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

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\section*{A R T I C L E   I N F O}
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\textbf{Keywords:}
- PM\textsubscript{2.5}
- Salivary agglutinin
- Airway antimicrobial defense
- Preschool children
- E-waste

\section*{A B S T R A C T}

Ambient fine particulate matter (PM\textsubscript{2.5}) is a risk factor for respiratory diseases. Previous studies suggest that PM\textsubscript{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\textsubscript{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: \textit{n} = 110 vs. Haojiang: \textit{n} = 112). Air PM\textsubscript{2.5} pollution data was collected to calculate individual PM\textsubscript{2.5} CDI. The mean concentration of PM\textsubscript{2.5} in Guiyu was higher than in Haojiang, resulting in a higher individual PM\textsubscript{2.5} CDI. Concomitantly, salivary 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 associated with CDI. Similar results were found for neutrophils and monocytes. To our knowledge, this is the first study on the relationship between PM\textsubscript{2.5} exposure and innate airway antimicrobial activity in children, in an e-waste area, showing that PM\textsubscript{2.5} pollution may weaken airway antimicrobial activity by down-regulation of saliva SAG levels, which might accelerate airway pathogen infection in children.

1. Introduction

Fine particulate matter (PM\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2.5} exposure and increased susceptibility to respiratory pathogen infection (Neupane et al., 2010; Strickland et al., 2016). PM\textsubscript{2.5} exposure may down-regulate airway antimicrobial proteins and peptides (AMPs), thereby accelerating airway pathogen infection (Apte et al., 2015; 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).
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 consequentially increase airway infection (Chen et al., 2018; Mushtaq 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) recycling areas in the world, which leads to decreased lung surfactant functions and increased lung function, acceleration of respiratory symptoms, and increased cardiovascular risk in children (Cong et al., 2018; Li et al., 2018; Lu et al., 2018; Wu et al., 2016; Zeng et al., 2016; Zeng et al., 2016; Zhang et al., 2017). Therefore, we hypothesize that PM$_{2.5}$ toxicity will alter the amount of AMPs in children living in e-waste areas, thereby affecting airway antimicrobial immune defense, ultimately raising the risk of respiratory infection. 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 socioeconomic 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 Chaonan 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$_{2.5}$, and E represents the deposition fraction of PM$_{2.5}$, 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 hematology 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 Combimune Panels according to the manufacturer’s instructions (Thermo Fisher Scientific Inc., Austria), and the assayed results were analyzed with a Lumines® 200™ (Lumines 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 remaining 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 education level was lower, and parents had a lower monthly household income in Guiyu (all P < 0.05).

3.2. Ambient PM$_{2.5}$ pollution and factors influencing individual CDI

The mean concentration of PM$_{2.5}$ in Guiyu was significantly higher than Haojiang (39.06 μg/m$^3$ vs. 26.68 μg/m$^3$, 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$_{2.5}$ concentrations in both areas exceed the current guidelines of the World Health Organization (WHO) for ambient PM$_{2.5}$ (10 μg/m$^3$ annual mean and 25 μg/m$^3$ 24-hour mean), as for the national ambient air quality standards of China, the average PM$_{2.5}$ level in Guiyu surpasses annual mean levels (level I and II, 15 μg/m$^3$ annually) 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

**General characteristics of the study population.**

<table>
<thead>
<tr>
<th></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*</td>
</tr>
<tr>
<td>Age (mean ± SD, years)</td>
<td>4.75 ± 0.82</td>
<td>4.71 ± 0.82</td>
<td>0.676*</td>
</tr>
<tr>
<td>Height (mean ± SD, cm)</td>
<td>108.44 ± 6.75</td>
<td>105.10 ± 6.35</td>
<td>0.000*</td>
</tr>
<tr>
<td>Weight (mean ± SD, kg)</td>
<td>18.19 ± 2.95</td>
<td>16.76 ± 2.29</td>
<td>0.000*</td>
</tr>
<tr>
<td>BMI (mean ± SD, kg/m$^2$)</td>
<td>15.40 ± 1.19</td>
<td>15.13 ± 1.17</td>
<td>0.115*</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*</td>
</tr>
<tr>
<td>Child outdoor time [n (%), hour]</td>
<td>0.000*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.5</td>
<td>3 (2.7)</td>
<td>14 (13.0)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>25 (22.3)</td>
<td>40 (37.0)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>48 (42.8)</td>
<td>28 (25.9)</td>
<td></td>
</tr>
<tr>
<td>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>0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>11 (10.1)</td>
<td>30 (28.8)</td>
<td></td>
</tr>
<tr>
<td>Family history of asthma [n (%)]</td>
<td>0.985*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2 (1.8)</td>
<td>2 (1.8)</td>
<td></td>
</tr>
<tr>
<td>Family member daily cigarette consumption [n (%)]</td>
<td>0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>56 (50.0)</td>
<td>31 (28.7)</td>
<td></td>
</tr>
<tr>
<td>1 to 2 cigarettes</td>
<td>14 (13.0)</td>
<td>7 (6.3)</td>
<td></td>
</tr>
<tr>
<td>3 to 4 cigarettes</td>
<td>16 (14.3)</td>
<td>28 (25.9)</td>
<td></td>
</tr>
<tr>
<td>&gt; 5 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>0.000*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 10</td>
<td>3 (2.7)</td>
<td>45 (45.4)</td>
<td></td>
</tr>
<tr>
<td>10 to 20</td>
<td>22 (19.6)</td>
<td>26 (24.1)</td>
<td></td>
</tr>
<tr>
<td>&gt; 20</td>
<td>27 (24.1)</td>
<td>22 (20.3)</td>
<td></td>
</tr>
<tr>
<td>Father’s educational level [n (%)]</td>
<td>0.000*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>College/university</td>
<td>51 (45.5)</td>
<td>8 (7.3)</td>
<td></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>0.000*</td>
<td></td>
<td></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>0.000*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 3000</td>
<td>12 (10.7)</td>
<td>21 (21.0)</td>
<td></td>
</tr>
<tr>
<td>3001 to 6000</td>
<td>18 (16.1)</td>
<td>23 (20.3)</td>
<td></td>
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<tr>
<td>6001 to 9000</td>
<td>19 (17.0)</td>
<td>30 (28.0)</td>
<td></td>
</tr>
<tr>
<td>&gt; 9000</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.

* Analysis by Pearson chi-square test.

b Analysis by independent-sample t-test.

Similarly, the median individual PM$_{2.5}$ CDI in Guiyu children was higher than Haojiang children (1.40 ng·kg$^{-1}$·day$^{-1}$ vs. 0.88 ng·kg$^{-1}$·day$^{-1}$, P < 0.001) (Fig. 1B). In addition, Pearson correlation analysis, applied to explore if there were certain factors related to individual PM$_{2.5}$ CDI, suggested that individual PM$_{2.5}$ 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$_{s}$ = 0.225, r$_{s}$ = 0.302, r$_{s}$ = 0.233, and r$_{s}$ = 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$_{s}$ = −0.327, r$_{s}$ = −0.358, r$_{s}$ = −0.413, r$_{s}$ = −0.134, r$_{s}$ = −0.472, r$_{s}$ = −0.419, r$_{s}$ = −0.350, and r$_{s}$ = −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 PM$_{2.5}$ 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 PM$_{2.5}$ exposure could weaken airway AMP defenses through downregulating expression of and adhesion to AMPs, thereby accelerating airway susceptibility to Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa and Mycobacterium tuberculosis (Chen et al., 2018; Mushtaq et al., 2011; Rivas-Santiago et al., 2015; Vargas Buongiorno et al., 2017). To determine the correlation between individual PM$_{2.5}$ 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) [B (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 [B (95% CI) = −1.215 (−2.293, −0.137), $P < 0.05$]. However, unexpectedly, the serum SPD concentration in Guiyu children was similar to Haojiang children (8.00 ng/mL vs. 7.48 ng/mL, $P > 0.05$) (Fig. 2). Impaired air-blood barrier integrity plays an elemental role in secreted lung protein translocation into the bloodstream (Hastings et al., 1992). PM$_{2.5}$ 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 × 10$^9$/L vs. 7.36 × 10$^9$/L, 4.22 × 10$^9$/L vs. 3.25 × 10$^9$/L, and 0.57 × 10$^9$/L vs. 0.37 × 10$^9$/L, respectively, all $P < 0.001$) (Fig. 3A). In addition, the concentrations of interleukin (IL)-8 and tumor necrosis factor (TNF)-α in Guiyu children were higher than Haojiang children (2.685 pg/mL vs. 1.847 pg/mL, and 5.206 pg/mL vs. 3.354 pg/mL, all $P < 0.05$) (Fig. 3B). The results suggest that PM$_{2.5}$ exposure could cause chronic inflammation.

---

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

<table>
<thead>
<tr>
<th>CDI</th>
<th>Ln-SAG</th>
<th>B (95% CI)</th>
<th>$\beta$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td>−0.796 (−1.369, −0.222)</td>
<td>−0.195</td>
<td>0.007</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td>−0.941 (−1.769, −0.113)</td>
<td>−0.228</td>
<td>0.026</td>
</tr>
<tr>
<td>Model 3</td>
<td></td>
<td>−0.982 (−1.864, −0.100)</td>
<td>−0.238</td>
<td>0.029</td>
</tr>
<tr>
<td>Model 4</td>
<td></td>
<td>−1.215 (−2.293, −0.137)</td>
<td>−0.294</td>
<td>0.027</td>
</tr>
</tbody>
</table>

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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, $***P < 0.001$.

**Fig. 1.** Ambient air PM$_{2.5}$ concentration and individual PM$_{2.5}$ chronic daily intake in preschool children.

(A): Data are presented as mean ± standard deviation, analyzed by independent-sample t-test, $***P < 0.001$.

(B): Data are presented as median (interquartile range), analyzed by the Mann-Whitney U test, $***P < 0.001$. 

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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 participate in antimicrobial defense in chronic sinusitis (Kim et al., 2007; Rosenstiel et al., 2007). In addition, increased SAG expression has been observed in infected tissue (Kang et al., 2002). To further understand the relationships of saliva SAG level and inflammatory biomarkers, a multivariable adjusted linear regression model was performed. Our results showed that there was no statistical correlation between saliva SAG level and pro-inflammatory cytokines, whereas a higher peripheral monocyte count was correlated with lower saliva SAG level [B (95% CI), 0.510 (−0.363, 0.657), P = 0.725] (Table 4). In this study, all subjects were healthy children, whereas the above referenced inflammatory cytokine up-regulation of SAG expression occurred in the disease state or in vitro (Kim et al., 2007; Rosenstiel et al., 2007; Kang et al., 2002). To further understand the relationships of saliva SAG level and inflammatory biomarkers, a multivariable adjusted linear regression model was performed. Our results showed that there was no statistical correlation between saliva SAG level and pro-inflammatory cytokines, whereas a higher peripheral monocyte count was correlated with lower saliva SAG level [B (95% CI), 0.510 (−0.363, 0.657), P = 0.725] (Table 4). In this study, all subjects were healthy children, whereas the above referenced inflammatory cytokine up-regulation of SAG expression occurred in the disease state or in vitro (Kim et al., 2007; Rosenstiel et al., 2007; Kang et al., 2002). To further understand the relationships of saliva SAG level and inflammatory biomarkers, a multivariable adjusted linear regression model was performed. Our results showed that there was no statistical correlation between saliva SAG level and pro-inflammatory cytokines, whereas a higher peripheral monocyte count was correlated with lower saliva SAG level [B (95% CI), 0.510 (−0.363, 0.657), P = 0.725] (Table 4). In this study, all subjects were healthy children, whereas the above referenced inflammatory cytokine up-regulation of SAG expression occurred in the disease state or in vitro (Kim et al., 2007; Rosenstiel et al., 2007; Kang et al., 2002). In addition, monocytes may reflect toxic changes (Chabot-Richards and George, 2014). This may be the reason for the results of the present study. Collectively, a high peripheral monocyte count may play a role in...
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### Appendix A. Supplementary data

Supplementary data to this article can be found online at [https://doi.org/10.1016/j.envint.2018.12.061](https://doi.org/10.1016/j.envint.2018.12.061).

### References


Fessler, M.B., Carney, M.U., Salo, P.M., Wilkerson, J., Cohn, R.D., King, D., Hoppin, J.A.,


Wang, B., Li, K., Jin, W., Lu, Y., Zhang, Y., Shen, G., Wang, R., Shen, H., Li, W., Huang, Y.,
Zhang, Y., Wang, X., Li, X., Liu, W., Cao, H., Tao, S., 2013. Properties and in-
flammatory effects of various size fractions of ambient particulate matter from
The acute airway inflammation induced by PM$_{2.5}$ exposure and the treatment of
related chronic mortality risk in China based on multi-model exposure data. Environ.
Int. 110, 105–112.
White, M.R., Crouch, E., van Eijk, M., Hartshorn, M., Pemberton, L., Tornoe, I., Holmskov,
288, L831–L840.
Wong, C.M., Lai, H.K., Tsang, H., Yeung, T.Q., Thomas, G.N., Lam, K.B., Chan, K.P., Yang,
Satellite-based estimates of long-term exposure to fine particles and association with
mortality in elderly Hong Kong residents. Environ. Health Perspect. 123, 1167–1172.
Wong, S.S.W., Rani, M., Dodagatta-Marri, E., Ibrahim-Granet, O., Kishore, U., Bayry, J.,
surfactant protein D-mediated opsonization of and host immune response to asper-
World Health Organization (WHO), 2016. Ambient air pollution: A global assessment of
exposure and burden of disease. http://www.who.int/phe/publications/air-
pollution-global-assessment/en/.
World Health Organization (WHO), 2018. Ambient (outdoor) air quality and health.
http://www.who.int/en/news-room/fact-sheets/detail/ambient-(outdoor)-air-
quality-and-health.
umbilical cord blood and relevant factors in neonates from Guiyu, China. Environ.
Yin, P., Brauer, M., Cohen, A., Burnett, R.T., Liu, J., Liu, Y., Liang, R., Wang, W., Qi, J.,
Wang, L., Zhou, M., 2017. Long-term fine particulate matter exposure and non-
accidental and cause-specific mortality in a large national cohort of Chinese men.
Environ. Health Perspect. 125, 117002.
Yorifuji, T., Kashiwa, S., Nishigaki, T., Sanada, S., Doi, H., 2017. Prenatal exposure to
outdoor air pollution and child behavioral problems at school age in Japan.
2013. Determining the relative importance of soil sample locations to predict risk of
child lead exposure. Environ. Int. 60, 7–14.
Zhang, Y., Wang, X., Chen, A., Huo, X., 2016. Heavy metals in PM$_{2.5}$ and in blood,
and children’s respiratory symptoms and asthma from an e-waste re-
function with mediation of blood parameters linked to e-waste lead and cadmium
Zeng, X., Xu, X., Qin, Q., Ye, K., Wu, W., Huo, X., 2018. Heavy metal exposure has adverse
effects on the growth and development of preschool children. Environ. Geoch.
Zhang, Y., Huo, X., Yao, J., Yang, T., Xu, L., Xu, X., 2016. Elevated lead levels and adverse
effects on natural killer cells in children from an electronic waste recycling area.
Environ. Pollut. 213, 143–150.
e-waste exposure are linked to decreased olfactory memory in children.
Environ. Pollut. 231, 1112–1121.
Ho, S.M., Lee, S.A., Reponen, T., Huo, X., 2016. Ambient air heavy metals in PM$_{2.5}$
and potential human health risk assessment in an informal electronic-waste recycling