E-waste polycyclic aromatic hydrocarbons (PAHs) exposure lead to child gut-mucosal inflammation and adaptive immune response

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Abstract

Polycyclic aromatic hydrocarbon (PAH) exposure alters immunological responses. Research concerning PAH exposure on intestinal immunity of children in electronic waste (e-waste) areas is scarce. The aim of this study was to evaluate the effects of polycyclic aromatic hydrocarbons (PAHs) pollutants on intestinal mucosal immunity of children in e-waste areas. Results showed higher hydroxylated PAH (OH-PAH) concentrations in e-waste-exposed children, accompanied with higher sialyl Lewis A (SLA) level, absolute lymphocyte and monocyte counts, decreased of percentage of $CD4^+$ T cells, and had a higher risk of diarrhea. OH-PAH concentrations were negative with child growth. 1-OHNap mediated through WBCs, along with 1-OHPyr were both correlated with an increase SLA concentration. 2-OHFlu, 1-OHPhe, 2-OHPhe, 1-OHPyr and 6-OHChr were positively correlated with secretory immunoglobulin A (sIgA) concentration. Our results indicated that PAH pollutants caused inflammation, affected the intestinal epithelium, and led to transformation of microfold cell (M cell). M cells initiating mucosal immune responses and the subsequent increasing sIgA production might be an adaptive immune respond of children in the e-waste areas. To our knowledge, this is the first study of PAH exposure on children intestinal immunity in e-waste area, showing that PAH exposure plays a negative role in child growth and impairs the intestinal immune function.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are listed as a kind of priority environmental pollutants with public health concerns (Ramirez et al., 2011). Existing studies show that diabetes, metabolic syndrome, cardiovascular disease, asthma, dyslipidemia, hematology,neurobehavioral disorders and cancer are related to exposure to PAHs (Hu et al., 2018a; Karimi et al., 2015; Kim et al., 2021; Manoli et al., 2016; Rengarajan et al., 2015; Roshandel et al., 2012; Yang et al., 2014; Yilmaz et al., 2007; Wang et al., 2020a; Zhang et al., 2020a). Studies also reveal a link between PAHs and gastrointestinal (GI) symptoms and diseases, and even GI tumors (Bansal and Kim, 2015; Diggs et al., 2011; Gunter et al., 2007; Henkler et al., 2012; Poirier et al., 2019; Prince, 2015; Roshandel et al., 2012). These findings strongly suggest an association between PAH exposure and impairment of the GI tract.

The GI tract is the main organ providing an internal barrier against environmental exposure, and plays an important role in the physical and immune barriers to entry of harmful compounds in the body (Arnal and Lalles, 2016; Ghosh et al., 2020). Breaking the epithelial barrier or even a minor disorder can lead to serious pathological consequences, including infection and inflammation (Citi, 2018). Mounting studies imply an association between PAHs and microflora. By altering bacterial communities and interrupting the function of the intestinal microflora, PAHs can cause intestinal inflammatory disorders and immune respond (Defois et al., 2018; Mantey et al., 2014; Roslund et al., 2019; Roslund et al., 2020). In vitro and in vivo studies have also shown that PAHs can affect the immune system (Abdel-Shafy and Mansour, 2016; Kim et al., 2013). However, very few studies have investigated alterations of the GI immune system (Abdel-Shafy and Mansour, 2016).

To maintain gut immune homeostasis, there are multiple layers of defense in the intestinal mucosa, including innate and adaptive defenses (Ren et al., 2016). M cells mediate antigen uptake and specific secretory immunoglobulin A (sIgA) production which play a vital role in adaptive defenses (Kobayashi et al., 2019). M cells initiate mucosal immune responses by active phagocytosis and transcytosis of luminal bacteria and antigen presentation to dendritic cells (DCs) in the underlying lymphoid follicles (Ohno, 2016). T cells and B cells are activated when DCs present antigen. Mucosal B cells then undergo IgA class switch recombination (CSR), migrate into the lamina propria and mature into IgA-producing plasma cells to produce IgA (Li et al., 2020; Liu et al., 2013). IgA binds to the polymeric immunoglobulin receptor (pIgR) and is transported across the cell to the lumen to form the molecule sIgA (Li et al., 2020).

SLA is a lectin largely restricted to M cells within epithelial tissues (Giannasca et al., 1999; Ragupathi et al., 2009). During inflammation, M cells are induced in the intestinal tract and their number increases in inflamed mucosa (Lugering et al., 2004). In vitro, epithelial cells are transformed to cells with an M-cell-like morphology and up-regulate SLA antigen production (Gullberg et al., 2000). It has been observed that an increase in IgA level is associated with the increase in SLA level in colon disease (Iarumov et al., 1998; Jasim et al., 2008). All these results suggest a link between M-cell differentiation and sIgA production in intestinal inflammation.

Although epidemiological studies have revealed a close link between PAH exposure and human digestive disease, the underlying mechanisms remain unexplored (Mantey et al., 2014; Shiue, 2016). Previous studies on PAH exposure and serum IgA expression are not consistent (Gao et al., 2014; Jeng et al., 2011; Karakaya et al., 1999; Szczeklik et al., 1994). Currently, limited studies of PAH exposure and human intestinal immunity have been about occupational exposure, and most of those studies have focused on IgA levels. Since serum IgA is monomolecular and sIgA is multimolecular, serum IgA cannot fully reflect the mucosal immunity (Li et al., 2020). According to the literature, most serum sIgA probably origin from the digestive tract and its levels of determination can be the most direct way to assess the amount of sIgA secretion in digestive tract (Pérez-Griera et al., 2017). The association between PAH exposure and serum sIgA level has not been studied, especially for the children in e-waste areas.

Guiyu is an e-waste recycling town located in Guangdong province, in southeast China, and has a more than 40-year history of e-waste disposal (Zeng et al., 2018). It has been reported that environmental medias surrounding e-waste dismantling areas extremely contaminated by PAHs from thermal recycling activities (Liu et al., 2020; Wang et al., 2020a). Our previous studies have shown that local residents in Guiyu are exposed to PAHs and have health problems (Guo et al., 2012; Huang et al., 2020; Wang et al., 2020a; Xu et al., 2013; Xu et al., 2015; Zeng et al., 2020; Zheng et al., 2019). Previous studies shows PAH exposure affects immune system (Abdel-Shafy and Mansour, 2016; Burchiel and Luster, 2001; Dupuy et al., 2014; Ekhator et al., 2018; Gou et al., 2017; Kim et al., 2013). To better understand the relationship between PAH exposure and intestinal immunity, we recruited children from Guiyu and Haojiang (as a reference area located 31.6 km to the east of Guiyu) for the current study. We hypothesize that PAH exposure may cause GI tract inflammation and lead to M cell differentiation, which may consequently alter intestinal immunity.

2. Materials And Methods

2.1. Study population

A total of 232 children (2–7 years old), all residents in Guiyu and Haojiang for more than one year, were included in this study (exposed group n=119 vs. reference group n=113). All children were recruited from two kindergartens during November to December 2018, and were free from general medical conditions and diseases. Apart from e-waste pollution, the two regions are very similar in ethnicity, cultural background and population. Informed consent with a questionnaire on general characteristics, dwelling environment, children's living habits, family history, monthly household income, and parental educational level was obtained from the parents or guardians of all participants. This study was approved by the Human Ethics Committee of Shantou University Medical College, China. As previously described, fasting venous blood was collected by a nurse. The whole blood was used to measure immune cells and serum was used to measure SLA and sIgA. The rest of the serum was aliquoted and stored at -80℃ until analysis (Zheng et al. , 2019).

2.2. Measurement of PAH metabolites in urine

Eleven urinary PAH metabolites [1-hydroxynaphthalene (1-OHNap), 2-hydroxynaphthalene (2-OHNap), 2-hydroxyfluorene (2-OHFlu), 9-hydroxyfluorene (9-OHFlu), 1-hydroxyphenanthrene (1-OHPhe), 2-hydroxyphenanthrene (2-OHPhe), 3-hydroxyphenanthrene (3-OHPhe), 4-hydroxyphenanthrene (4-OHPhe), 9-hydroxyphenanthrene (9-OHPhe), 1-hydroxypyrene (1-OHPyr) and 6-hydroxychrysene (6-OHChr)] were measured by gas chromatography/mass spectrometry (GC/MS, 7890A-5975C Agilent Technologies) according to previous studies, with electron ionization used in selected ion monitoring mode (Campo et al., 2008; Cheng et al., 2020; Dai et al., 2019; Huang et al., 2020; Huo et al., 2019; Wang et al., 2020b; Zheng et al., 2019). Methods for QA/QC were based on our previously published methods with minor modifications (Cheng et al., 2020; Dai et al., 2019). SOH-PAHs was defined as the sum of the eleven congeners in urine. SOHNap was defined as the sum of 1-OHNap and 2-OHNap. SOHFlu was defined as the sum of 2-OHFlu and 9-OHFlu. SOHPhe was defined as the sum of five OHPhe congeners in urine.

2.3. General physical tests and biological measurements

General physical examinations, including height, weight and chest circumference, were performed by trained physician as described previously (Dai et al., 2019; Wang et al., 2020b; Xu et al., 2015; Zeng et al., 2020). A Sysmex XE-2100 automatic hematology analyzer was used for determining the white blood cell count in peripheral blood. The serum levels of SLA and sIgA were measured with a CA19-9/Sialyl Lewis A (Human) ELISA Kit (KA0207, Abnova, Taiwan) and a Secretory IgA (Human) ELISA Kit (KA3980, Abnova, Taiwan). Sensitivity was 10 U /mL and 0.6 μg /mL, respectively. ELISAs were performed following the manufacturer's instructions.

2.4. Flow Cytometry

To determine the B lymphocytes ($CD3^-CD19^+$) and $CD3^+CD4^+CD8^-$T ($CD4^+$T cells) cells phenotype, 100 μL whole blood was mixed with appropriate volume of the following monoclonal antibodies: CD3-APC-Cy7, CD4-PE-CF594, CD8-FITC and CD19-PE-Cy7 (BD Bioscience, USA), and incubated for 15 min away from light at room temperature, then 2 mL of 1 × lysing solution (BD Bioscience, USA) was added and vortexed gently, and incubated for 10 min away from light at room temperature. After centrifugation at 500 g for 5 min, the supernatant was discarded, the cells were washed twice with 2 mL of 1× PBS, followed by resuspension in 500 μL of 1× PBS. Cells were analyzed by FACS using an CYTEK Aurora flowcytometer (CYTEK Biosciences inc., USA). Data was analyzed with Spectro Flo (CYTEK Biosciences inc., USA).

2.5. Statistical analysis

Data were analyzed by SPSS (Statistical Package for Social Sciences) version 22. All data were expressed as median and ranges or mean and standard deviation. We used the Pearson chi-square test, independent sample *t*-test, or Mann-Whitney *U* test to assess demographic and other characteristic differences between the two groups. Spearman's correlation analysis was performed to assess the relevant factors contributing to urinary PAH metabolites and the effects of PAH metabolites on the other indicators. Variables with skewed distributions were ln-transformed prior to regression and mediation analysis. $P < 0.05$ was set as the significance level in a two-tailed test.

3. Results

3.1. Basic characteristics of the study population

A total of 232 children were enrolled in the study (Table 1). Children in the two groups have no significant differences in gender and age ($P > 0.05$). The weight, height, chest circumference and BMI of children are lower in the exposed group ($P < 0.05$). Children in the exposed group reside in a poorer residential environment, and the majority children reside in a residence near a road or an e-waste site, or live in family workshops and have a family member who smoking (all $P < 0.05$). All these provide potential exposure to environmental pollution. The education level of the parents and monthly household income are lower for exposed children. Moreover, children in the exposed group more commonly display irritable bowel symptoms, diarrhea (more than three movements of loose stools a day) compared to the reference [odds ratio (OR) = 2.21; 95% CI: 1.16, 4.21; p-value = 0.014].

3.2. Urinary PAH metabolite concentrations and factors influencing OH-PAHs

Except for 9-OHFlu, 2-OHPhe, 3-OHPhe and 9-OHPhe, the other seven urinary OH-PAH concentrations are significantly higher in the exposed than the reference group (Table 2). Spearman correlation analysis showed that most urinary PAH metabolites are highly correlated with BMI, height, weight, and chest circumference (Table 3). In order to investigate the potential influencing factors of PAH exposure, a Spearman correlation analysis is performed (Table 4). Results show that urinary 1-OHNap concentration is positively correlated with e-waste contact, family workshops, residence within 50 m from an e-waste site and family member smoking. Urinary 1-OHNap, SOHNap and SOHPAHs concentrations are negatively

correlated with distance between residence and road, and urinary 1-OHNap, 1-OHPyr, SOHNap, SOHPhe, and SOHPAH concentrations are negatively correlated with the educational level of children's parents.

3.3. Peripheral leukocyte count and associations between urinary OH-PAHs

As shown in Fig. 1, the absolute lymphocyte and monocyte counts in the exposed group children are significantly higher than the reference group (both $P < 0.05$). Both white blood cells (WBCs) and absolute neutrophil counts of children in the exposed group tended to be higher than the reference group, but there is no significant difference (both $P > 0.05$). Spearman correlation analysis show that urinary 1-OHNap is positive correlated with WBCs and the absolute lymphocyte and monocyte counts ($r_s = 0.142$, $r_s = 0.147$ and $r_s = 0.206$ respectively, all $P < 0.05$), urinary 1-OHPyr is positive correlated with the absolute lymphocyte counts ($r_s = 0.132$, $P < 0.05$)

3.4. Comparison of SLA, sIgA concentration and immune cells

The SLA concentration of children in the exposed group is significantly higher compared with the reference group (Fig. 2, $P < 0.05$). The percentage of $CD4^+$ T cells is lower in the exposed group than the reference group ($P < 0.05$). The percentage of B cells tended to be higher in the exposed children, but no significance difference is obtained when compared with the reference (49.83 % vs. 46.58 %, $P > 0.05$).

3.5. SLA and sIgA concentration of the 4 and 5-year-old children

Due to the fact that children included in this study had sample bias, we only analyzed the subgroup of 4 and 5-year-old children from the two areas (Fig.3). Results show that for the 4-year-old children, both SLA and sIgA are both higher in the exposed group than reference (both $P < 0.05$), but no significance difference in the 5-year-old children. The sIgA concentration of 5-year-old children is lower than 4-years in the exposed group ($P < 0.05$), but no significance difference in the reference group.

3.6. Urinary PAH metabolite concentrations of the 4- and 5-year-old children

In the reference group, urinary SOHPAHs, 2-OHNap, 2-OHFlu, 1-OHPhe, 2-OHPhe, 4-OHPhe, 9-OHPh, 1-OHPyr, 6-OHCh, SOHNap and SOHPhe concentrations are higher in the 4-year-old group compared with the 5-year-old group (all $P < 0.05$). In the exposed area, the urinary OH-PAH concentrations between the two age groups show no significant difference (Fig.4).

3.7. Associations between urinary OH-PAHs with sIgA, SLA, and B cell percentage

A multivariable linear regression model was performed to identify the contributions of OH-PAHs to sIgA, SLA and B cell percentage in children (Fig. 5). Unadjusted regression analysis shows that 2-OHFlu, 1-OHPhe, 2-OHPhe, 1-OHPyr and 6-OHChr are positively correlated with sIgA concentration, 1-OHPyr is positively correlated with SLA concentration and 1-OHNap, 2-OHNap, 1-OHPyr, SOHNap and SOH-PAHs are positively correlated with the percentage of B cells. The correlations between 1-OHPhe, 2-OHPhe, 1-OHPyr, 6-OHChr and sIgA concentration, 1-OHPyr level and SLA concentration, and 1-OHNap and B cell

percentage remain significant after further adjustment for gender, age, BMI, contact with e-waste, parental educational level, and monthly household income.

3.8. Mediation effect analysis

Mediation model analysis shows that WBC concentration is a mediator in the correlation of 1-OHNap and SLA level (Fig. 6). In increasing WBC concentration, each 1-unit-increase in 1-OHNap concentration is estimated to be correlated with a 0.020 μg/mL increase in SLA level. However, the direct effect of 1-OHNap on SLA level is not statically significant in mediation effect analysis, indicating that WBC concentration is completely responsible for the mediation.

4. Discussion

In this study, we find that exposure group children had higher risk for diarrhea [odds ratio (OR) = 2.21] compared to the reference children. PAH exposure is negatively correlates with BMI, height, weight and chest circumference of the children. Most of hydroxylated polycyclic aromatic hydrocarbons (OH-PAHs) concentrations of children were higher in the exposed group and positively correlates with inflammation cells and intestinal immune biomarkers. Our results demonstrate that PAH exposure may be associated with gastrointestinal inflammation and immune responses. To our knowledge, this is the first study to provide evidence about the relationship between PAH exposure and intestinal immunity.

Urinary levels of OH-PAHs are widely used as a biomarker for estimating human exposure to PAHs from all routes of exogenous compounds (Lu et al., 2016; Yang et al., 2016). In this study, eight of eleven urinary OH-PAH concentrations of children are significantly higher in the exposed group than in the reference group, which is consistent with our previous studies indicating that children from e-waste recycling areas have elevated OH-PAH levels (Dai et al., 2019; Wang et al., 2020b; Xu et al., 2015; Zeng et al., 2020; Zheng et al., 2019). We find that 1-OHNap is negative with BMI, whereas most of the OH-PAHs negatively correlate with height, weight, and chest circumference, suggesting that PAH exposure has adverse effects on the development of children. As the recent studies described, we used biomonitoring studies to reveal the relationships between urinary PAH metabolite levels and several lifestyle and/or demographic variables (Keir et al., 2020; Oliveira et al., 2020). The results show that urinary 1-OHNap metabolites are positively correlated with e-waste contact, family workshops, residence within 50 m from an e-waste site and family member smoking. We also find that OH-PAHs are negatively correlated with parental education levels, which corroborates previous studies suggesting that child health is associated with parental educational attainment reflecting knowledge-related assets, as well as other health-related characteristics (Carozza et al., 2010; Faught et al., 2019) . In total, our findings indicate that pollution in e-waste areas and living habits affect children's urinary OH-PAH levels and have adverse effects on child growth.

Many studies have reported associations of PAH exposure and inflammation (Alshaarawy et al., 2013; Kamal et al., 2014; Zhang et al., 2020b). PAH exposure is known to induce oxidative stress and promote the production of reactive oxygen species (Dupuy et al., 2014; Huang et al., 2018; Jeng et al., 2011; Lu et

al., 2016; Yang et al., 2014; Yilmaz et al., 2007; Zhang et al 2020b). The results of the present study showed that 1-OHNap and 1-OHPyr correlate with inflammatory cells and both compounds are elevated in the exposed group, consistent with our prior studies demonstrating that PAH exposure is associated with inflammation (Cheng et al., 2020; Dai et al., 2019; Zheng et al., 2019) .

Relationship between PAH exposure and GI inflammation has been poorly studied in human. An animal study has indicated that PAHs can be metabolized and the metabolic products secreted into the GI tract to cause toxicity to epithelial cells (Mantey et al., 2014) . Except for the direct impact on the digestive tract, PAHs can also affect intestinal flora to cause intestinal inflammatory disorders by secreting toxic metabolic products, altering bacterial communities and interrupting the functions of the intestinal microflora (Defois et al., 2018; Mantey et al., 2014; Roslund et al., 2019; Roslund et al., 2020). Under inflammatory conditions, intestinal epithelial cells may be converted to M cells (Gullberg and Soderholm, 2006; Lugering et al., 2004). We applied a linear mediation model with adjustment for factors to quantify the association between PAH exposure and SLA expression. Results show that 1-OHPyr affects the SLA level directly while 1-OHNap affects the SLA level through WBC mediation. For each 1-unit increase in concentration of 1-OHPyr and 1-OHNap, the SLA concentration increases 0.093 μg / mL and 0.020 μg/mL, respectively. Collectively, our results suggest that PAH exposure may be linked to GI inflammation and leads to M cell differentiation. Previous studies indicated that 1-OHNap present almost exclusively in vapor phase and is associated with inhalation exposure while 1-OHPyr as particulate matter with dietary exposure (Kim et al.,2021; Lao et al. , 2018; Manoli et al., 2016; Nethery et al., 2012; Onyemauwa et al., 2009). We speculate that atmospheric PAH exposure causes a systemic inflammatory reaction and impairs the epithelium of the GI tract, whereas dietary PAH exposure directly modulates the gastrointestinal immune response, and both lead to M cell differentiation in the intestinal epithelium, as manifested by increased SLA concentrations in children.

Diarrhea is the manifestation of a disturbed gut environment as a symptom of an intestinal tract infection usually caused by a host of pathogens, which most likely results from disturbances in antigen-specific mucosal immune responses (Dong et al., 2017; Nagai et al., 2019; Yaya et al., 2018). We find that children in exposure group had higher risk for diarrhea [odds ratio (OR) = 2.21] compared to the reference. Previous studies have shown that PAH exposure was associated with suppression of T-cell proliferation and decreased the percentage of $CD4^+$ T cells, while lower numbers of $CD4^+$ T cells are predictive of chronic diarrhea (Gou et al., 2017; Lauer et al., 2019; Navin et al., 1999). In this study, the percentage of $CD4^+$ T cells decrease in the exposed group may associate with PAH exposure, which may be the reason for the diarrhea in exposed children. The correlation and regression analysis for OHPAHs and B cells is also consistent with a prior study reporting that exposure to PAHs might affect the differentiation of B cells (Huang et al., 2018). Together, our results suggest that PAH exposure may impair intestinal immune function, raise the risk of GI tract pathogen infection, lead children diarrhea and favor B-cell differentiation as an adaptive response.

Previous studies indicate that PAH exposure alters immunological responses and changes the expression of serum IgA. However, those results are not consistent. An increase of serum IgA level has been

suggested in bitumen workers exposed to PAHs compared to the control group, but this disparity was not significant (Karakaya et al., 1999). By contrast, Jeng et al. (2011) found an inverse association between levels of PAHs and IgA (Jeng et al., 2011). Szczeklik et al. (1994) also found that workers chronically exposed to PAHs had depression of mean IgA levels (Szczeklik et al., 1994). Gao et al. (2014) showed that individuals exposed to high levels of PAHs had significantly lower mean IgA level (Gao et al., 2014). Limited studies about associations between PAH exposure and serum IgA expression have yielded inconsistent findings. All of those studies focused on adult occupational exposure, by analyzing the association between PAH exposure with serum IgA, which cannot totally reflect the mucosal immunity. According to the literature, serum sIgA is presumed to be a reliable indicator of mucosal immunity and the increases levels are evidence of subclinical intestinal compromise (Arias et al., 2020; Pérez-Griera et al., 2017). Here we explored the relationship of PAH exposure and serum sIgA in the children in an e-waste area, which has not been studied. Results show that 1-OHPhe, 2-OHPhe, 1-OHPyr and 6-OHChr are estimated to be correlated with an increase in sIgA level, suggesting that PAH exposure might affect child mucosal immunity and elevate the level of serum sIgA.

Younger children are more seriously exposed to PAHs because they prefer to play and crawl around on the floor and ground, and display hand-to-mouth behavior (Huang et al., 2019; Oliveira et al., 2019). Our results showing that the concentrations of most OHPAHs decrease in the 5-year-old children than 4-year-old children in the reference group, supports this suggestion. However, in the exposed group, no significant difference of OHPAH concentration was observed between the 4- and 5-year-old children. We speculate that the high urine OHPAH levels of children in e-waste area are associated with the high concentration of PAHs in the environment. Even though behavioral changes with age can reduce PAH exposure, the urinary OH-PAH levels of older children remain high, indicating that environmental PAH pollution continues to pose a long-term serious threat to local children.

M cells have critical roles in intestinal sIgA production (Ren et al., 2016). For the 4-year-old, both SLA and sIgA are significantly elevated in the exposed group. Depending on various parameters, PAHs exert complex effects on the immune system resulting in immune-suppression or immune-potentiation (Abdel-Shafy and Mansour, 2016). Low levels of PAH exposure may lead to immune enhancement or an adjuvant effect (Burchiel and Luster, 2001). In the current study, we found that PAH exposure increases children’s sIgA levels. The reason may be that occupational PAH exposure is more serious than life-style exposure of children in e-waste areas. In addition, children enrolled in this study are all healthy individuals, and the concentrations of PAHs are estimated to be low, even in the exposed group. The effects of low levels of PAHs on human health, particularly in children, are unknown (Ekhator et al., 2018). We speculate that the increase level of sIgA might be an adaptive protective response of children to the external PAHs related to toxic intestinal inflammation, suggesting that mucosal immunity strengthens, to some degree, the protective mechanism against environmental irritants.

The limitations of this study are as follows. Firstly, though our study provides an association between PAHs and intestinal immune responses, this may not necessarily indicate a cause-and-effect relationship between PAH exposure and intestinal immune-mediated inflammation. Secondly, children included in this

study have sample bias, due to the insufficient number of samples of 2-, 3- and 6-year-old children, we only compare the subgroups of 4- and 5-year-old children for some parameters. We are not able to determine the trend of the influence of PAHs on mucosal immunity, so a large-sample follow-up observation is necessary. Thirdly, there is a wide variety of toxic substances in e-waste site. We only analyze the effect of PAH metabolites on mucosal immunity, and only collected morning urine samples at one point. To confirm our findings, more samples of contaminants and multiple measurements of PAH metabolites are needed for analyze.

5. Conclusion

In summary, this is the first study to identify the relationship between mucosal immune response and PAH exposure of children from an e-waste area. The results show diarrhea occurs more often in e-waste-exposed children, and PAH exposure has adverse effects on child growth. We also find that PAH exposure causes inflammation and leads to M cell differentiation, with subsequently initiating an adaptive immune response by secreting sIgA. Younger children are more susceptible to PAH exposure, especially in e-waste areas. These available data support the hypothesis that for young children in e-waste areas, low level of PAH exposure may lead to intestinal inflammation and alter the intestinal immune response, which may raise the risk of GI tract pathogen infection, lead children diarrhea and affect development. The elevation of sIgA levels may be a protective immune response to PAH exposure. Even behavioral changes with age can reduce PAH exposure, urinary OH-PAH levels remain high in older children in e-waste areas, suggesting PAH exposure poses a long-term health threat to the local children. It is necessary to take more preventive measures to further reduce organic pollutant exposure in e-waste areas and pay more attention to protect children from e-waste contamination.

Declarations

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Author contributions

GC: Conceptualization, Investigation, Formal analysis, Writing Original Draft. XL: Investigation, Formal analysis. ZC: Data Curation, Investigation. YZ: Investigation, Project administration. XX and XH: Review & Editing, Supervision, Project administration, Funding acquisition, Formal analysis. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest

Ethics approval

This study was approved by the Human Ethics Committee of Shantou University Medical College, China. Informed consent was obtained from each child's parents or guardians.

Consent to participate All authors were participated in this work

Consent to publish All authors agree to publish.

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Tables

Table 1

General characteristics of the study population

	N	Reference group	N	Exposed group	*p*
Gender (boys/girls)	113	68（60.2%）/ 45（39.8%）	119	66（55.5%）/ 53（44.5%）	0.467[a]
Age (median (IQR), years)	113	4.88 (4.37, 5.80)	119	5.11 (4.39, 5.76)	0.431[b]
Height (mean ± SD, cm)	113	109.29 ± 7.24	119	107.13 ± 7.52	0.027*[b]
Weight (median (IQR), kg)	113	18.50 (16.50, 20.25)	119	16.50 (15.00, 19.00)	0.000*[b]
BMI (median (IQR), kg/m2)	113	15.49 (14.71, 16.30)	118	14.93 (13.87, 15.75)	0.000*[b]
Chest circumference (median (IQR), cm)	113	52.50 (50.40, 54.95)	116	51.25 (49.63, 53.58)	0.026*[b]
Contact with electronic waste（yes/no）	113	12（11.65 %）/101（89.38 %）	119	33（27.73 %）/86（72.27 %）	0.002*[a]
Diarrhea (never/1~2 times monthly)	109	91（83.5 %）/18（16.5 %）	115	80（70.7 %）/35（29.3 %）	0.020*[a]
Distance between residence and road [n (%), m]	111		119		0.000*[a]
<10		15（13.52 %）		48（40.3 %）	
~50		30（27.03 %）		34（28.6 %）	
~100		24（21.62 %）		22（18.5 %）	
>100		42（37.83 %）		15（12.6 %）	
Residence within 50m from an e-waste site(yes/no)	112	3（2.68 %）/109（97.32 %）	116	27（23.28 %）/ 89（76.72 %）	0.000*[a]
Residence as a workshop(yes/no)	110	6（5.5%）/ 104（94.5 %）	119	32（26.9 %）/ 87（73.1 %）	0.000*[a]
Family member daily cigarette consumption [n (%)]	112		118		0.032*[a]
Non-smoking		56（50.0 %）		40（33.9 %）	
~ 2 cigarettes		12（10.7 %）		24（20.3 %）	
~10 cigarettes		15（13.4 %）		20（17.0 %）	
~20 cigarettes		25（22.3 %）		23（19.5 %）	
>20 cigarettes		4（3.6 %）		11（9.3 %）	
Father's educational level [n (%)]	113		119		0.000*[a]
Middle school or lower		23（20.4 %）		90（75.6 %）	
Secondary school		19（16.8 %）		8（6.7 %）	
High school		17（15.0 %）		12（10.1 %）	
College/university		54（47.8 %）		9（7.6 %）	
Mother's educational level [n (%)]	113		118		0.000*[a]
Middle school or lower		31（27.4 %）		87（73.7 %）	
Secondary school		21（18.6%）		10（8.5%）	
High school		15（13.3%）		9（7.6%）	
College/university		46（40.7%）		12（10.2%）	
Monthly household income [n (%), Yuan]	111		112		0.000*[a]

< 3000	12（10.8%）	11（ 9.8%）
~4500	27（ 24.3%）	26（23.2%）
~6000	19（17.1%）	46（41.1%）
> 6000	53（47.8%）	29 （25.9%）

BMI, body mass index. SD, standard deviation. Statistical significance,

* $P < 0.05$.

[a]Analysis by Pearson chi-square test.

[b]Analysis by independent-sample *t*-test.

Table 2

Urinary PAH metabolite concentrations in e-waste-exposed and reference groups

	Reference group (N = 113)	Exposed group (N = 119)	*p*
OH-PAH (μmol/mmol Cr) /median (25th, 75th)			
Urine-Cre	12.48 (7.25, 26.07)	12.27 (6.89, 24.12)	0.777
1-OHNap	0.18 (0.08, 0.34)	0.85 (0.47, 1.51)	0.000*
2-OHNap	3.16 (1.58, 5.48)	4.49 (2.57, 6.99)	0.008*
2-OHFlu	0.54 (0.26, 0.93)	0.64 (0.34, 1.11)	0.046*
9-OHFlu	1.86 (0.82, 4.59)	2.31 (1.13, 5.25)	0.167
1-OHPhe	0.77 (0.38, 1.40)	1.18 (0.59, 2.05)	0.001*
2-OHPhe	0.89 (0.46, 1.53)	1.04 (0.59, 1.82)	0.067
3-OHPhe	1.66 (1.12, 2.53)	1.85 (1.22, 3.17)	0.081
4-OHPhe	0.87 (0.46, 1.50)	1.06 (0.63, 1.85)	0.015*
9-OHPhe	0.79 (0.36, 1.37)	0.87 (0.48, 1.73)	0.117
1-OHPyr	1.26 (0.60,3.40)	2.49 (1.27, 5.49)	0.000*
6-OHChr	0.44 (0.23, 0.81)	0.56 (0.33, 0.95)	0.033*
SOHNap	3.46 (1.72, 5.82)	5.43 (3.14, 7.91)	0.000*
SOHFlu	2.50 (1.11, 5.64)	2.93 (1.47, 6.06)	0.136
SOHPhe	4.98 (2.75, 8.32)	6.15 (3.55, 10.80)	0.027*
SPHAs	13.77 (6.65, 23.01)	19.20 (11.18, 32.16）	0.002*

Cre: creatinine

Analysis by independent-sample *t*-test.

*$P < 0.05$.

**$P < 0.01$.

Table 3

Spearman analysis of the association between urinary PAH metabolites and characteristics of children

	BMI	High	Weight	Head circumference	Chest circumference
1-OHNap	-0.219**	-0.211**	-0.307**	0.002	-0.199**
2-OHNap	-0.090	-0.245**	-0.250**	-0.091	-0.222**
2-OHFlu	-0.078	-0.178**	-0.182**	-0.036	-0.162*
9-OHFlu	-0.077	-0.083	-0.099	0.025	-0.084
1-OHPhe	-0.043	-0.232**	-0.219**	-0.061	-0.180**
2-OHPhe	-0.067	-0.228**	-0.221**	-0.090	-0.200**
3-OHPhe	-0.075	-0.196**	-0.201**	-0.051	-0.167*
4-OHPhe	-0.092	-0.205**	-0.216**	-0.051	-0.187**
9-OHPhe	-0.060	-0.204**	-0.190**	-0.068	-0.175**
1-OHPyr	-0.075	-0.237**	-0.254**	-0.092	-0.187**
6-OHChr	-0.077	-0.223**	-0.220**	-0.091	-0.186**
SOHNap	-0.115	-0.253**	-0.272**	-0.083	-0.230**
SOHFlu	-0.074	-0.096	-0.107	0.022	-0.092
SOHPhe	-0.070	-0.221**	-0.218**	-0.064	-0.190**
SOHPHAs	-0.085	-0.213**	-0.219**	-0.048	-0.182**

*$P < 0.05$.

**$P < 0.01$.

Table 4

Spearman correlation analysis between urinary metabolites of PAHs and related factors

Related factors	1-OHNap	1-OHPyr	S OHNap	S OHPhe	SOHPAHs
	r_s	r_s	r_s	r_s	r_s
Electronic waste contact	0.207**	0.095	0.105	0.058	0.095
Residence as a workshop	0.237**	0.119	0.126	0.078	0.119
Distance between residence and road	-0.271**	-0.129	-0.138*	-0.093	-0.133*
Residence within 50 m from an e-waste site	0.161*	0.092	0.063	0.065	0.070
Family member cigarette smoker	0.136*	0.057	0.079	0.041	0.061
Father's educational level	-0.416**	-0.217**	-0.214**	-0.149*	-0.201**
Mother's educational level	-0.343**	-0.183**	-0.210**	-0.133**	-0.186**
Monthly household income	0.073	0.095	0.095	0.101	0.097

* $P < 0.05$.

** $P < 0.01$

Figures

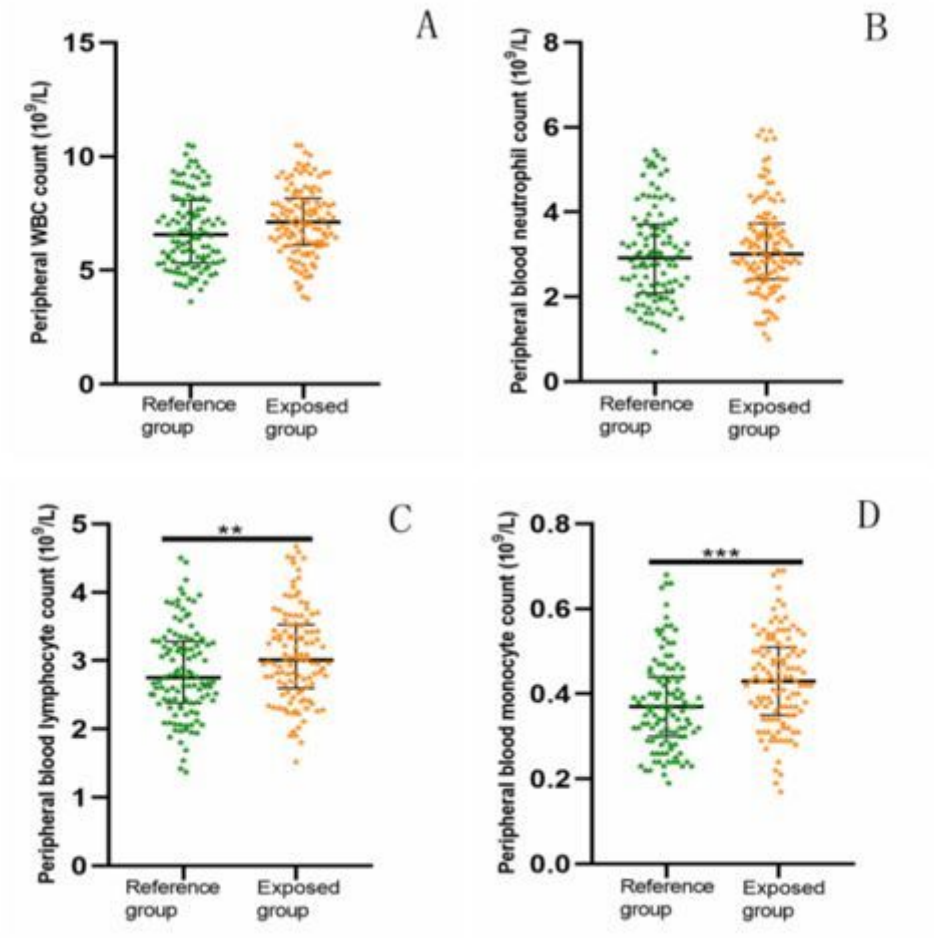

Figure 1

White blood cells, neutrophils, lymphocytes and monocytes between the two groups Reference group, n =113; exposed group, n = 119. A: Results are presented as mean ± standard deviation, analyzed by independent-sample t-test. B, C and D: Results are presented as median (interquartile range), analyzed by the Mann-Whitney U test. Values of *P < 0.05, **P < 0.01, ***P < 0.001 were considered statistically significant.

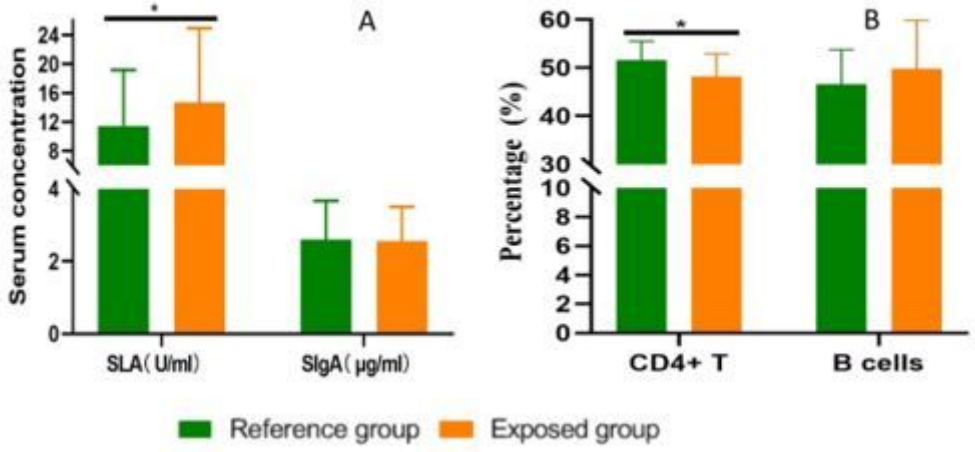

Figure 2

Biomarkers in exposed and reference groups A: Serum SLA and sIgA concentration in the two groups (SLA: exposed group, n = 108; reference group, n = 105; sIgA: exposed group, n = 113; reference group, n = 113); B: Percentage of CD4+ and B cells between the two groups (exposed group, n = 113; reference group, n = 119). Results are presented as mean ± standard deviation (median interquartile range) , obtained with an independent-sample t-test; values of *P < 0.05 were considered statistically significant.

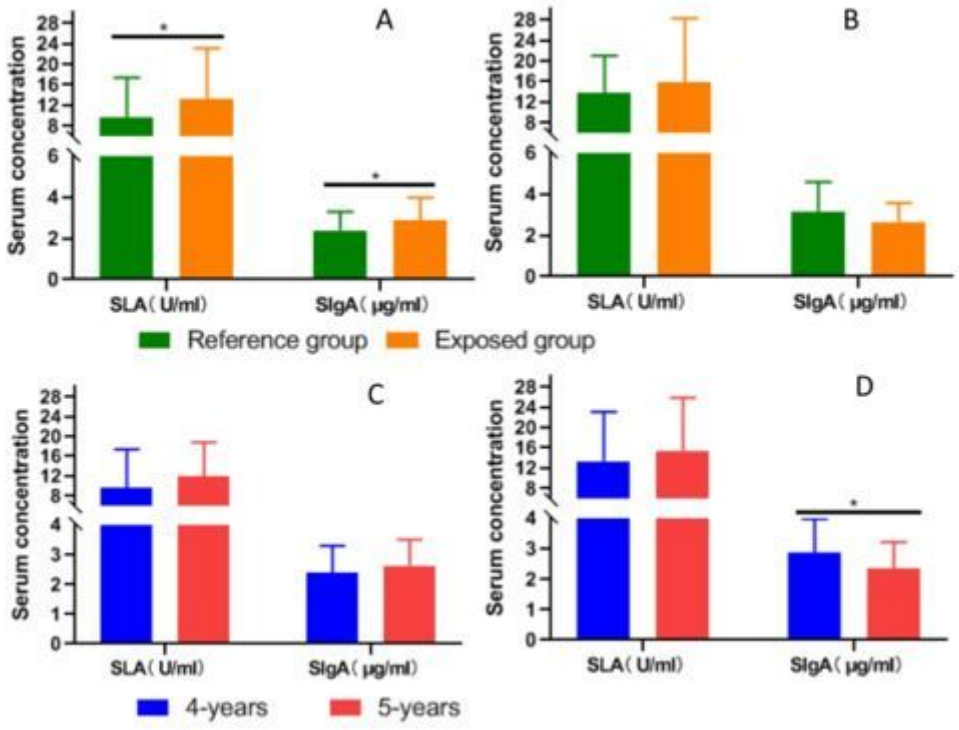

Figure 3

Subgroup analysis Serum SLA and sIgA concentration of 4- and 5-year-old children. A: 4-year-oldgroup (SLA: exposed group, n = 31; reference group, n = 42; sIgA: exposed group, n = 38; reference group, n =45), B: 5-year-old group (SLA: exposed group, n = 46; reference group, n = 31; sIgA: exposed group, n = 40; reference group, n =34). C: Reference group (SLA: 4-year-old group, n = 42; 5-year-old group, n = 31; sIgA: 4-year-old group, n = 45; 5-year-old group, n = 34), D: Exposed group (SLA: 4-year-old group, n = 31; 5-year-old group, n = 46; sIgA: 4-year-old group, n = 38; 5-year-old group, n = 40). Results are presented as median (interquartile range), analyzed by the Mann-Whitney U test. Values of *P < 0.05 indicate statistical significance.

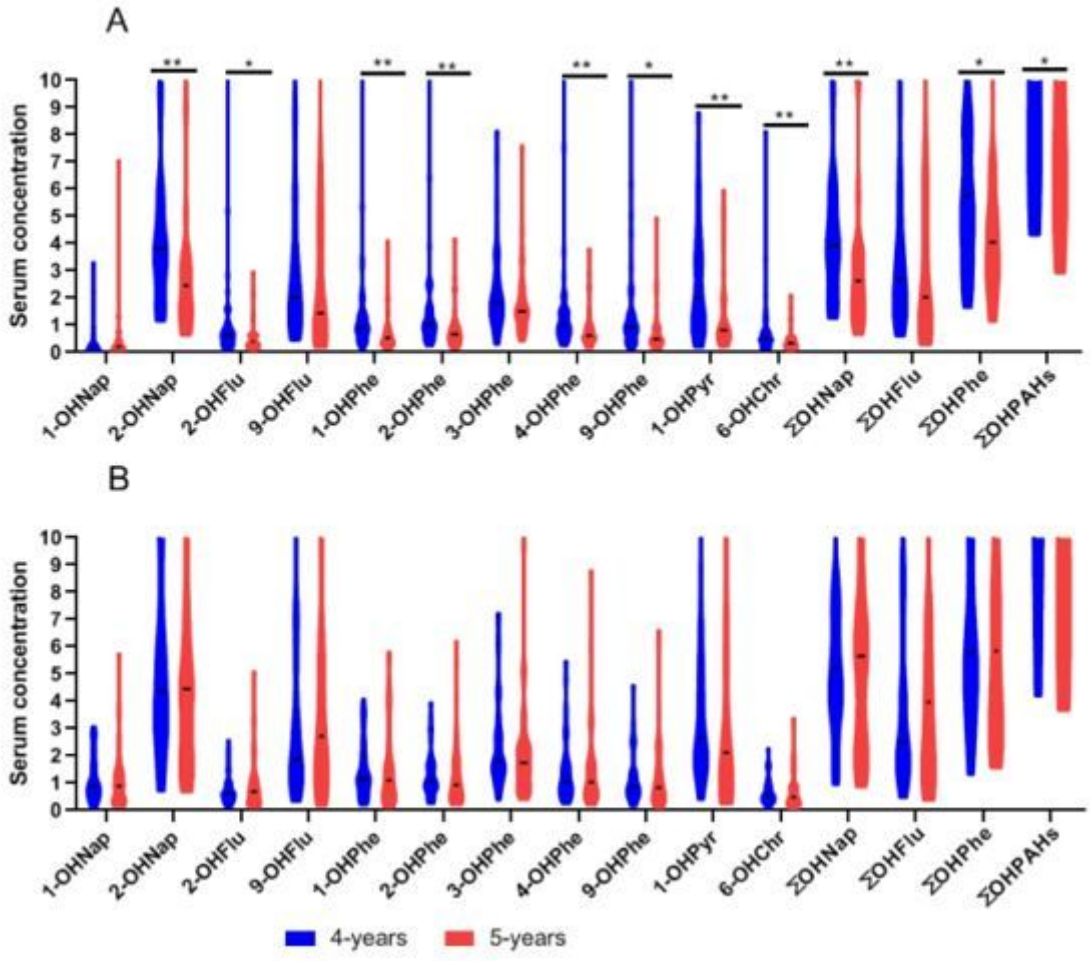

Figure 4

Subgroup analysis of urinary PAH metabolite concentrations (μg/g Cre) of 4- and 5-year-old children. A:Reference group (4-year-old group, n = 45; 5-year-old group, n = 36). B: Exposed group (4-year-old group, n = 38; 5-year-old group, n = 46). Analysis by independent-sample t-test. Values of *P < 0.05, **P < 0.01 were considered statistically significant

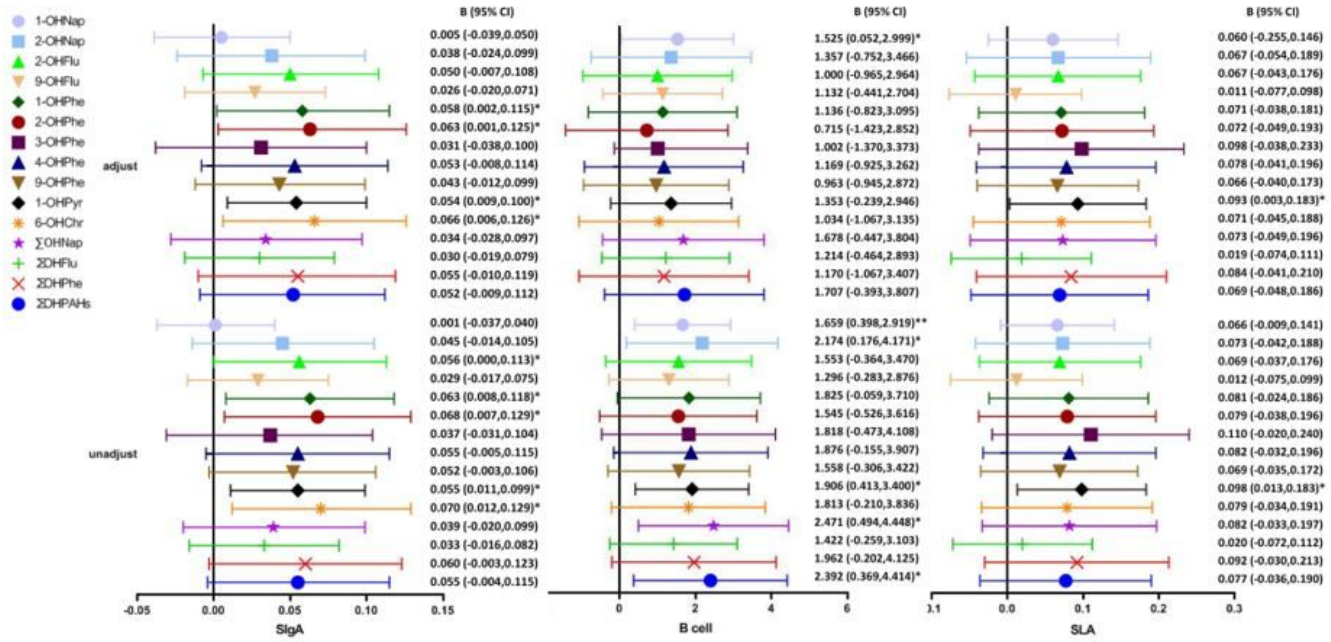

Figure 5

Effect estimates and 95% confidence intervals for OH-PAHs with sIgA, SLA and B cells. Adjusted model adjusting for gender, age, BMI, contact with e-waste, parental educational level, and monthly household income. BMI, body mass index; B, unstandardized coefficient; CI, confidence interval. Values of *P < 0.05, **P < 0.01 indicate statistical significance.

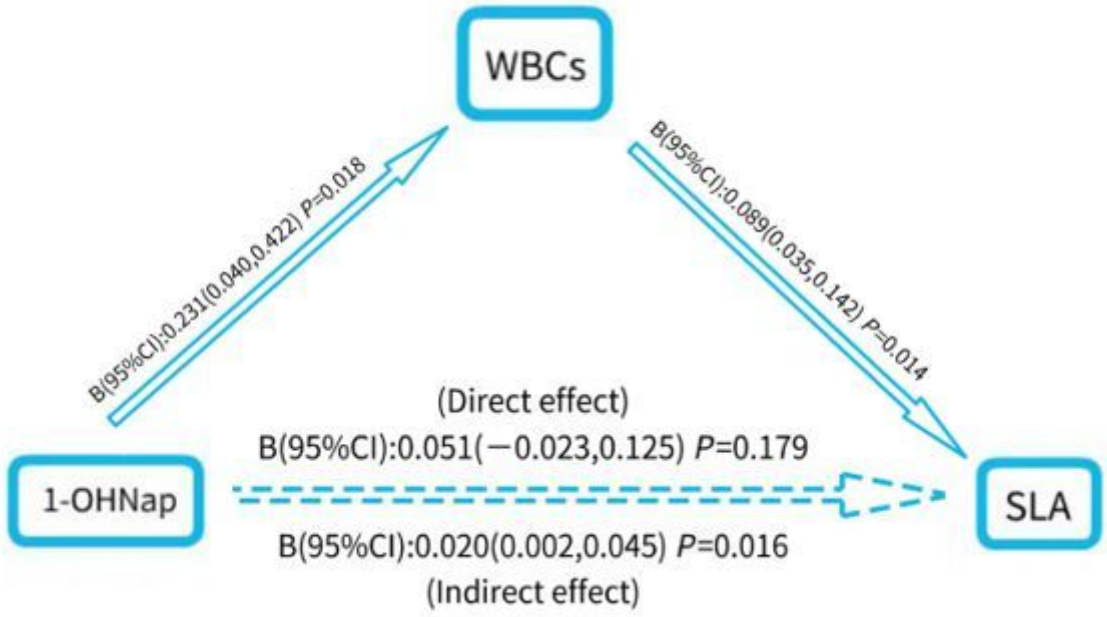

Figure 6

Mediation effect of WBCs on the relationship between 1-OHNap and SLA. B, unstandardized coefficient; CI, confidence interval；BMI; 5000 bootstrap samples; n= 203. Statistical significance, $P < 0.05$.