Aerosol and Air Quality Research

ORIGINAL RESEARCH
https://doi.org/10.4209/aaqr.220288

Associations between Children's Exposure to $PM_{2.5}$ and their Serum Inflammatory Responses in Taiwan

Wei-Jung Tseng[1], Jian-He Lu[2], How-Ran Chao[1,2,3,4*†], Ming-Hsien Tsai[2,5], Yu-Ting Chang[1], Liang-Jen Wang[6*†], Chih-Cheng Chen[7†], Wan-Nurdiyana-Wan Mansor[8], Juliana Jalaludin[9], Chih-Lung Wang[10,11], Ying-I Tsai[12]

[1] Department of Environmental Science and Engineering, College of Engineering, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan
[2] Emerging Compounds Research Center, General Research Service Center, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan
[3] Institute of Food Safety Management, College of Agriculture, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan
[4] School of Dentistry, College of Dental Medicine, Kaohsiung Medical University, Kaohsiung 80708, Taiwan
[5] Department of Child Care, College of Humanities and Social Sciences, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan
[6] Department of Child and Adolescent Psychiatry, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 83301, Taiwan
[7] Department of Pediatrics, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung 83301, Taiwan
[8] Faculty of Ocean Engineering Technology & Informatics, Universiti Malaysia Terengganu, 21300, Malaysia
[9] Faculty of Medicine and Health Science, Universiti Putra Malaysia, 43400, Malaysia
[10] Department of Civil Engineering and Geomatics, Cheng Shiu University, Kaohsiung 83347, Taiwan
[11] Center for Environmental Toxin and Emerging-contaminant Research, Cheng Shiu University, Kaohsiung 83347, Taiwan
[12] Department of Environmental Engineering and Science, Chia Nan University of Pharmacy & Science, Tainan 71710, Taiwan

OPEN ACCESS

Received: July 31, 2022
Revised: October 19, 2022
Accepted: October 30, 2022

* **Corresponding Authors:**
How-Ran Chao
hrchao@mail.npust.edu.tw
Liang-Jen Wang
wangliangjen@gmail.com

† These authors contributed equally to this work

Publisher:
Taiwan Association for Aerosol Research
ISSN: 1680-8584 print
ISSN: 2071-1409 online

Copyright: The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are cited.

ABSTRACT

$PM_{2.5}$-induced inflammation have been demonstrated in the cellular and animal models, but few studies reported the associations of schoolchildren exposure to $PM_{2.5}$ with their serum inflammatory biomarkers. Our goal was to examine whether serum inflammation was activated after children with long-term exposure to $PM_{2.5}$ in an industrialized city of Taiwan. Schoolchildren (n = 183) between the ages of 6 and 12 years living in southern Taiwan were recruited to measure their serum inflammation including interferon-γ (IFN-γ), IFN-α2, interleukin 1b (IL-1b), IL-2, IL-6, IL-10, IL-13, IL-17, monocyte chemotactic protein (MCP)-1, and tumor necrosis factor (TNF-α). The subjects were sorted into three groups based on residential addresses in low-$PM_{2.5}$, high-$PM_{2.5}$, and traffic-related-air-pollution (TRAP)-$PM_{2.5}$ areas on the basis of long-term $PM_{2.5}$ pollution data from air monitoring sites collected over the past decade. Children living in the TRAP-$PM_{2.5}$ areas had significantly higher TNF-α serum levels than those living in the low-$PM_{2.5}$ areas. Although serum levels of MCP-1 in children exposed to low $PM_{2.5}$ concentrations were lower than those in children exposure to high and TRAP $PM_{2.5}$ levels, the differences were nonsignificant. Principal component (PC) analyses revealed a close association between serum MCP-1 and outdoor $PM_{2.5}$ (Rotated PC2 (RPC2) and percentage of variance = 10.5%), whereas serum IFN-γ, IFN-α2, IL-1b, IL-2, IL-6, IL-10, IL-13, IL-17, and TNF-α were highly correlated (RPC1 and percentage of variance = 45.5%). Children's serum TNF-α was significantly linked to $PM_{2.5}$ exposure scenarios (p = 0.031)

by the test of the multivariate analysis (adjusted R^2 = 0.033, p = 0.039), but the other variables of age (p = 0.147) and gender (p = 0.291) were not statistically significant. In conclusion, serum TNF-α might be positively and significantly correlated with the longitudinal exposure of schoolchildren to $PM_{2.5}$, especially among children living in TRAP-$PM_{2.5}$ region.

Keywords: $PM_{2.5}$, Tumor necrosis factor (TNF-α), Inflammatory responses, Traffic-related air pollution (TRAP), Health risk

1 INTRODUCTION

Airborne particulate, particularly respirable or fine particle matter (PM) whose aerodynamic diameter is less than 2.5 μm ($PM_{2.5}$), has been recognized as the main contribution to the worst air pollution in decades. The global concern towards $PM_{2.5}$ pollution due to the increase in adverse health effects including heart attack, cardiovascular disease, pulmonary disease including chronic obstructive pulmonary disease (COPD), lung cancer, chronic kidney disease (CKD), diabetes mellitus (DM), and neurological disorders is increasing (Shah *et al.*, 2013; Meier *et al.*, 2014; Wang *et al.*, 2015; Feng *et al.*, 2016; Kao *et al.*, 2019; Shou *et al.*, 2019; Grande *et al.*, 2021; Li *et al.*, 2021). $PM_{2.5}$ is emitted from vehicular engines, industrial processing, open biomass burning, chemical industries, cooking fume, resuspended road dust, incomplete combustion from fossil fuels, and agricultural and construction processing (Fann *et al.*, 2012; Aunan and Wang, 2014; Loftus *et al.*, 2015; Chao *et al.*, 2018; Mukherjee and Agrawal, 2018; Chen *et al.*, 2019; Yu *et al.*, 2021). $PM_{2.5}$ from the long-range atmospheric transport (LRAT) or transboundary transportation through Asian dust storms or monsoon mainly contributed to $PM_{2.5}$ pollution in the winter and spring seasons of Taiwan (Liu *et al.*, 2017; Shen *et al.*, 2020; Yu *et al.*, 2021). According to the report "Particulate Matters ($PM_{2.5}$) Precursor Demonstration Guidance" by the United States Environmental Protection Agency (U.S. EPA) (Mathias and Wayland, 2019), nitrogen oxides, sulfur dioxide, volatile organic compounds, aldehydes or ketones, and ammonia, which are the precursors of $PM_{2.5}$ associated with the traffic-related air pollutants (TRAPs), which was easily developed or converted into $PM_{2.5}$. TRAP-$PM_{2.5}$-bound chemicals might be linked with several inorganic or organic pollutants, such as carbon black, polycyclic aromatic hydrocarbons (PAHs), heavy metals, carbonaceous compounds, and chlorinated dioxins/furans (Chao *et al.*, 2016). The $PM_{2.5}$ is characterized by large surface area and small particle diameter, which enables it to induce a variety of toxic responses (Xing *et al.*, 2017; Chung *et al.*, 2019, 2020). Several environmental factors such as temperature, relative humidity, transboundary pollution, and season variation might be related to the $PM_{2.5}$ concentrations in the local region of Taiwan (Chen *et al.*, 2019). Compared with PM_{10} (PM diameter less than 10 μm), the small size and large surface area of $PM_{2.5}$ render potentially harmful chemicals to adhere to these particles (Hassanvand *et al.*, 2015; Pun *et al.*, 2017; Xing *et al.*, 2017; Chen *et al.*, 2019; Hao *et al.*, 2022).

$PM_{2.5}$ inhaled directly through the airway will deposit and penetrate the lung alveoli into the blood circulation (Han and Zhuang, 2021; Xie *et al.*, 2021). TRAP $PM_{2.5}$ poses potential threats to human health by further activating the inflammatory responses, leading to DNA damage or adducts, and causing epigenetics modifications, which is detrimental to lung function (Li *et al.*, 2014; Chao *et al.*, 2018; Hisamuddin *et al.*, 2020; Suhaimi *et al.*, 2021; Putri Anis Syahira *et al.*, 2020). In our previous study (Chao *et al.*, 2018), compared with policemen working office jobs, policemen employed as traffic conductors exhibited significantly higher levels of serum proinflammatory biomarkers, such as tumor necrosis factor (TNF-α), which was attributed to heavy exposure to TRAP $PM_{2.5}$. The value of TNF-α equivalence (TNFα-EQ) in the serum from the exposed group (the traffic conductors) was 0.0302 ± 0.0048 pg TNFα-EQ mL^{-1} was significantly higher than that whose level was 0.00440 ± 0.000800 pg TNFα-EQ mL^{-1} from the reference control (the office policemen) (Chao *et al.*, 2018). Taxi drivers exhibited systematic inflammation and high concentrations of serum cytokines (e.g., TNF-α) after they were exposed to TRAP (Brucker *et al.*, 2013). $PM_{2.5}$-bound hazardous air pollutants (HAPs) (e.g., PAHs induced the activation of systematic inflammatory responses, including TNF-α, C-reactive protein (CRP), interleukin-1b (IL-1b), intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1), in the blood (Huang *et al.*, 2013; Liu *et al.*, 2017; Chao *et al.*, 2018; Wang *et al.*, 2018; Rahmatinia *et al.*, 2021). $PM_{2.5}$ also induced

cytokine release and oxidative stress to be involved lung cancer and chronic airway inflammatory diseases like asthma and COPD (Li *et al.*, 2018).

$PM_{2.5}$ may trigger neurodevelopmental disruption or delay and may affect neurological behavior in humans. Recent studies also reported a negative association of TRAP $PM_{2.5}$ with neurological development or function, such as function deterioration of the central nervous system, delay of neurobehavior, and autism spectrum disorder (ASD) in the early age of life (Becerra *et al.*, 2013; Raz *et al.*, 2015; Sram *et al.*, 2017). Aside from TRAP $PM_{2.5}$, ambient $PM_{2.5}$ is also a neurotoxicant that triggers harmful effects on the brain, especially during the utero, infancy, and early childhood (Sassá *et al.*, 2011). According to the current epidemiology, prenatal or postnatal children exposed to severe $PM_{2.5}$ has a high risk of developing ASD and disrupting the motor and cognitive development of infants (Lertxundi *et al.*, 2015; Talbott *et al.*, 2015; Xi and Wu, 2021). A review article exploring the relationship of ASD and attention deficit hyperactivity disorder (ADHD) with $PM_{2.5}$ exposure concluded that although the evidence was insufficient to establish a causal relationship between ADHD and $PM_{2.5}$, ADHD development was linked to several factors, such as genome (e.g., transgenerational effects), *in-utero* exposure, and home environment that were easily influenced by $PM_{2.5}$, whereas high levels of $PM_{2.5}$ were associated with acceleration of ASD development (Xi and Wu, 2021). In an animal model, decreased responses of brain-derived neurotrophic factor (BDNF) expression and cAMP response element binding protein (CREB) phosphorylation were observed in the immature rats with high-dose exposure to $PM_{2.5}$ (Liu *et al.*, 2021). $PM_{2.5}$ might also disrupt emotional and cognitive development (Liu *et al.*, 2021). The evidence suggests that children's health is susceptible to $PM_{2.5}$ exposure and $PM_{2.5}$ is a potentially toxic precursor that causes neurological and neurobehavioral impairment.

2 METHODS

2.1 The Studying Participants

This cross-sectional study was followed up an established cohort from the Kaohsiung Chang Gung Memorial Hospital (KCGMH) ADHD case-control (KCGMH-ADHD cohort) study. The KCGMH-ADHD cohort was recruited from the Department of Child Psychiatry of KCGMH in southern Taiwan. Specially, patients with ADHD were recruited from the outpatient Department of Child Psychiatry of KCGMH, and children without ADHD were recruited from the communities surrounding KCGMH to serve as controls (Wang *et al.*, 2022a). Of the 234 participants aged 6–12 years from the KCGMH-ADHD cohort, 183 children, comprising 115 ADHD patients (case) and 68 children without ADHD (control), were selected to participate in the present study based on completed questionnaires, inflammatory biomarker data, and submission of a complete and correct residential address. The study protocol was approved by the Institutional Review Boards (IRB) of the Human Ethical Committee of the KCGMH. All ethical standards outlined in the Declarations of Helsinki were followed. After receiving a detailed explanation of the study and its potential consequences, all participants' parents or guardians signed written informed consent forms before the enrolment. The subjects were selected according to the criteria described in our previous study (Wang *et al.*, 2022a). The inclusion criteria for the patients with ADHD were as follows: (1) verified clinical diagnosis of ADHD according to Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) at the age of 6–12 years; (2) drug-naïve; and (3) no recorded history of comorbid neurodevelopmental or psychiatric disorder, such as ASD, intellectual disability, psychotic disorder, bipolar disorder, epilepsy, or brain injury. The children without ADHD were selected based on the following requirements: (1) age-matched to the study group; (2) without diagnosis of ADHD; and (3) no recorded history of major neurological, neurobehavioral developmental, or psychiatric disorder including ASD, intellectual disability, psychotic disorder, major depressive disorder, substance dependence, or severe head trauma.

2.2 Neurological Measurements

The neurological and neurobehavioral status of the participants from the KCGMH-ADHD cohort was determined by an experienced child psychologist by the Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV) (Baron, 2005; Wang *et al.*, 2022a). The behavioral symptoms were examined using the standard questionnaires of the Swanson, Nolan, and Pelham Version IV

Scale (SNAP-IV), specifically the SNAP-IV parent form (answered by the child's parents) and the SNAP-IV teacher form (answered by the child's homeroom teacher).

The parent and teacher SNAP-IV forms each comprised a 26-item questionnaire evaluating ADHD symptoms, severity, and needs (Zhang *et al.*, 2005; Bussing *et al.*, 2008). These 26 items included symptoms of inattention (n = 9), hyperactivity/impulsivity (n = 9), and oppositional defiant disorder (n = 8). Each item was scored on a Likert scale ranging from 0 to 3. The present study only selected the core ADHD symptoms (inattention and hyperactivity/impulsivity subscales) for further evaluation.

2.3 Biochemical Tests of Inflammatory Markers

Blood samples from the participants were collected between 8:00 and 9:00 AM after an overnight fast. Approximately 2–4 c.c. of serum was collected from each participant. Each blood sample was centrifuged at 3000 rpm for 5 min and then stored at −80°C. Pre-inflammatory biomarkers including TNF-α, monocyte chemotactic protein (MCP)-1, interleukin 1b (IL-1b), IL-2, IL-6, IL-10, IL-13, IL-17, interferon-γ (IFN-γ), and IFN-α2 in the serum were determined using a custom multiplex Laminex immunoassay (Luminex, Austin, TX). Recombinant cytokines pre-diluted in pooled blank plasma from HCs were used as standards for measurement. To detect antigen-reactive IgG, 50 μL of diluted Microparticle Cocktail was mixed with 50 μL of diluted serum and standard, and then incubated with detection antibodies for 2 h at room temperature (22 ± 3°C) with shaking at 800 rpm. After washing, 50 μL well^{-1} of diluted Biotin-Antibody Cocktail was added and incubated for 1.5 h at room temperature with shaking. The plates were washed again and 50 μL well^{-1} of phycoerythrin-conjugated streptavidin was added and incubated for 30 min at room temperature with shaking. Finally, the concentration of cytokines in the bead array was measured based on the fluorescence intensity calculated using the Flowmetrix software. All the biomarker measurements exceeded the limits of detection (LODs). The relative standard deviations of intra-assay precision and inter-assay precision were within 10% for all measured markers.

2.4 $PM_{2.5}$ in the Ambient Air

The long-term data of $PM_{2.5}$ in the ambient air from 12 TEPA air monitoring sites (Ciaotou, Nanzi, Mino, Linyuan, Fuxing, Fengshan, Qianzhen, Qianjin, Zuoying, Renwu, Xiaogang, and Daliao) during 2010−2019 and one air monitoring station (Ai-Gou) during 2016-2019 were obtained from the Environmental Protection Bureau Kaohsiung Government (KEPB) (Fig. 1 and Table S1). The historical $PM_{2.5}$ levels were recorded from the websites of TEPA and KEPB. Finally, three types of $PM_{2.5}$ exposure scenarios were determined.

2.5 Statistical Analysis

Further statistical analysis was conducted on biomarkers with measurements exceeding the LODs. Descriptive analysis was used to determine the means and standard deviations (SD) of demographics, ADHD symptoms, SNAP-IV forms, IFN-α, IFN-γ, IL-16, IL-2, IL-6, IL-10, IL-13, IL-17, MCP-1, and TNF-α stratified by three $PM_{2.5}$ exposure scenarios. Evaluation of normality was performed using the Shapiro-Wilks or Kolmogorov-Smirnov method. An unpaired independent two-tailed student's *t*-test assessed the differences in measured parameters among the three $PM_{2.5}$ exposure scenario groups. A logistic regression was used to examine levels of inflammatory biomarkers (e.g., TNF-α) among the three $PM_{2.5}$ exposure scenarios. The multivariate analysis was used to predict inflammatory biomarker levels by the $PM_{2.5}$ exposure scenarios after the age, gender, or ADHD were adjusted (Model 1 was adjusted for age and gender, Model 2 was adjusted for age, gender, and ADHD.). Model 3 was used to determine the association between ADHD and inflammatory responses with adjustment of age, gender, and $PM_{2.5}$ exposure scenarios. All statistical analyses were performed using Statistical Product and Service Solutions, version 12.0 (Chicago, Ill., USA). A *p*-value of less than 0.05 represented statistical significance.

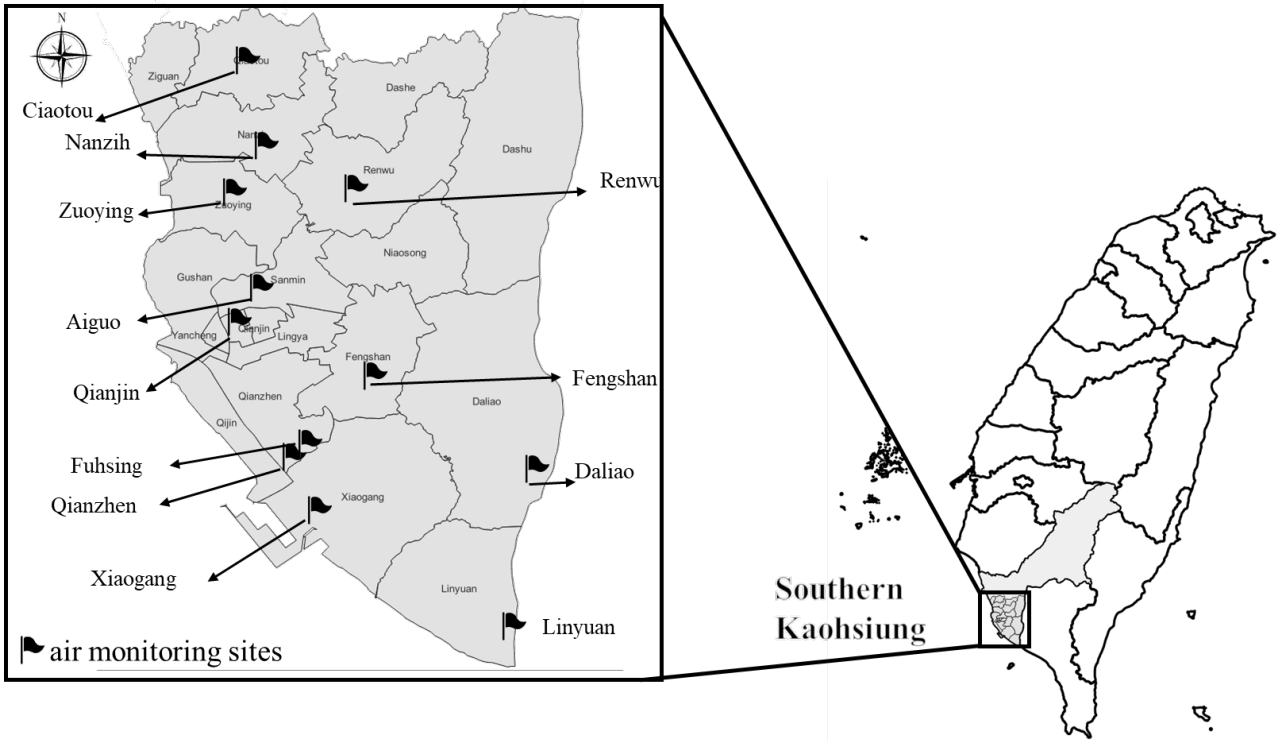

Fig. 1. $PM_{2.5}$ concentrations in 12 air monitoring sites from TEPA or KEPB.

3 RESULTS AND DISCUSSION

3.1 The Historical Data of $PM_{2.5}$ in the Studying Region

Upon examining the historical records and long-term exposure data for $PM_{2.5}$ in the study area (Kaohsiung City), we observed a substantial reduction (almost 50%) in $PM_{2.5}$ from 2010 to 2019 (Table S2). $PM_{2.5}$ emissions improved in the study area over the past decade. The study area is the economic center of southern Taiwan and is location of several key industries including manufacturing, steel-making, oil refining, freight transport, and shipbuilding. According to the big data analysis of $PM_{2.5}$ detected by $PM_{2.5}$ sensors in Kaohsiung from our previous study (Chen *et al.*, 2019), four main patterns of $PM_{2.5}$ were concentrated in the industrial zones or the harbors in the northern and southern Kaohsiung. The TRAP $PM_{2.5}$ pattern was independent of the four main types of $PM_{2.5}$ patterns (Chen *et al.*, 2019; Yu *et al.*, 2021). In the present study, the Sanmin and Fengshan districts located in the central Kaohsiung were selected as the typical TRAP $PM_{2.5}$ areas in the present study. According to the historical and annual records of $PM_{2.5}$ by TEPA and KEPB and the $PM_{2.5}$ patterns in the studying areas from our previously published paper (Chen *et al.*, 2019), $PM_{2.5}$ levels in the study areas could be categorized into three types, specifically low (Ciaotou, Nanzi, Mino, and Linyuan), high (Fuxing, Qianzhen, Qianjin, Zuoying, Renwu, Xiaogang, and Daliao), and TRAP (Sanmin and Fengshan) $PM_{2.5}$. Further statistical analyses were stratified by these three types of $PM_{2.5}$ exposure scenarios in the present study.

3.2 $PM_{2.5}$ and Serum Cytokines in School-children

The schoolchildren in the present study were categorized into three groups, namely low-$PM_{2.5}$, high-$PM_{2.5}$, and TRAP-$PM_{2.5}$ exposure groups, on the basis of their residential addresses (Table 1). Descriptive analyses of neurological or neurobehavioral development and inflammatory biomarkers were stratified by the three types of $PM_{2.5}$ exposure scenarios (low-$PM_{2.5}$, high-$PM_{2.5}$, and TRAP-$PM_{2.5}$ areas). Most of the SNAP-IV scores from the parent and teacher questionnaires displayed the significant differences ($p < 0.05$) among the three $PM_{2.5}$ exposure groups. ADHD symptoms were highly correlated with SNAP-IV records in the $PM_{2.5}$ exposure groups. Because the participants in this study were from the KCGMH-ADHD cohort (Wang *et al.*, 2022a), the ratio of children with

Table 1. Neurological or neurological behavioral development and inflammatory responses in children at the age between 5 and 16 with exposure to three scenario types of $PM_{2.5}$ (n = 183).

Characteristics	Low $PM_{2.5}$[a]	High $PM_{2.5}$[b]	TRAP $PM_{2.5}$[c]	p [ab]	p [ac]	p [bc]
	Mean ± SD	Mean ± SD	Mean ± SD			
Number (person)	32	80	71	0.842	0.535	0.312
Age (year)	9.18 ± 2.32	9.08 ± 2.63	9.48 ± 2.21			
Gender (person)				0.334	0.072#	0.174
Male	26	59	47			
Female	6	21	24			
Height (cm)	134.2 ± 14.5	135.3 ± 14.2	135.8 ± 13.9	0.954	0.983	0.996
Weight (kg)	34.5 ± 14.6	33.8 ± 12.9	33.5 ± 11.9	0.928	0.932	0.945
ADHD (person)				0.005**	0.001**	0.693
NO	3	32	33			
YES	29	48	38			
Parents' form (score)						
HI	13.7 ± 7.09	10.5 ± 8.18	9.18 ± 7.75	0.060#	0.008**	0.296
Inattention	15.7 ± 6.69	11.5 ± 7.48	11.1 ± 8.04	0.010*	0.006**	0.747
Opposition	11.3 ± 6.87	9.09 ± 6.83	7.91 ± 6.39	0.114	0.019*	0.289
Teachers' form (score)						
HI	10.1 ± 7.45	8.70 ± 7.58	6.53 ± 6.92	0.359	0.024*	0.081#
Inattention	13.4 ± 1.07	10.9 ± 7.37	8.41 ± 7.09	0.093#	0.001**	0.046*
Opposition	7.22 ± 7.03	5.95 ± 6.23	4.97 ± 5.83	0.336	0.096#	0.357
Inflammatory makers (ρg mL^{-1})						
IFN-α2	27.2 ± 63.1	20.5 ± 47.6	44.3 ± 196	0.540	0.631	0.294
IFN-γ	18.3 ± 20.2	27.3 ± 49.8	23.4 ± 48.4	0.324	0.568	0.626
IL-1b	1.90 ± 1.02	2.14 ± 1.80	4.30 ± 19.5	0.492	0.490	0.325
IL-2	1.16 ± 0.998	2.06 ± 5.20	3.75 ± 19.1	0.337	0.448	0.448
IL-6	1.63 ± 1.56	2.80 ± 6.11	5.94 ± 20.9	0.290	0.088#	0.225
IL-10	2.23 ± 3.09	3.69 ± 6.14	10.1 ± 49.5	0.098#	0.374	0.285
IL-13	44.3 ± 105	41.4 ± 101	34.2 ± 92.8	0.891	0.625	0.651
IL-17	10.2 ± 13.8	12.3 ± 21.8	11.8 ± 23.4	0.624	0.718	0.908
MCP-1	436 ± 162	454 ± 151	503 ± 190	0.582	0.089#	0.081#
TNF-α	10.7 ± 4.70	12.6 ± 7.36	20.2 ± 31.7	0.181	0.016*	0.053#

# $p < 0.1$; * $p < 0.05$; ** $p < 0.01$.
[a] Low $PM_{2.5}$ exposure scenario region.
[b] High $PM_{2.5}$ exposure scenario region.
[c] Traffic-related-air-pollution (TRAP) $PM_{2.5}$ exposure scenario region.

ADHD symptoms (62.8%) in this study was higher than that in the general Taiwanese population (3%–7%). The sex ratios (male/female) of the cohort in the present study, the prevalence of Taiwanese ADHD cases, and the Taiwanese general population were 2.59, 3.0, and 1.08, respectively. Xi and Wu (2021) found that air pollutants, including $PM_{2.5}$, was significantly associated with the development of ASD, but the occurrence of ADHD appeared to be connected to genetics, daily diet and nutrition, perinatal conditions, and home environment. Dunn *et al.* (2019) indicated that ADHD etiology was related to increased serum cytokine levels and the genetic polymorphism of relevant inflammatory pathways, which exacerbated ADHD through inflammatory mechanisms after children were exposed to environmental toxicants. In our previous study, we observed that, compared with children in the control group, children with ADHD had significantly lower plasma TNF-α levels (Wang *et al.*, 2022b). Although $PM_{2.5}$ exposure could not be definitively linked to ADHD based on the current evidence, several ADHD patients might be comorbid with inflammatory and autoimmune disorders; therefore, the possible contribution of $PM_{2.5}$ exposure to ADHD was also adjusted for in the present study. $PM_{2.5}$ penetrates the alveolar cells of the lung directly through the airway and is further transported to the circulatory system by alveolar cells (Han and Zhuang, 2021; Xie *et al.*, 2021). In addition to causing neurological disorders, $PM_{2.5}$ can lead to

premature death caused by inflammatory responses or local or systematic disorders such as lung cancer, cardiovascular diseases, COPD, respiratory disease, and pneumonia (Pun *et al.*, 2017; Mukherjee and Agrawal, 2018; Chowdhury *et al.*, 2019).

In the present study, the IL-6 levels in the low-$PM_{2.5}$ participants were margin-significantly higher than those in the TRAP-$PM_{2.5}$ participants ($p = 0.088$). Compared with the high-$PM_{2.5}$ participants, the low-$PM_{2.5}$ participants had a borderline-significantly lower IL-10 level ($p = 0.098$). MCP-1 concentrations in the higher exposure groups including the high-$PM_{2.5}$ ($p = 0.089$) and TRAP-$PM_{2.5}$ ($p = 0.081$) participants were higher than those in the low-$PM_{2.5}$ exposure group, with borderline significance. Finally, the participants in the TRAP-$PM_{2.5}$ exposure group had the significantly higher TNF-α levels than those in the low-$PM_{2.5}$ exposure group ($p = 0.016$), whereas the participants in the high-$PM_{2.5}$ group exhibited higher TNF-α concentrations than the participants in the low-$PM_{2.5}$ area did, with the margin significance ($p = 0.053$).

Several articles have addressed the associations of $PM_{2.5}$ with the levels of inflammatory cytokines such as TNF-α in schoolchildren. A Mexican study investigated the systematic inflammation in the schoolchildren living in urban (Mexico City: high $PM_{2.5}$ area) and rural (Polotitlán: low $PM_{2.5}$ area) areas. They reported that children living in Polotitlán exhibited significantly lower serum levels of TNF-α (26.3 pg mL^{-1}, $p = 0.005$), IL-1b (4.43 pg mL^{-1}, $p = 0.02$), and IL-10 (3.54 pg mL^{-1}, $p < 0.001$) than those living in Mexico City (TNF-α, IL-1b, and IL-10: 66.5, 19.2, and 5.97 pg mL^{-1}, respectively) (Calderón-Garcidueñas *et al.*, 2008). The present study focused on the relationship between $PM_{2.5}$ exposure and serum cytokine levels in children; our results are consistent with those reported in literatures, particularly regarding the associations between the adults' exposure to $PM_{2.5}$ and their serum cytokine concentrations (e.g., TNF-α). In our previous study (Chao *et al.*, 2016), the police officers who worked as traffic conductors were found to have significantly higher levels of serum TNF-α-EQ than the officers who worked in the office by testing an NF-κB reporter gene assay when they were exposed to $PM_{2.5}$ (the police officers carried $PM_{2.5}$ personal pumps for estimation of personal $PM_{2.5}$ exposure). Brucker *et al.* (2013) found that the serum TNF-α levels in taxi drivers ($n = 39$) were significantly higher than those in individuals with nonoccupational $PM_{2.5}$ exposure ($n = 21$) possibly due to the taxi drivers with occupational exposure to TRAP $PM_{2.5}$. For diabetes patients, the increased serum levels of IL-6 and TNF-α were positively related to higher levels of $PM_{2.5}$ (Schneider *et al.*, 2010). Shakya *et al.* (2019) investigated air pollutants, including $PM_{2.5}$, in Kathmandu Valley, Nepal, during the spring and summer of 2014 and found $PM_{2.5}$ emissions of 124 and 45.2 μg m^{-3} in spring and summer, respectively. They observed that the serum levels of inflammatory cytokines, such as TNF-α and IL-1β, 2, 4, 6, 8, 10, 12, and 13, in traffic officers and train conductors were significantly lower in spring (high $PM_{2.5}$ period) than in summer (low $PM_{2.5}$ period), as revealed by an independent *t*-test and Wilcoxon test ($p < 0.05$). These findings of Shakya's report conflict with those of other studies and the present report (Brucker *et al.*, 2013; Calderón-Garcidueñas *et al.*, 2008; Chao *et al.*, 2016; Schneider *et al.*, 2010; Shakya *et al.*, 2019).

In Fig. 2, the PCA test with varimax rotation was used to examine the associations between systematic inflammation, schoolchildren's age and gender, and three types of $PM_{2.5}$ exposure scenarios. Four principal components (PCs) could account for 73.9% of the total variances if the eigenvalue was set to > 1.00. Rotated PC1 (RPC1) accounted for 45.5% of variance in IL-1b, IL-2, IL-10, IFN-α2, IL-6, IFN-γ, TNF-α, IL-13 levels. RPC2, comprising MCP-1 and the type of $PM_{2.5}$ exposure, accounted for 10.5% of variance. RPC3 and RPC4 were only associated with the variables of age (9.10% of variance) and sex (8.86% of variance), respectively. PCA tests revealed that the $PM_{2.5}$ exposure type and serum MCP-1 were closely correlated. Few studies have reported whether serum MCP-1 in children is affected by $PM_{2.5}$ exposure. Several cellular studies using RAW264.7 cells have revealed that $PM_{2.5}$ triggers the phosphorylation of NF-κB to activate the gene and protein expression of inflammatory responses (e.g., MCP-1 or TNF-α) (Fu *et al.*, 2020; He *et al.*, 2017). A Chinese research team revealed that BALB/c mice with continuous exposure to $PM_{2.5}$ exhibited increased MCP-1 expression from the bronchoalveolar lavage fluid (BALF), indicating that the expression of inflammatory cytokines in the lung was substantially induced by $PM_{2.5}$ (Yang *et al.*, 2020).

Table 2 presents the odds ratios (ORs) of inflammatory biochemical markers for high-$PM_{2.5}$ and TRAP-$PM_{2.5}$ exposure groups in comparison with the reference group of children with low-$PM_{2.5}$ exposure group. The TRAP-$PM_{2.5}$ exposure group exhibited significantly higher ORs of IL-2 (OR =

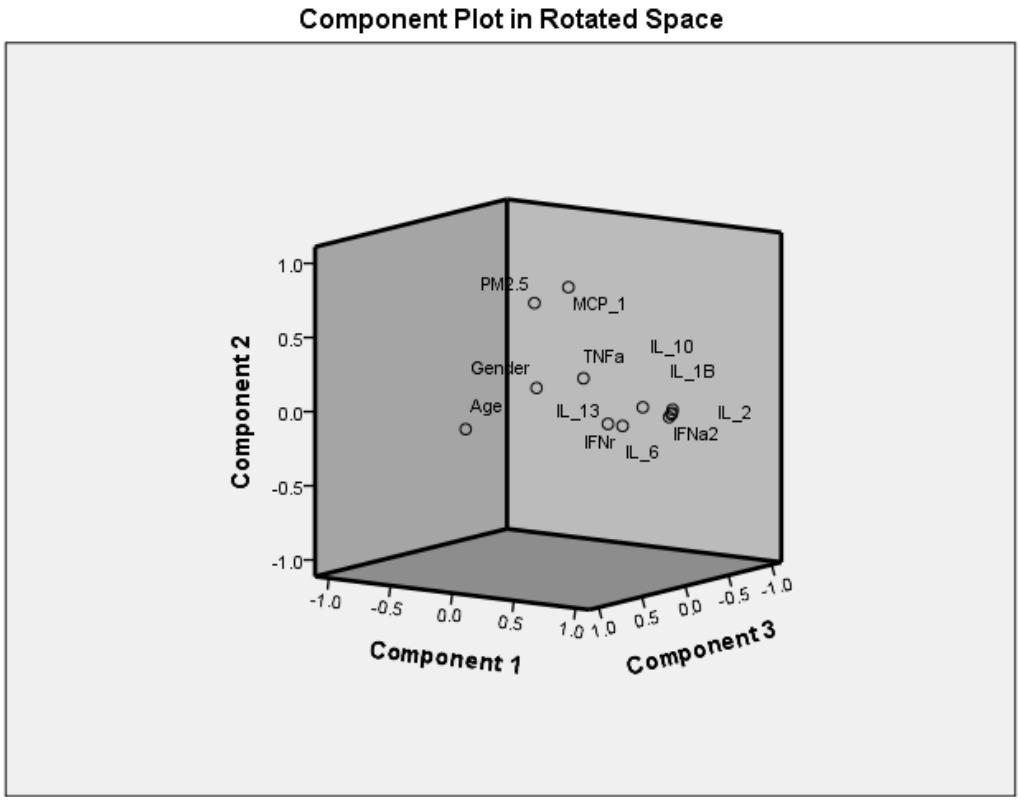

Fig. 2. The associations between inflammatory biomarkers, age, and $PM_{2.5}$ scenarios groups were examined by the test of the principal component analysis.

Table 2. Odds ratios of inflammatory responses among three $PM_{2.5}$ exposure scenario groups.

Characteristics	Odds ratio	95% CI	*p*-value
Low-$PM_{2.5}$			
Reference	1.00	1.00	-
High-$PM_{2.5}$			
IFN-α2	0.988	0.974–1.00	0.117
IFN-γ	1.01	0.986–1.04	0.362
IL-10	1.03	0.902–1.18	0.645
IL-13	0.999	0.994–1.00	0.598
IL-17	0.992	0.948–1.04	0.735
IL-1b	1.01	0.685–1.49	0.962
IL-2	1.21	0.634–2.31	0.563
IL-6	1.12	0.786–1.59	0.545
MCP-1	1.00	0.998–1.00	0.455
TNF-α	1.03	0.938–1.12	0.570
TRAP-$PM_{2.5}$			
IFN-α2	0.995	0.983–1.01	0.387
IFN-γ	1.01	0.973–1.05	0.638
IL-10	1.04	0.938–1.14	0.490
IL-13	0.991	0.983–0.999	0.022*
IL-17	0.971	0.915–1.029	0.318
IL-1b	0.483	0.205–1.14	0.095#
IL-2	3.61	1.19–11.0	0.023*
IL-6	1.12	0.867–1.45	0.385
MCP-1	1.00	0.999–1.006	0.104
TNF-α	1.03	0.965–1.11	0.352

# $p < 0.1$; * $p < 0.05$.

3.61, 95% confidence interval (CI): 1.19–11.0, p = 0.023) and lower ORs of IL-13 (OR = 0.991, 95% CI: 0.983–0.999, p = 0.022) than the reference group. The OR of IL-1b (OR = 0.483 with 95%CI between 0.205 and 1.14, p = 0.095) in TRAP-$PM_{2.5}$ exposure group had the margin significantly higher than that of the reference control.

A few epidemiological studies have examined the associations between children's exposure to $PM_{2.5}$ and cytokine levels in their serum or blood. Calderón-Garcidueñas *et al.* (2008) showed that childhood exposure to high levels of $PM_{2.5}$ had significantly higher levels of IL-1β and IL-10 than those exposed to low levels of $PM_{2.5}$. For Nepalese traffic officers, serum levels of IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12, IL-13, and TNF-α were significantly higher during summer (TRAP-low-$PM_{2.5}$ season) than during spring (TRAP-high-$PM_{2.5}$ season) (Shakya *et al.*, 2019). In the present study, our findings indicated that the children living in the TRAP-$PM_{2.5}$ areas had lower ORs of serum IL-13 than the controls living in the low-$PM_{2.5}$ areas, which is consistent with the result of the aforementioned Nepalese study (Shakya *et al.*, 2019).

The inflammatory cytokines in children's serum could be predicted by the independent variables using the general linear models (GLM) in the present study (Tables 3 and 4). In the univariate analysis, serum TNF-α in children could be significantly and weakly explained by the variable of $PM_{2.5}$ exposure scenarios without adjusting for confounders factors such as age, gender, and ADHD (adjusted R^2 = 0.027, p = 0.032). As presented in Table 3, only two inflammatory biomarkers (i.e., MCP-1 and TNF-α) were statistically or marginally significant predictors in Model 1 (the variable of $PM_{2.5}$ exposure scenarios and the confounders of age and gender) and Model 2 (the variables of $PM_{2.5}$ exposure scenarios and the confounders of age, gender, and ADHD). In Model 1, TNF-α could be partially explained (adjusted R^2 = 0.033, p = 0.039) by the independent variables of age (p = 0.147), gender (p = 0.291), and $PM_{2.5}$ exposure (p = 0.031) in the multivariate analysis. The children's serum TNF-α levels were significantly linked to their $PM_{2.5}$ exposure scenarios in Model 1. In Model 2, the children's serum TNF-α levels could also be significantly predicted (adjusted R^2 = 0.055, p = 0.010) by age (p = 0.290), gender (p = 0.130), $PM_{2.5}$ exposure scenarios (p = 0.070), and ADHD (p = 0.025). ADHD was significantly correlated with serum TNF-α levels, and the correlation between TNF-α levels and $PM_{2.5}$ exposure scenarios was marginally or borderline significant in

Table 3. The multivariate analyses of inflammatory responses were predicted by the $PM_{2.5}$ exposure scenario groups with the adjustment of children's age, gender, or ADHD

Dependence	Independence	R^2	Adjusted R^2	p value
Model 1			0.033	0.039*
TNF-α		0.055		
	Age			0.147
	Gender			0.291
	$PM_{2.5}$ [a]			0.031*
MCP-1		0.028	0.006	0.285
	Age			0.530
	Gender			0.850
	$PM_{2.5}$			0.092#
Model 2				
TNF-α		0.081	0.055	0.010*
	Age			0.290
	Gender			0.130
	ADHD			0.025*
	$PM_{2.5}$			0.070#
MCP-1		0.028	0.01	0.406
	Age			0.568
	Gender			0.897
	ADHD			0.792
	$PM_{2.5}$			0.091#

# $p < 0.1$; * $p < 0.05$.

[a] $PM_{2.5}$ exposure scenarios including low-$PM_{2.5}$, high-$PM_{2.5}$, and TRAP-$PM_{2.5}$.

Table 4. The multivariate analyses of inflammatory responses were predicted by ADHD with the adjustment of children's age, gender, and $PM_{2.5}$ exposure scenario groups.

Dependence	Independence	R^2	Adjusted R^2	*p* value
Model 3				
TNF-α		0.074	0.054	0.008**
	Age			0.253
	Gender			0.138
	$PM_{2.5}$ [a]			0.044*
	ADHD			0.035*
MCP-1		0.026	0.004	0.313
	Age			0.595
	Gender			0.910
	$PM_{2.5}$			0.034*
	ADHD			0.729

*$p < 0.05$; **$p < 0.01$.
[a] $PM_{2.5}$ exposure scenarios including low-$PM_{2.5}$, high-$PM_{2.5}$, and TRAP-$PM_{2.5}$.

Model 2 in multivariate analysis. Gender was slightly and significantly correlated with ADHD occurrence ($R^2 = 0.052$, $p = 0.002$), and boys had a significantly higher ADHD prevalence (OR: 2.80, 95% CI: 1.44–5.43, $p = 0.002$) than girls. On the basis of the results of the multivariate analysis, ADHD was a major variable that significantly affected the GLM of Model 2. In Table 4, the results of Model 3 presented that ADHD is a significant predictor of serum TNF-α in children ($p = 0.035$), with adjustment for the confounders of age ($p = 0.253$), gender ($p = 0.138$), and $PM_{2.5}$ exposure scenarios ($p = 0.044$; adjusted $R^2 = 0.054$, $p = 0.008$). In Model 3, ADHD and $PM_{2.5}$ exposure scenarios were significantly associated with serum TNF-α. The environmental epidemiological studies have yet to provide adequate evidence on the association of ADHD occurrence in children with the severity of exposure to $PM_{2.5}$ (Suades-González *et al.*, 2015; Xi and Wu, 2021); rather, $PM_{2.5}$ exposure scenarios and ADHD occurrence were found to be independent. In the present study, ADHD prevalence and SNAP-IV scores were inversely and significantly correlated with heavily $PM_{2.5}$ exposure scenarios in Table 1, and ADHD prevalence was significantly correlated with gender among the children. On the basis of our results, we determined that serum TNF-α in our cohort might be slightly or weakly correlated with living in the TRAP-$PM_{2.5}$ areas, and the influence of ADHD on inflammatory responses must also be adjusted for.

The findings of the present study were limited because this is an environmental epidemiological study and the *in-vitro* and *in-vivo* tests are not included. Firstly, the statistics were only used to examine the cause-effect with the inflammation in children's serum after they were longitudinally exposed to $PM_{2.5}$. This cause-effect relationship was still needed to be tested by the cellular and animal experiments. Secondly, the three $PM_{2.5}$ exposure scenarios were determined by the long-term $PM_{2.5}$ data from TEPA air monitor sites. The subjects were assigned into the different $PM_{2.5}$ exposure scenarios based on the children's household address. Our subjects might have complicated $PM_{2.5}$ exposure scenarios. Finally, compared with the general population in Taiwan, the KCGMH-ADHD cohort might have some sampling bias to influence the study's outcomes.

According to most *in-vitro*, *in-vivo*, and epidemiological studies (Brucker *et al.*, 2013; Calderón-Garcidueñas *et al.*, 2008; Chao *et al.*, 2016; Chen *et al.*, 2018; Fu *et al.*, 2020; He *et al.*, 2017; Schneider *et al.*, 2010; Yang *et al.*, 2020), the elevated levels of inflammatory cytokines are linked to the severe $PM_{2.5}$ exposure. On the basis of our results, certain cytokine biomarkers in the serum, such as IL-2, IL-13, and TNF-α, in the schoolchildren were associated with exposure to $PM_{2.5}$, particularly for those children in the TRAP areas. The *in-vitro* and *in-vivo* studies usually used the high doses of $PM_{2.5}$ to test their models. Compared with the doses used in the *in-vitro* and *in-vivo* models, our epidemiological report used the relatively low doses to examine the cause-effect relationship in the present study. Several environmental epidemiological studies have demonstrated that $PM_{2.5}$ induces asthma morbidity in both children and adults, especially among those living in TRAP $PM_{2.5}$ areas (Loftus *et al.*, 2015; Klümper *et al.*, 2015; Orellano *et al.*, 2017; Rosenquist *et al.*, 2020; Jalaludin *et al.*, 2014). Several review articles have reported that $PM_{2.5}$ might be involved in triggering and aggravating asthma and COPD through $PM_{2.5}$-induced

cytokine release and oxidative stress production (Orellano *et al.*, 2017; Li *et al.*, 2018; Liu *et al.*, 2022). Because few epidemiological studies have focused on the relationship between serum cytokines and $PM_{2.5}$ exposure in children, the hypothesis of $PM_{2.5}$-induced activation of systematic inflammation that causes asthma symptoms in schoolchildren is difficult to examine or evaluate. Further studies are warranted to examine the relationships between inflammatory responses and $PM_{2.5}$ exposure in children.

4 CONCLUSIONS

Currently, the evidence from these epidemiological studies was inadequate to verify the correlations between serum inflammatory biomarkers (e.g., inflammatory cytokines) in schoolchildren and outdoor $PM_{2.5}$. In this study, we examined the serum levels of cytokines in schoolchildren exposed to $PM_{2.5}$. Several inflammatory biomarkers except for TNF-α in schoolchildren's serum were not linked with children exposure to $PM_{2.5}$. Furthermore, our results demonstrated that the serum levels of TNF-α in children were significantly correlated with living in high-$PM_{2.5}$ exposure areas, particularly TRAP exposure areas. The *in-vitro* and *in-vivo* studies were shown that pre-inflammatory and inflammatory responses were activated by the cells or animals after they were exposed to severe $PM_{2.5}$, but few epidemiological studies examined the cause-effect relationship between serum inflammation and children exposure to heavy $PM_{2.5}$ pollution. This study might provide the observation to show that TRAP $PM_{2.5}$ induced the activation of serum TNF-α after children were longitudinally exposed to TRAP.

ACKNOWLEDGMENTS

Our study was supported by grants from the Ministry of Science and Technology (MOST 109-2221-E-020-009-MY3 and MOST 111-2314-B-182-018-MY3), Chang Gung Memorial Hospital (CMRPG8K1281 and CMRPG8K1282), and Pingtung Christian Hospital (PS110005). This manuscript was edited by Wallace Academic Editing. The authors wish to thank the joined research teams in the Environmental Health and Risk Assessment Laboratory, National Pingtung University of Science and Technology and Department of Child and Adolescent Psychiatry, and Department of Pediatrics in Kaohsiung Chang Gung Memorial Hospital for their assistance with this study.

DISCLAIMER

The authors declare no competing interest.

SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version at https://doi.org/10.4209/aaqr.220288

REFERENCES

Aunan, K., Wang, S. (2014). Internal migration and urbanization in China: Impacts on population exposure to household air pollution (2000–2010). Sci. Total Environ. 481, 186–195. https://doi.org/10.1016/j.scitotenv.2014.02.073

Baron, I.S. (2005). Test review: Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV). Child Neuropsychol. 11, 471–475. https://doi.org/10.1080/09297040590951587

Becerra, T.A., Wilhelm, M., Olsen, J., Cockburn, M., Ritz, B. (2013). Ambient air pollution and autism in Los Angeles County, California. Environ. Health Perspect. 121, 380–386. https://doi.org/10.1289/ehp.1205827

Brucker, N., Moro, A.M., Charão, M.F., Durgante, J., Freitas, F., Baierle, M., Nascimento, S., Gauer, B., Bulcão, R.P., Bubols, G.B., Ferrari, P.D., Thiesen, F.V., Gioda, A., Duarte, M.M., de Castro, I.,

Saldiva, P.H., Garcia, S.C. (2013). Biomarkers of occupational exposure to air pollution, inflammation and oxidative damage in taxi drivers. Sci. Total Environ. 463–464, 884–893. https://doi.org/10.1016/j.scitotenv.2013.06.098

Bussing, R., Fernandez, M., Harwood, M., Wei, H., Garvan, C.W., Eyberg, S.M., Swanson, J.M. (2008). Parent and teacher SNAP-IV ratings of attention deficit hyperactivity disorder symptoms: Psychometric properties and normative ratings from a school district sample. Assessment 15, 317–328. https://doi.org/10.1177/1073191107313888

Calderón-Garcidueñas, L., Villarreal-Calderon, R., Valencia-Salazar, G., Henríquez-Roldán, C., Gutiérrez-Castrellón, P., Torres-Jardón, R., Osnaya-Brizuela, N., Romero, L., Torres-Jardón, R., Solt, A., Reed, W. (2008). Systemic inflammation, endothelial dysfunction, and activation in clinically healthy children exposed to air pollutants. Inhalation Toxicol. 20, 499–506. https://doi.org/10.1080/08958370701864797

Chao, H.R., Hsu, J.W., Ku, H.Y., Wang, S.L., Huang, H.B., Liou, S.H., Tsou, T.C. (2018). Inflammatory response and $PM_{2.5}$ exposure of urban traffic conductors. Aerosol Air Qual. Res. 18, 2633–2642. https://doi.org/10.4209/aaqr.2018.04.0132

Chao, H.R., Que, D.E., Gou, Y.Y., Chuang, C.Y., Chang, T.Y., Hsu, Y.C. (2016). Indoor and Outdoor concentrations of polybrominated diphenyl ethers on respirable particulate in Central and Southern Taiwan. Aerosol Air Qual. Res. 16, 3187–3197. https://doi.org/10.4209/aaqr.2016.11.0472

Chen, S., Cui, K., Yu, T.Y., Chao, H.R., Hsu, Y.C., Lu, I.C., Arcega, R.D., Tsai, M.H., Lin, S.L., Chao, W.C., Chen, C., Yu, K.L.J. (2019). A big data analysis of $PM_{2.5}$ and PM_{10} from low cost air quality sensors near traffic areas. Aerosol Air Qual. Res. 19, 1721–1733. https://doi.org/10.4209/aaqr.2019.06.0328

Chen, T., Zhang, J., Zeng, H., Zhang, Y., Zhang, Y., Zhou, X., Zhao, D., Feng, Y., Zhou, H. (2018). The impact of inflammation and cytokine expression of $PM_{2.5}$ in AML. Oncol. Lett. 16, 2732–2740. https://doi.org/10.3892/ol.2018.8965

Chowdhury, P.H., Honda, A., Ito, S., Okano, H., Onishi, T., Higashihara, M., Okuda, T., Tanaka, T., Hirai, S., Takano, H. (2019). Effects of ambient $PM_{2.5}$ collected using cyclonic separator from Asian cities on human airway epithelial cells. Aerosol Air Qual. Res. 19, 1808–1819. https://doi.org/10.4209/aaqr.2019.01.0016

Chung, M.C., Tsai, M.H., Que, D.E., Bongo, S.J., Hsu, W.L., Tayo, L.L., Chao, H.R., Lin, Y.H., Lin, S.L., Gou, Y.Y., Hsu, Y.C., Hou, W.C., Huang, K.L. (2019). Fine particulate matter-induced toxic effects in an animal model of *Caenorhabditis elegans*. Aerosol Air Qual. Res. 19, 1068–1078. https://doi.org/10.4209/aaqr.2019.03.0127

Chung, M.C., Huang, K.L., Avelino, J.L., Tayo, L.L., Lin, C.C., Tsai, M.H., Lin, S.L., Mansor, W.N.W., Su, C.K., Huang, S.T. (2020). Toxic assessment of heavily traffic-related fine particulate matter using an *in-vivo* wild-type *Caenorhabditis elegans* Model. Aerosol Air Qual. Res. 20, 1974–1986. https://doi.org/10.4209/aaqr.2020.05.0192

Dunn, G.A., Nigg, J.T., Sullivan, E.L. (2019). Neuroinflammation as a risk factor for attention deficit hyperactivity disorder. Pharmacol. Biochem. Behav. 182, 22–34. https://doi.org/10.1016/j.pbb.2019.05.005

Fann, N., Baker, K.R., Fulcher, C.M. (2012). Characterizing the $PM_{2.5}$-related health benefits of emission reductions for 17 industrial, area and mobile emission sectors across the U.S. Environ. Int. 49, 141–151. https://doi.org/10.1016/j.envint.2012.08.017

Feng, S., Gao, D., Liao, F., Zhou, F., Wang, X. (2016). The health effects of ambient $PM_{2.5}$ and potential mechanisms. Ecotoxicol. Environ. Saf. 128, 67–74. https://doi.org/10.1016/j.ecoenv.2016.01.030

Fu, H., Liu, X., Li, W., Zu, Y., Zhou, F., Shou, Q., Ding, Z. (2020). $PM_{2.5}$ exposure induces inflammatory response in macrophages via the TLR4/COX-2/NF-κB pathway. Inflammation 43, 1948–1958. https://doi.org/10.1007/s10753-020-01269-y

Grande, G., Wu, J., Ljungman, P.L.S., Stafoggia, M., Bellander, T., Rizzuto, D. (2021). Long-term exposure to $PM_{2.5}$ and cognitive decline: A longitudinal population-based study. J. Alzheimers Dis. 80, 591–599. https://doi.org/10.3233/jad-200852

Han, X., Zhuang, Y. (2021). $PM_{2.5}$ induces autophagy-mediated cell apoptosis via PI3K/AKT/mTOR signaling pathway in mice bronchial epithelium cells. Exp. Ther. Med. 21, 1. https://doi.org/10.3892/etm.2020.9433

Hao, G., Zuo, L., Xiong, P., Chen, L., Liang, X., Jing, C. (2022). Associations of $PM_{2.5}$ and road traffic

noise with mental health: Evidence from UK Biobank. Environ. Res. 207, 112221. https://doi.org/10.1016/j.envres.2021.112221

Hassanvand, M.S., Naddafi, K., Faridi, S., Nabizadeh, R., Sowlat, M.H., Momeniha, F., Gholampour, A., Arhami, M., Kashani, H., Zare, A., Niazi, S., Rastkari, N., Nazmara, S., Ghani, M., Yunesian, M. (2015). Characterization of PAHs and metals in indoor/outdoor PM_{10}/$PM_{2.5}$/PM_1 in a retirement home and a school dormitory. Sci. Total Environ. 527–528, 100–110. https://doi.org/10.1016/j.scitotenv.2015.05.001

He, M., Ichinose, T., Yoshida, S., Ito, T., He, C., Yoshida, Y., Arashidani, K., Takano, H., Sun, G., Shibamoto, T. (2017). $PM_{2.5}$-induced lung inflammation in mice: Differences of inflammatory response in macrophages and type II alveolar cells. J. Appl. Toxicol. 37, 1203–1218. https://doi.org/10.1002/jat.3482

Hisamuddin, N.H., Jalaludin, J., Yusof, A.N., Tualeka, A.R. (2020). Genotoxic effects of exposure to urban traffic related air pollutants on children in Klang Valley, Malaysia. Aerosol Air Qual. Res. 20, 2614–2623. https://doi.org/10.4209/aaqr.2020.03.0114

Huang, H.B., Chen, G.W., Wang, C.J., Lin, Y.Y., Liou, S.H., Lai, C.H., Wang, S.L. (2013). Exposure to heavy metals and polycyclic aromatic hydrocarbons and DNA damage in Taiwanese traffic conductors. Cancer Epidemiol. Biomarkers Prev. 22, 102–108. https://doi.org/10.1158/1055-9965.Epi-12-0706

Jalaludin, J., Noh, S.N.S., Suhaimi, N.F., Akim, A.M. (2014). Tumor necrosis factor-alpha as biomarkers of exposure to indoor pollutants among primary school children in Klang Valley. American J. Appl. Sci. 11, 1616–1630. https://doi.org/10.3844/AJASSP.2014.1616.1630

Kao, C.C., Chen, C.C., Avelino, J.L., Cortez, M.P., Tayo, L.L., Lin, Y.H., Tsai, M.H., Lin, C.W., Hsu, Y.C., Hsieh, L.T., Lin, C., Wang, L.C., Yu, K.L.J., Chao, H.R. (2019). Infants' neurodevelopmental effects of $PM_{2.5}$ and persistent organohalogen pollutants exposure in Southern Taiwan. Aerosol Air Qual. Res. 19, 2793–2803. https://doi.org/10.4209/aaqr.2019.10.0550

Klümper, C., Krämer, U., Lehmann, I., von Berg, A., Berdel, D., Herberth, G., Beckmann, C., Link, E., Heinrich, J., Hoffmann, B., Schins, R.P. (2015). Air pollution and cytokine responsiveness in asthmatic and non-asthmatic children. Environ. Res. 138, 381–390. https://doi.org/10.1016/j.envres.2015.02.034

Lertxundi, A., Baccini, M., Lertxundi, N., Fano, E., Aranbarri, A., Martínez, M.D., Ayerdi, M., Álvarez, J., Santa-Marina, L., Dorronsoro, M., Ibarluzea, J. (2015). Exposure to fine particle matter, nitrogen dioxide and benzene during pregnancy and cognitive and psychomotor developments in children at 15 months of age. Environ. Int. 80, 33–40. https://doi.org/10.1016/j.envint.2015.03.007

Li, G., Huang, J., Wang, J., Zhao, M., Liu, Y., Guo, X., Wu, S., Zhang, L. (2021). Long-term exposure to ambient $PM_{2.5}$ and increased risk of CKD prevalence in China. J. Am. Soc. Nephrol. 32, 448–458. https://doi.org/10.1681/asn.2020040517

Li, P., Zhao, J., Gong, C., Bo, L., Xie, Y., Kan, H., Song, W. (2014). Association between individual $PM_{2.5}$ exposure and DNA damage in traffic policemen. J. Occup. Environ. Med. 56, e98–e101. https://doi.org/10.1097/jom.0000000000000287

Li, R., Zhou, R., Zhang, J. (2018). Function of $PM_{2.5}$ in the pathogenesis of lung cancer and chronic airway inflammatory diseases. Oncol. Lett. 15, 7506–7514. https://doi.org/10.3892/ol.2018.8355

Liu, C., Cai, J., Qiao, L., Wang, H., Xu, W., Li, H., Zhao, Z., Chen, R., Kan, H. (2017). The acute effects of fine particulate matter constituents on blood inflammation and coagulation. Environ. Sci. Technol. 51, 8128–8137. https://doi.org/10.1021/acs.est.7b00312

Liu, J., Liu, B., Yuan, P., Cheng, L., Sun, H., Gui, J., Pan, Y., Huang, D., Chen, H., Jiang, L. (2021). Role of PKA/CREB/BDNF signaling in $PM_{2.5}$-induced neurodevelopmental damage to the hippocampal neurons of rats. Ecotoxicol. Environ. Saf. 214, 112005. https://doi.org/10.1016/j.ecoenv.2021.112005

Liu, K., Hua, S., Song, L. (2022). $PM_{2.5}$ exposure and asthma development: The key role of oxidative stress. Oxid. Med. Cell. Longevity 2022, 3618806. https://doi.org/10.1155/2022/3618806

Loftus, C., Yost, M., Sampson, P., Arias, G., Torres, E., Vasquez, V.B., Bhatti, P., Karr, C. (2015). Regional $PM_{2.5}$ and asthma morbidity in an agricultural community: A panel study. Environ. Res. 136, 505–512. https://doi.org/10.1016/j.envres.2014.10.030

Mathias, S., Wayland, R. (2019). Fine Particulate Matter ($PM_{2.5}$) Precursor Demonstration Guidance.

Office of Air Quality Planning and Standards, United States Environmental Protection Agency. https://www.epa.gov/sites/default/files/2019-05/documents/transmittal_memo_and_pm25_precursor_demo_guidance_5_30_19.pdf (accessed July 2022).

Meier, R., Cascio, W.E., Ghio, A.J., Wild, P., Danuser, B., Riediker, M. (2014). Associations of short-term particle and noise exposures with markers of cardiovascular and respiratory health among highway maintenance workers. Environ. Health Perspect. 122, 726–732. https://doi.org/10.1289/ehp.1307100

Mukherjee, A., Agrawal, M. (2018). A Global Perspective of Fine Particulate Matter Pollution and Its Health Effects, in: de Voogt, P. (Ed.), Reviews of Environmental Contamination and Toxicology Volume 244, Springer International Publishing, Cham, pp. 5–51. https://doi.org/10.1007/398_2017_3

Orellano, P., Quaranta, N., Reynoso, J., Balbi, B., Vasquez, J. (2017). Effect of outdoor air pollution on asthma exacerbations in children and adults: Systematic review and multilevel meta-analysis. PLoS One 12, e0174050. https://doi.org/10.1371/journal.pone.0174050

Pun, V.C., Kazemiparkouhi, F., Manjourides, J., Suh, H.H. (2017). Long-term $PM_{2.5}$ exposure and respiratory, cancer, and cardiovascular mortality in older US adults. Am. J. Epidemiol. 186, 961–969. https://doi.org/10.1093/aje/kwx166

Putri Anis Syahira, M.J., Karmegam, K., Nur Athirah Diyana, M.Y., Irniza, R., Shamsul Bahri, M.T., Vivien, H., Nurul Maizura, H., Sivasankar, S. (2020). Impacts of $PM_{2.5}$ on respiratory system among traffic policemen. WORK 66, 25–29. https://doi.org/10.3233/WOR-203147

Rahmatinia, T., Kermani, M., Farzadkia, M., Nicknam, M.H., Soleimanifar, N., Mohebbi, B., Jafari, A.J., Shahsavani, A., Fanaei, F. (2021). Potential cytotoxicity of $PM_{2.5}$-bound PAHs and toxic metals collected from areas with different traffic densities on human lung epithelial cells (A549). J. Environ. Health Sci. Eng. 19, 1701–1712. https://doi.org/10.1007/s40201-021-00724-8

Raz, R., Roberts, A.L., Lyall, K., Hart, J.E., Just, A.C., Laden, F., Weisskopf, M.G. (2015). Autism spectrum disorder and particulate matter air pollution before, during, and after pregnancy: A nested case-control analysis within the Nurses' Health Study II Cohort. Environ. Health Perspect. 123, 264–270. https://doi.org/10.1289/ehp.1408133

Rosenquist, N.A., Metcalf, W.J., Ryu, S.Y., Rutledge, A., Coppes, M.J., Grzymski, J.J., Strickland, M.J., Darrow, L.A. (2020). Acute associations between $PM_{2.5}$ and ozone concentrations and asthma exacerbations among patients with and without allergic comorbidities. J. Exposure Sci. Environ. Epidemiol. 30, 795–804. https://doi.org/10.1038/s41370-020-0213-7

Sassá, A.H., Higarashi, I.H., Bercini, L.O., Arruda, D.C.d., Marcon, S.S. (2011). Bebê de risco: Acompanhando o crescimento infantil no primeiro ano de vida. Acta Paul Enferm. 24, 541–549. https://doi.org/10.1590/S0103-21002011000400015

Schneider, A., Neas, L.M., Graff, D.W., Herbst, M.C., Cascio, W.E., Schmitt, M.T., Buse, J.B., Peters, A., Devlin, R.B. (2010). Association of cardiac and vascular changes with ambient $PM_{2.5}$ in diabetic individuals. Part. Fibre Toxicol. 7, 14. https://doi.org/10.1186/1743-8977-7-14

Shah, A.S.V., Langrish, J.P., Nair, H., McAllister, D.A., Hunter, A.L., Donaldson, K., Newby, D.E., Mills, N.L. (2013). Global association of air pollution and heart failure: A systematic review and meta-analysis. Lancet 382, 1039–1048. https://doi.org/10.1016/S0140-6736(13)60898-3

Shakya, K.M., Peltier, R.E., Zhang, Y., Pandey, B.D. (2019). Roadside exposure and inflammation biomarkers among a cohort of traffic police in Kathmandu, Nepal. Int. J. Environ. Res. Public Health 16, 377. https://doi.org/10.3390/ijerph16030377

Shen, H., Yang, T.M., Lu, C.C., Yuan, C.S., Hung, C.H., Lin, C.T., Lee, C.W., Jing, G., Hu, G., Lo, K.C. (2020). Chemical fingerprint and source apportionment of $PM_{2.5}$ in highly polluted events of southern Taiwan. Environ. Sci. Pollut. Res. Int. 27, 6918–6935. https://doi.org/10.1007/s11356-019-07328-8

Shou, Y., Huang, Y., Zhu, X., Liu, C., Hu, Y., Wang, H. (2019). A review of the possible associations between ambient $PM_{2.5}$ exposures and the development of Alzheimer's disease. Ecotoxicol. Environ. Saf. 174, 344–352. https://doi.org/10.1016/j.ecoenv.2019.02.086

Sram, R.J., Veleminsky, M., Jr., Veleminsky, M., Sr., Stejskalová, J. (2017). The impact of air pollution to central nervous system in children and adults. Neuro Endocrinol. Lett 38, 389–396.

Suades-González, E., Gascon, M., Guxens, M., Sunyer, J. (2015). Air pollution and neuropsychological development: A review of the latest evidence. Endocrinology 156, 3473–3482. https://doi.org/10.1210/en.2015-1403

Suhaimi, N.F., Jalaludin, J., Abu Bakar, S. (2021). The influence of traffic-related air pollution (TRAP) in primary schools and residential proximity to traffic sources on histone H3 level in selected Malaysian children. Int. J. Environ. Res. Public Health 18, 7995. https://doi.org/10.3390/ijerph18157995

Talbott, E.O., Arena, V.C., Rager, J.R., Clougherty, J.E., Michanowicz, D.R., Sharma, R.K., Stacy, S.L. (2015). Fine particulate matter and the risk of autism spectrum disorder. Environ. Res. 140, 414–420. https://doi.org/10.1016/j.envres.2015.04.021

Wang, C., Tu, Y., Yu, Z., Lu, R. (2015). $PM_{2.5}$ and cardiovascular diseases in the elderly: An overview. Int. J. Environ. Res. Public Health 12, 8187–8197. https://doi.org/10.3390/ijerph120708187

Wang, C., Chen, R., Shi, M., Cai, J., Shi, J., Yang, C., Li, H., Lin, Z., Meng, X., Liu, C., Niu, Y., Xia, Y., Zhao, Z., Kan, H., Weinberg, C.R. (2018). Possible mediation by methylation in acute inflammation following personal exposure to fine particulate air pollution. Am. J. Epidemiol. 187, 484–493. https://doi.org/10.1093/aje/kwx277

Wang, L.J., Li, S.C., Li, S.W., Kuo, H.C., Lee, S.Y., Huang, L.H., Chin, C.Y., Yang, C.Y. (2022a). Gut microbiota and plasma cytokine levels in patients with attention-deficit/hyperactivity disorder. Transl. Psychiatry 12, 76. https://doi.org/10.1038/s41398-022-01844-x

Wang, L.J., Huang, Y.H., Chou, W.J., Lee, S.Y., Chang, H.Y., Chen, C.C., Chao, H.R. (2022b). Interrelationships among growth hormone, thyroid function, and endocrine-disrupting chemicals on the susceptibility to attention-deficit/hyperactivity disorder. Eur. Child Adolesc. Psychiatry. https://doi.org/10.1007/s00787-021-01886-4

Xi, T., Wu, J. (2021). A review on the mechanism between different factors and the occurrence of autism and ADHD. Psychol. Res. Behav. Manage. 14, 393–403. https://doi.org/10.2147/prbm.S304450

Xie, W., You, J., Zhi, C., Li, L. (2021). The toxicity of ambient fine particulate matter ($PM_{2.5}$) to vascular endothelial cells. J. Appl. Toxicol. 41, 713–723. https://doi.org/10.1002/jat.4138

Xing, J., Tang, H., Lee, W.J., Wang, L.C., Zhu, J., Huang, Q., Cui, K. (2017). Part II: $PM_{2.5}$ and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) in the ambient air of northern China. Aerosol Air Qual. Res. 17, 2010–2026. https://doi.org/10.4209/aaqr.2017.06.0211

Yang, Y., Li, X., An, X., Zhang, L., Li, X., Wang, L., Zhu, G. (2020). Continuous exposure of $PM_{2.5}$ exacerbates ovalbumin-induced asthma in mouse lung via a JAK-STAT6 signaling pathway. dv. Clin. Exp. Med. 29, 825–832. https://doi.org/10.17219/acem/121009

Yu, T.Y., Chao, H.R., Tsai, M.H., Lin, C.C., Lu, I.C., Chang, W.H., Chen, C.C., Wang, L.J., Lin, E.T., Chang, C.T., Chen, C., Kao, C.C., Wan Mansor, W.N., Yu, K.L.J. (2021). Big data analysis for effects of the COVID-19 outbreak on ambient $PM_{2.5}$ in areas that were not locked down. Aerosol Air Qual. Res. 21, 210020. https://doi.org/10.4209/aaqr.210020

Zhang, S., Faries, D.E., Vowles, M., Michelson, D. (2005). ADHD Rating Scale IV: Psychometric properties from a multinational study as a clinician-administered instrument. Int. J. Methods Psychiatr. Res. 14, 186–201. https://doi.org/10.1002/mpr.7