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Inflammatory Response and $PM_{2.5}$ Exposure of Urban Traffic Conductors

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ABSTRACT

Human exposure to airborne $PM_{2.5}$ has been linked to an increased risk of respiratory and cardiovascular diseases, possibly via the activation of systemic inflammation. However, the associations between airborne $PM_{2.5}$ and systemic inflammation in humans remain inconclusive. Traffic-related air pollutants (TRAPs) are the major source of $PM_{2.5}$ in urban areas; the adverse health effect of $PM_{2.5}$ from TRAPs is currently a critical issue of public concern. The present cross-sectional study examines the relationship between $PM_{2.5}$ exposure and systemic inflammation in order to consider the health impacts of TRAP $PM_{2.5}$ on urban traffic conductors. All study participants, viz., office-based police officers (the reference) and traffic conductors (the exposure), were requested to carry a personal sampler to determine individual $PM_{2.5}$ exposure. An adenovirus-based NF-κB luciferase reporter assay was used to determine the proinflammatory activity in serum samples collected from the study participants. The blood proinflammatory activity was presented as tumor necrosis factor-α (TNFα) equivalence (TNFα-EQ), which was extrapolated from the sigmoidal semi-logarithmic dose-response curve of the NF-κB reporter assay by TNFα. The levels of both personal $PM_{2.5}$ exposure and blood proinflammatory activity (TNFα-EQ) in the exposure group (traffic conductors) were significantly higher than in the reference group (office-based police officers) ($p < 0.05$). The present study reveals a positive and significant association between personal $PM_{2.5}$ exposure levels and blood TNFα-EQ levels in a linear regression model of $y = 0.511x - 3.062$ (y = log TNFα-EQ and x = log $PM_{2.5}$; R = 0.231 and $p = 0.047$); the results suggest that exposure to TRAP $PM_{2.5}$ significantly contributes to increased systemic inflammation in humans. This research provides clear evidence that long-term occupational exposure to TRAPs causes adverse health impacts, i.e., inflammation, on traffic conductors.

***Keywords*:** Air pollution; Health effects/risks; Human exposure; Personal exposure; Toxicology.

INTRODUCTION

For decades, particulate matter (PM), particularly fine PM, has been identified to have marked contribution to air pollution. Upon inhalation, PM tends to accumulate in human respiratory tract and thus is classified as a severe health hazard (Bilal *et al.*, 2017; Cai *et al.*, 2017; Morales Betancourt *et al.*, 2017). According to U.S. Environmental Protection Agency (EPA) (2016), $PM_{2.5}$ is the fine particle with a diameter of 2.5 micrometers or less, such as the emissions from construction sites, unpaved roads, smokestacks, fire, and vehicles. Both toxicological and clinical studies revealed that acute exposure to high levels of PM led to immediate physiological changes (Osornio-Vargas *et al.*, 2003; Chow *et al.*, 2015; McGrath *et al.*, 2017). The peak concentration of air pollutants in the transportation environment could be up to three times higher than that in the background (Morales Betancourt *et al.*, 2017). Therefore, traffic related air pollutants (TRAPs) may cause significant health impacts especially on those with routine exposure, e.g., drivers, commuters, and traffic conductors.

Atmospheric $PM_{2.5}$ has been recognized as one of the major air pollutants in urban areas because of its influence on public health, visibility deterioration, and global climate

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change (Liang *et al.*, 2015; Chew *et al.*, 2016; Li *et al.*, 2016). $PM_{2.5}$ emission from internal combustion engines represents a major source of TRAPs in urban areas with heavy traffic (Matawle, 2015; Lu *et al.*, 2016; Tseng, 2016; Fan *et al.*, 2017; Fujitani *et al.*, 2017). It was found that the $PM_{2.5}$-bound pollutants included polycyclic aromatic hydrocarbons (PAHs), carbonaceous species, heavy metals, carbon black, and halogen persistent organic chemicals (e.g., polybrominated diphenyl ethers (PBDEs)) in heavy-traffic areas (Chen *et al.*, 2016; Chao *et al.*, 2016; Wang *et al.*, 2018). Importantly, there is evidence that airborne PM of TRAPs is linked to increased levels of inflammatory response (Kannan *et al.*, 2006; Ritz and Wilhelm, 2008; Liu *et al.*, 2017).

Epidemiological studies have demonstrated an increased risk of pulmonary disease, lung cancer, cardiovascular disease, or DNA damage in humans with long-term exposure to airborne $PM_{2.5}$ (Pope *et al.*, 2002; Vinzents *et al.*, 2005; Miller *et al.*, 2007; Cao *et al.*, 2012; Chu *et al.*, 2015; Wang *et al.*, 2015). During cold weather, high levels of outdoor $PM_{2.5}$ were associated with increased emergency visits for cardiovascular and respiratory diseases, particularly hypertension, heart failure, and asthma (Rodopoulou *et al.*, 2015). Results from animal model studies revealed the activation of lung inflammation in response to atmospheric PM (Mantecca *et al.*, 2010) or $PM_{2.5}$ collected in a residential area (Park *et al.*, 2011). Upon inhalation, $PM_{2.5}$ is more capable than PM_{10} of reaching distal regions of the lung, where $PM_{2.5}$ may trigger the inflammatory response (Osornio-Vargas *et al.*, 2003; Ferguson *et al.*, 2013).

Epidemiological studies have also revealed the association between TRAPs and systemic inflammation. Occupational exposure of taxi drivers with air pollutants resulted in elevated levels of proinflammatory cytokines, e.g., TNFα, in blood (Brucker *et al.*, 2013). Effects of $PM_{2.5}$/$PM_{2.5}$-bound chemicals on activation of systemic inflammation were observed in urban residents (Liu *et al.*, 2017; Wang *et al.*, 2018). However, it was also noted that some epidiological results did not support the idea that TRAPs could cause inflammation. A study in healthy adults in commuting indicated that TRAPs exposure was not consistently associated with acute changes in serum inflammation markers, i.e., IL6, IL8, TNFα, and C-reactive protein (CRP) (Zuurbier *et al.*, 2011). Another study in highway maintenance workers suggested that $PM_{2.5}$ exposure was positively associated with CRP, but was negatively associated with TNFα (Meier *et al.*, 2014). Moreover, short-term diesel exhaust exposure caused no significant effects on proinflammatory cytokines (i.e., IL6 and TNFα) in healthy adults (Cliff *et al.*, 2016). Indeed, exposure scenarios of TRAPs, such as dosage, exposure duration, pollutant types, etc., may partly explain the difference of these studies. Importantly, plasma samples collected from human volunteers with diesel exhaust exposure were demonstrated to enhance inflammatory gene expression *in vitro*, suggesting the elevated proinflammatory factors in circulating (Channell *et al.*, 2012). Therefore, improving measurement of the *total* proinflammatory activity, instead of a selected proinflammatory marker or a panel of inflammatory cytokines, in blood samples is a promising alternative to further justify the finding.

In the present cross-sectional study in traffic conductors (the exposure) and office-based police officers (the reference), personal airborne $PM_{2.5}$ sampling and an adenovirus-based NF-κB luciferase reporter assay were used to determine the individual $PM_{2.5}$ exposure and the total proinflammatory activity in blood, respectively. Results of the study clearly demonstrated the association between personal $PM_{2.5}$ exposure and systemic proinflammatory activity in humans with occupational exposure to TRAPs.

METHODS

Study Participants

The cross-sectional study was designed in this research. Study participants were invited to have a health examination survey of the Taipei polices (HESTP) from April 2009 to June 2011. The HESTP cohort information was previously described in detail (Huang *et al.*, 2012; Huang *et al.*, 2013). Briefly, there was a total of 144 participants in the HESTP cohort, including 91 traffic conductors as the exposure group (case) and 53 indoor office police officers as the reference group (control), at ages between 20 and 63 years old. The HESTP cohort were healthy and had been in their current job for more than 3 months. With their agreement, the HESTP cohort were required to complete self-administered questionnaires (including demographic parameters, lifestyle, smoking/drinking habit, and disease history), to undergo health examination, and to collect urine and serum samples.

The present study participants were selected using convenience sampling. As summarized in Table 1, Population 1 (N = 115), i.e., 69 traffic conductors (the exposure group) and 46 office police officers (the reference group), was sampled from the HESTP cohort. Population 2 (N = 75), i.e., 35 traffic conductors (the exposure group) and 40 office police officers (the reference group), was sampled from the Population 1. The study protocol was reviewed and approved by the Institutional Review Board of the Human Ethical Committees in National Health Research Institutes, Taiwan, in 2009. Ethical standards formulated in Declarations of Helsinki in 1964 and revised in 2008 (sixth revision) were followed. The informed consent was written by the participants after receiving detailed explanation of the study and potential consequences prior to enrollment (Huang *et al.*, 2012, 2013).

Airborne $PM_{2.5}$ Sampling and Personal $PM_{2.5}$ Exposure Determination

Following the standard method by United States Environmental Protection Agency (U.S. EPA) (EPA Method IP-10A), personal airborne $PM_{2.5}$ sampling was conducted to determine individual $PM_{2.5}$ exposure in the daily work shift as previously described (Huang *et al.*, 2012). Briefly, the Personal Environmental Monitor (PEM) (761-203) (SKC Inc., PA, USA), with a 2.5-μm single-stage impactor for $PM_{2.5}$ air sampling, was connected to a Gilian GilAir 5 pump (Sensidyne Inc., Clearwater, FL, USA); before each test, the pump was calibrated at a flow rate of 2 L min^{-1}.

Table 1. Descriptive analysis of demographic characteristics of study participants.

Variables	Population 1 (N = 115) [a]			Population 2 (N = 75) [a]		
	Exposure group (N = 69)	Reference group (N = 46)	*p* values	Exposure group (N = 35)	Reference group (N = 40)	*p* values
	Mean (SD) or N (%) [b]	Mean (SD) or N (%) [b]		Mean (SD) or N (%) [b]	Mean (SD) or N (%) [b]	
Age in years [c]	48.9 (9.08)	42.8 (8.84)	< 0.001	47.5 (9.33)	42.6 (7.09)	0.036
BMI in kg m^{-2} [c]	25.1 (3.54)	29.1 (6.49)	< 0.001	25.0 (3.59)	29.1 (6.49)	< 0.001
Gender [d]			< 0.001			< 0.001
Male	52 (75.4)	10 (21.7)		26 (74.3)	9 (22.5)	
Female	17 (24.6)	36 (78.3)		9 (25.7)	31 (77.5)	
Education levels [d]			< 0.001			< 0.001
College	26 (37.7)	38 (82.6)		13 (37.1)	33 (82.5)	
High school	43 (62.3)	8 (17.4)		22 (62.9)	7 (17.5)	
Smoking habit [d]			0.196			0.223
Smokers	16 (23.2)	6 (13.0)		8 (22.9)	6 (15.0)	
Nonsmokers	53 (76.8)	40 (87.0)		27 (77.1)	34 (85.0)	
Cooking habit [d]			0.107			0.134
Yes	29 (42.0)	24 (52.2)		15 (42.9)	21 (52.5)	
No	40 (58.0)	22 (47.8)		20 (57.1)	19 (47.5)	
Drinking alcohol [d]			0.997			0.997
Yes	12 (17.4)	8 (17.4)		6 (17.1)	7 (17.5)	
No	57 (82.6)	38 (82.6)		29 (82.9)	33 (82.5)	
Vitamin supplement			0.151			0.284
Yes	39 (56.5)	19 (41.3)		20 (57.1)	17 (42.5)	
No	30 (43.5)	27 (58.7)		15 (42.9)	23 (57.5)	

[a] Population 1 (N = 115), i.e., 69 traffic conductors (the exposure group) and 46 office policemen (the reference group), was sampled from the HESTP cohort. Population 2 (N = 75), i.e., 35 traffic conductors (the exposure group) and 40 office policemen (the reference group), was sampled from the Population 1.
[b] Mean (SD) or N (%): mean (standard deviation) or number (percentage).
[c] Mean (standard deviation).
[d] Number (percentage).

For airborne $PM_{2.5}$ sampling, the study participants were equipped with a PEM for 9–10 working hours per day. Airborne fine particulate ($PM_{2.5}$) was collected on a 2.5-μm 50%-cutting-size Teflon filter (37-mm diameter) (Biotech Line, Lynge, Denmark). To reduce sampling bias, the Teflon filters were conditioned in a temperature/humidity-controlled space (before and after each sampling) before weighing on a Micro Balance MT5 (Mettler-Toledo, Glostrup, Denmark). Personal $PM_{2.5}$ exposure (μg m^{-3}) was defined as the collected $PM_{2.5}$ mass on the filter divided by the sampled air volume.

Reagents and Cell Culture

RPMI medium 1640, fetal bovine serum (FBS) (10091-148), and penicillin/streptomycin (15140-122) were purchased from Gibco/Invitrogen (Carlsbad, CA, USA). Sodium bicarbonate (S5761) was from Sigma Aldrich and tumor necrosis factor-α (TNFα) (1371843) was from Roche. Human promonocytic leukemia HL-CZ cells (BCRC-60043) was purchased from Bioresource Collection and Research Center (BCRC) (Hsinchu, Taiwan). HL-CZ cells were cultured in RPMI supplemented with 10% FBS, 1% penicillin/streptomycin, and 1.5 mg mL^{-1} sodium bicarbonate.

Adenovirus-based NF-κB Luciferase Reporter Assay

Blood samples were collected from the study participants within half an hour after the end of a work shift on two consecutive days. Following centrifugation, serum was collected and kept at –20°C until use. NF-κB luciferase reporter assay was performed by using the recombinant adenovirus AdV-NFκB-Luc as previously described in detail (Tsou *et al.*, 2011). Briefly, HL-CZ cells (1×10^4 cells per well in 96-well plates) were infected with AdV-NFκB-Luc at multiplicity of infection (MOI) of 0.2 pfu $cell^{-1}$ for 16 hours. Then, the infected cells were treated with serum samples or different levels of TNFα for 6 hours. Luciferase activity was determined with the Luciferase Assay System (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Data and Statistical Analysis

Experimental data were presented as means ± standard error (SE). Both personal $PM_{2.5}$ exposure and blood proinflammatory activity levels were compared between reference and exposure groups by the non-parametric Mann-Whitney *U* test due to the non-normal distribution of data. *P*-values less than 0.05 were considered statistically significant. After logarithmic transformation, levels of personal $PM_{2.5}$ exposure and blood proinflammatory activity were fitted into a normal (Gaussian) distribution for correlation analysis. All statistical analyses were carried out with the IBM SPSS Statistics 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Descriptive Analysis of Demographic Characteristics of Study Participants

Descriptive analysis of demographic characteristics of study participants was summarized in Table 1. In Population 1 (N = 115), the mean ages of the exposed and reference groups were 48.9 and 42.8 years old, respectively; in Population 2 (N = 75), the mean ages of the exposed and reference groups were 47.5 and 42.6 years old, respectively. In Population 1, body mass index (BMI) of the exposure group (25.1 kg m^{-2}) was significantly lower than that of the reference group (29.1 kg m^{-2}); in Population 2, BMI of the exposure group (25.0 kg m^{-2}) was significantly lower than that of the reference group (29.1 kg m^{-2}). Gender was significantly different between the exposed and reference groups, with 74.3% to 75.4% of males in the exposure group and 77.5% to 78.3% of females in the reference group. In both Population 1 and Population 2, the reference group had higher education levels than the exposure group ($p < 0.001$); no significant difference was observed in smoking habit, cooking habit, drinking alcohol, and vitamin supplement consumption between the two groups. Regarding the variables listed in Table 1, no significant difference was detected between Population 1 and Population 2 and both populations showed similar characteristics with the previous HESTP cohort studies (Huang *et al.*, 2012, 2013).

Establishment of a Dose-response Curve of NF-κB Luciferase Activation by TNFα

NF-κB, a pivotal transcription factor of inflammatory responses, regulates multiple aspects of innate and adaptive immune functions. Recent *in vitro* evidence revealed the cause-effect relationship between $PM_{2.5}$ and inflammatory responses, where $PM_{2.5}$ treatments induce gene expression of NF-κB family and activate NF-κB signaling (Marano *et al.*, 2002; Dou *et al.*, 2018; Zhang *et al.*, 2018). In this study, an adenovirus-based NF-κB luciferase reporter assay (Tsou *et al.*, 2011) was adopted to determine the total proinflammatory activity in serum samples collected from the study participants. Because NF-κB transcription factors responds to most proinflammatory stimuli, the NF-κB luciferase reporter assay provides a superior alternative to the conventional enzyme-linked immunosorbance assay (ELISA), which allows detection of only one or a panel of selected cytokines.

First of all, the responsiveness of the NF-κB luciferase reporter assay to proinflammatory stimuli was validated by using TNFα, a multifunctional proinflammatory cytokine, i.e., a positive mediator of inflammation. The AdV-NFκB-Luc-infected HL-CZ cells were treated with TNFα (0, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 pg mL^{-1}) for 6 hours and then luciferase activity was determined. Results in Fig. 1 summarized the dose-dependent activation of NF-κB luciferase reporter gene by TNFα; the sigmoidal semi-logarithmic dose-response curve ($R^2 > 0.95$, $p < 0.001$) was fitted by using a non-linear equation, $y = a_0 + (c_0 - a_0)/(1 + 10^{[(\log EC50 - x) \times \theta]})$ (see figure legend for details). The relative standard deviations (RSD) from triplicate measurements in each test was below 20%. The limit of detection (LOD) for TNFα was 0.00087 pg mL^{-1}, as defined by 3 times SD above the average RLU value of the zero standard. It was noted that the LOD of our NF-κB reporter assay was lower than those of ELISA (Dieme *et al.*, 2012; Brucker *et al.*, 2013; Hüls *et al.*, 2017; Zhou *et al.*, 2017). Taken together,

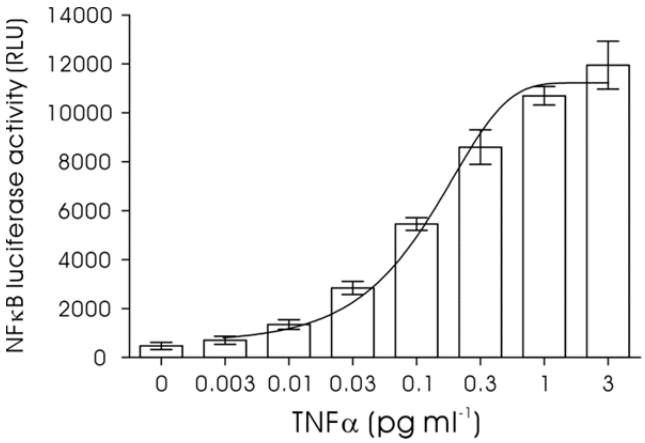

Fig. 1. Sigmoidal semi-logarithmic dose-response curve of NF-κB luciferase induction by TNFα. NF-κB luciferase activity is expressed as relative light units (RLU). Data from three independent experiments (n = 3) are presented in a bar chart with means ± SE. The dose-response curve (R^2 > 0.95, $p < 0.001$) was fitted by using a non-linear equation: $y = a_0 + (c_0 - a_0)/(1 + 10^{[(\log EC50 - x)\times\theta]})$, in which y is the NF-κB luciferase activity, c_0 is the maximal NF-κB luciferase activity, a_0 is the basal NF-κB luciferase activity, EC_{50} or EC50 is the half-maximal effective TNFα concentration, x is the TNFα concentration, and θ is the hillslope.

the results suggest that the NF-κB luciferase reporter assay is very sensitive to proinflammatory stimuli. Moreover, with TNFα as a reference proinflammatory cytokine, the reporter assay was used thereafter to determine the total proinflammatory activity in serum samples collected from the study participants.

Personal $PM_{2.5}$ Exposure and Blood Proinflammatory Activity

Taxi drivers are constantly exposed to TRAPs, a heterogeneous mixture of hazardous chemicals, and have been demonstrated to have significantly higher levels of proinflammatory biomarkers, such as TNFα, in blood (Brucker *et al.*, 2013). For the present HESTP cohort, we used personal airborne $PM_{2.5}$ sampling to determine individual $PM_{2.5}$ exposure and NF-κB luciferase reporter assay to determine the blood proinflammatory activity. It was of importance that the personal sampling and the reporter assay in the present study provided high-quality data of the $PM_{2.5}$ exposure and the total proinflammatory activity, respectively, of each study participant for further analysis.

Results in Fig. 2(A) indicated that the personal $PM_{2.5}$ exposure of the exposure group (150 ± 15.4 μg m^{-3}) (mean ± SE) was significantly higher than that of the reference group (82.0 ± 4.53 μg m^{-3}) ($p < 0.001$). A previous study in the same HESTP cohort revealed that the median values of $PM_{2.5}$ (82.9 μg m^{-3}) and $PM_{2.5}$-bound PAHs (13.1 ng m^{-3}) of the exposure group were significantly higher than that of the reference group ($PM_{2.5}$ = 70.8 μg m^{-3} and PAHs = 8.24 ng m^{-3}); in the exposure group, a statistically significant positive correlation between the personal $PM_{2.5}$ exposure and the $PM_{2.5}$-bound PAHs was observed (R = 0.42, $p <$ 0.001) (Huang *et al.*, 2012). The NF-κB luciferase reporter assay was used here to determine total proinflammatory activity in blood samples. The resulted induction of NF-κB activity (RLU) by blood samples was converted into TNFα equivalence (TNFα-EQ) (pg mL^{-1}) with the equation shown in Fig. 1. The blood proinflammatory activity in the exposure group (0.0302 ± 0.0048 pg mL^{-1} TNFα-EQ) (mean ± SE) was significantly higher than that of the reference group (0.00440 ± 0.000800 pg mL^{-1} TNFα-EQ) ($p < 0.001$) (Fig 2(B)). For example, blood samples with proinflammatory activity of 0.1 pg mL^{-1} TNFα-EQ could induce the same levels of NF-κB luciferase activity as 0.1 pg mL^{-1} TNFα. Results in Fig. 2 together showed that the exposure group exhibited higher levels of $PM_{2.5}$ exposure and blood proinflammatory activity than the reference group, suggesting the potential positive association between TRAPs (i.e., $PM_{2.5}$) and systemic inflammation (i.e., NF-κB luciferase activity or TNFα-EQ).

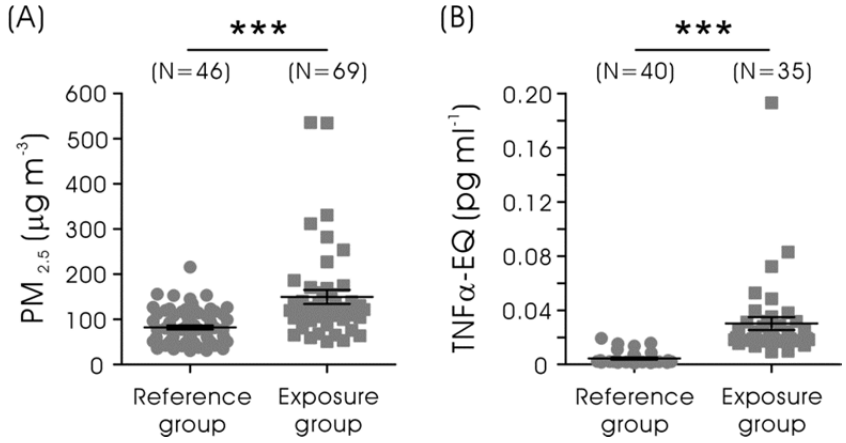

Fig. 2. Comparisons of personal $PM_{2.5}$ exposure and blood proinflammatory activity between reference and exposure groups. (A) Personal $PM_{2.5}$ exposure of Population 1 (N = 115) and (B) blood proinflammatory activity in Population 2 (N = 75) were shown in scatterplots with means and SE error bars. Number in parentheses means sample size. ***$p <$ 0.0001, with the non-parametric Mann-Whitney U test.

Associations between Blood Proinflammatory Activity and Personal $PM_{2.5}$ Exposure

However, it was noted that both personal $PM_{2.5}$ exposure and blood proinflammatory activity levels exhibited a non-normal distribution (Figs. 3(A) and 3(B)). Following logarithmic transformation, both results were shown in Figs. 3(C) and 3(D). The normality of data in Fig. 3 was assessed with skewness and kurtosis as previously described (Kim, 2013), where data were considered as a normal distribution when both $Z_{skewness}$ and $Z_{kurtosis}$ scores were between –3.29 and 3.29. The skewness, kurtosis, and Z scores of both $PM_{2.5}$ and blood proinflammatory activity (TNFα-EQ) before and after logarithmic transformation were summarized in Table 2. Clearly, the log-transformed data of both $PM_{2.5}$ exposure ($Z_{skewness}$ = 2.29 and $Z_{kurtosis}$ = 1.61) and blood TNFα-EQ ($Z_{skewness}$ = –0.390 and $Z_{kurtosis}$ = –1.903) were considered as a normal distribution.

Results in Fig. 4 revealed a statistically significant positive association between blood proinflammatory activity and personal $PM_{2.5}$ exposure after logarithmic transformation. The relationship between log TNFα-EQ and log $PM_{2.5}$ was fitted into the linear equation $y = 0.511x - 3.062$ (y = log TNFα-EQ and x = log $PM_{2.5}$), with R = 0.231 and p = 0.047. As the scatterplot summarized in Fig. 4, most data in the exposure group were above the regression line, whereas most data in the reference group were below the line. The present finding supports the idea that TRAPs are able to cause systemic inflammation in urban traffic conductors.

TNFα has been used as a biomarker to evaluate proinflammatory response by $PM_{2.5}$ exposure both *in vitro* and *in vivo*. $PM_{2.5}$ exposure markedly induced the expression and release of TNFα in culture cells (Dieme *et al.*, 2012; Liu *et al.*, 2014; Pardo *et al.*, 2015; Pope *et al.*, 2016; Yan *et al.*, 2016; Niu *et al.*, 2017). In rodents with $PM_{2.5}$ exposure, increased TNFα levels were detected in blood circulation, hippocampus, and prefrontal cortex (Li *et al.*, 2015; Hu *et al.*, 2017; Li *et al.*, 2018). Therefore, the NF-κB luciferase activity in this study was converted to TNFα-EQ for evaluation of proinflammatory activation in response to TRAPs. In the process, the $PM_{2.5}$-bound metals (Dieme *et al.*, 2012; Liu *et al.*, 2014; Pardo *et al.*, 2015; Yan *et al.*, 2016) and PAHs (Dieme *et al.*, 2012; Niu *et al.*, 2017) could be the active components for TNFα induction.

TRAP $PM_{2.5}$-bound chemicals contributed to the formation of reactive oxygen species (ROS)-related biomarkers (i.e., 8-oxo-7,8-dihydroguanine (8-oxodG) and 1-hydroxypyrene glucuronide (1-OHPG)) and activation of inflammatory mediators (i.e., interleukin 6 and TNFα) (Zuurbier *et al.*, 2011; Brucker *et al.*, 2013; Meier *et al.*, 2014; Cliff *et al.*, 2016; Hüls *et al.*, 2017; Dai *et al.*, 2018). Our previous studies in HESTP cohort revealed that $PM_{2.5}$-bound PAHs were significantly correlated with both 1-OHPG (a PAH metabolite) and 8-oxodG (an oxidative DNA damage biomarker) in urine (Huang *et al.*, 2012), and urinary 8-oxodG was significantly associated with urinary levels of cadmium and 1-OHPG (Huang *et al.*, 2013). The present study in healthy adults with occupational TRAP exposure indicated that the personal $PM_{2.5}$ exposure was significantly

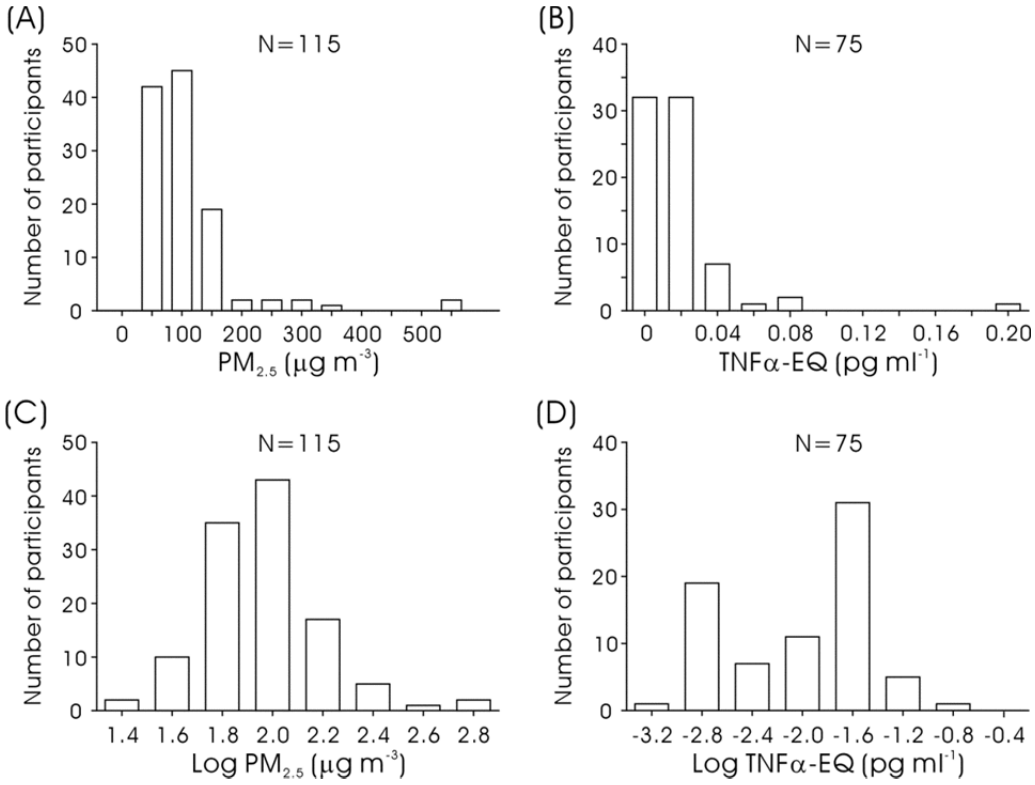

Fig. 3. Distribution of $PM_{2.5}$ exposure and proinflammatory activity of the study participants. Histograms of (A) $PM_{2.5}$ exposure of Population 1 (N = 115) and (B) TNFα-EQ levels in Population 2 (N = 75) were summarized. Logarithmic (C) $PM_{2.5}$ exposure and (D) TNFα-EQ levels were respectively converted from the original data in (A) and (B).

Table 2. Skewness, kurtosis, and Z scores for personal $PM_{2.5}$ exposure (in Population 1) and blood proinflammatory activity (TNFα-EQ) (in Population 2) before and after logarithmic transformation

	N	Skewness	$SE_{Skewness}$	$Z_{Skewness}$ [a]	Kurtosis	$SE_{Kurtosis}$	$Z_{Kurtosis}$ [a]
Population 1	115						
$PM_{2.5}$		3.22	0.226	14.3	14.1	0.447	31.4
Log $PM_{2.5}$		0.517	0.226	2.29	0.766	0.447	1.61
Population 2	75						
TNFα-EQ		4.62	0.277	16.7	28.5	0.548	52.0
Log TNFα-EQ		–0.108	0.277	–0.390	–1.043	0.548	–1.903

[a] $Z_{Skewness}$ = Skewness/$SE_{Skewness}$; $Z_{Kurtosis}$ = Kurtosis/$SE_{Kurtosis}$.

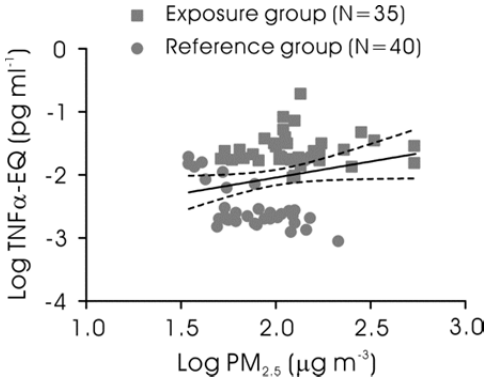

Fig. 4. Association between blood proinflammatory activity (in TNFα-EQ) and personal $PM_{2.5}$ exposure. Both TNFα-EQ and $PM_{2.5}$ data of the 75 participants in Population 2 (40 in reference group and 35 in exposure group) were summarized in the scatterplot of log TNFα-EQ versus log $PM_{2.5}$. The relationship between log TNFα-EQ and log $PM_{2.5}$ was fitted into the linear equation $y = 0.511x - 3.062$ (y = log TNFα-EQ and x = log $PM_{2.5}$), with R = 0.231, F(1,73) = 4.097, * $p = 0.047$, and the 95% confidence interval for mean of slope between 0.008 and 1.015.

associated with the blood proinflammatory activity (TNFα-EQ). Moreover, it is noted that NF-κB is a redox-sensitive transcription factor involved in regulating metabolism gene expression in response to heavy metal exposure (Korashy and El-Kadi, 2008). These studies together suggest that cellular metabolism of $PM_{2.5}$-bound pollutants, e.g., heavy metals and PAHs, from TRAPs may contribute to the induction of proinflammatory cytokine genes via ROS production and/or NF-κB activation. Results of previous (Huang *et al.*, 2012, 2013) and present studies in the HESTP cohort highlight the finding that long-term occupational exposure to TRAPs is able to cause adverse health impacts, e.g., inflammation and oxidative stress, on traffic conductors.

The limitations of this study are mentioned here for further consideration of research. Firstly, the $PM_{2.5}$ levels collected by personal samplers of both exposure and reference groups in the present study were pretty high. When the airborne $PM_{2.5}$ levels meet the indoor air quality standards (IAQs), the present NF-κB luciferase reporter system may not be sensitive enough to differentiate the difference of blood proinflammatory activity between exposure and reference groups. Secondly, PM-bound chemicals of different characteristics may activate different biological responses; therefore, chemicals of $PM_{2.5}$ emitted from the other sources may not be able to activate NF-κB as well as those of TRAP $PM_{2.5}$. Thirdly, convenience sampling, instead of random sampling, was used for recruiting the study participants in the present study.

CONCLUSIONS

The study of traffic conductors (the exposure group) and office-based police officers (the reference group) revealed that the exposure group exhibited elevated levels of both $PM_{2.5}$ exposure and proinflammatory activity compared to the reference group. A significant positive association between personal $PM_{2.5}$ exposure and blood proinflammatory activity was observed. These results suggest the potential involvement of TRAPs in the activation of systemic inflammation in urban traffic conductors.

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DISCLAIMER

The authors declare no conflicts of interest.

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