activation by releasing chemokines, such as IL-8 and TNF-α. Moreover, there is a correlation between reactive monocytes and chronic pathogen infection (Chabot-Richards and George, 2014; Kim and Bae, 2016; Kumar and Sharma, 2010; Tsuda et al., 2004). On the other hand, PM2.5 exposure could up-regulate IL-8 expression and enhance plasma TNF-α level (Liu et al., 2017; Wang et al., 2013). Previous studies, based on diseases or short-term exposure, showed no significant correlation between PM2.5 air pollution and WBC count, while others have suggested ambient PM2.5 exposure may increase the absolute counts of WBCs, neutrophils, and monocytes in healthy adults (Dabass et al., 2018; Huttunen et al., 2012; Poursafa et al., 2011; Rich et al., 2012; Steenhof et al., 2014). This study was conducted in healthy preschool children and showed that the peripheral cell counts of WBCs, neutrophils, and monocytes are elevated in Guiyu children, which is consistent with the findings of studies in healthy adults.

We identified the correlations between the absolute count of WBCs, as well as WBC subtypes, IL-8, TNF-α and individual PM2.5 CDI. Results showed a statistically significant, positive correlation of CDI to absolute counts of WBCs, neutrophils, monocytes, IL-8, and TNF-α, suggesting that with each one-fold increase in CDI, the absolute counts of WBCs, neutrophils, and monocytes increase 4.226 (10^9/L), 2.925 (10^9/L), and 0.510 (10^9/L), and IL-8 and TNF-α will increase by 1.370 pg/mL and 5.434 pg/mL, respectively, in children (Table 3).

### 3.5. Associations among saliva SAG level, peripheral leukocyte counts and pro-inflammatory cytokines

PM2.5 toxicity may induce alterations in inflammatory cytokines (such as IL-8) and AMPs (Chen et al., 2018; Rivas-Santiago et al., 2015; Vargas Buonfiglio et al., 2017). A previous study has indicated that SAG expression is up-regulated to respond to inflammation and participates in antimicrobial defense in chronic sinusitis (Kim et al., 2007). In addition, increased SAG expression has been observed in affected tissue with pro-inflammatory stimuli, including lipopolysaccharide and TNF-

**Table 3** Associations between CDI and absolute counts of total WBCs and WBC subtypes, as well as pro-inflammatory cytokine levels in preschool children.

<table>
<thead>
<tr>
<th>CDI</th>
<th>B (95% CI)</th>
<th>β</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>4.226 (2.381, 6.071)</td>
<td>0.540</td>
<td>0.000</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.925 (1.537, 4.314)</td>
<td>0.506</td>
<td>0.000</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.704 (−0.203, 1.705)</td>
<td>1.610</td>
<td>0.195</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.510 (0.363, 0.657)</td>
<td>0.725</td>
<td>0.000</td>
</tr>
<tr>
<td>IL-8</td>
<td>1.370 (0.446, 2.295)</td>
<td>0.483</td>
<td>0.004</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5.434 (2.933, 7.935)</td>
<td>0.539</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Adjusted for gender, age, height, chest circumference, pencil biting, contact with e-waste, distance between residence and road, residence within 50 m of an e-waste site, family history of asthma, family smoking daily cigarette consumption, parental educational level, and monthly household income.

Note: CDI, PM2.5 chronic daily intake; WBC, white blood cell; IL, interleukin; TNF-α, tumor necrosis factor-alpha; B, unstandardized coefficient; CI, confidence interval; β, standardized coefficient. Statistical significance, P < 0.05.
We, in summary, conducted the first study on the relationship between ambient PM$_{2.5}$ exposure and airway innate antimicrobial activity in preschool children in an e-waste area. The current results show severe PM$_{2.5}$ pollution in e-waste areas results in a heavy inside of asthma, family member daily cigarette consumption, parental educational level, and monthly household income. Note: SAG, salivary agglutinin; IL-8, interleukin; TNF-α, tumor necrosis factor-alpha; BMI, body mass index; B, unstandardized coefficient; CI, confidence interval.

**P** < 0.05.

**P** < 0.01.

abnormal airway antimicrobial activity.

Several limitations in the present study should be considered. We conducted a cross-sectional study that provides a correlation of ambient PM$_{2.5}$ exposure and airway innate antimicrobial activity, but does not prove causality. In addition, the sample size was small, and we did not obtain accurate PM$_{2.5}$ data through personal monitoring equipment or sensors, because the study populations were too young to use the equipment. Lastly, we failed to measure the concentration of SP in bronchoalveolar lavage fluid, nor did we culture and identify airway microbes, due to the difficulties of sampling. Therefore, future studies should pay more attention to large sample size, accuracy of PM$_{2.5}$ measurement, and effects of PM$_{2.5}$ on differences in airway microbial distribution.

**4. Conclusion**

We, in summary, conducted the first study on the relationship between ambient PM$_{2.5}$ exposure and airway innate antimicrobial activity in preschool children in an e-waste area. The current results show severe PM$_{2.5}$ pollution in e-waste recycling areas results in a heavy individual CDI in preschool children, accompanied by a decreased saliva SAG level, elevated peripheral absolute counts of WBCs, neutrophils and monocytes, and enhanced concentrations of serum IL-8 and TNF-α. Overall, our findings support the hypothesis that ambient PM$_{2.5}$ pollution may reduce airway antimicrobial activity by down-regulating saliva SAG levels, which might accelerate airway pathogen infection in children in e-waste areas. In addition, despite recent reductions, ambient PM$_{2.5}$ pollution still threatens the health of preschool children in e-waste areas. To protect children from the toxic effects of air PM$_{2.5}$ pollution caused by e-waste, stronger management by related government sectors should be carried out in the future.

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**Conflicts of interest**

The authors declare they have no conflict of interests.

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