Relationship Between Exposure To Particulate Matter And Biomarkers Among Bus Drivers In Klang Valley, Malaysia

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ABSTRACT: This cross-sectional comparative study investigates the association between particulate matters (PM; PM_{10}, $PM_{2.5}$ and ultrafine particle (UFP) and concentration of biomarkers; Interleukin-6 (IL-6) and Tumor Necrosis Factor- Alpha (TNF-α) using 62 bus drivers as exposed group and 62 administrative staff as comparative group in Klang Valley, Malaysia. T-test results showed that the mean exposure level of PM_{10} ($t = 8.14$, $p<0.01$), $PM_{2.5}$ ($t = 9.95$, $p<0.01$) and UFP ($t = 19.61$, $p<0.01$) were significantly higher among the bus drivers compared to comparative group. Mann-Whitney U test of IL-6 ($z = -2.43$, $p<0.05$) and TNF-α ($z = -5.88$, $p<0.01$) were also found to be significantly higher in the bus drivers. Positive correlations were found between the exposure level of PM and concentration of biomarkers. In conclusion, the bus drivers showed higher concentration of IL-6 and TNF-α and were at a higher risk of getting respiratory illnesses compared to comparative group. Thus, more attention should be given on the control of high level of exposure to PM in order to minimize the adverse health effects among the groups at risk.

Keywords: $PM_{10,}$ $PM_{2.5,}$ UFP, IL-6, TNF-α, respiratory symptoms, bus drivers

Introduction

Increase in the population explosion, vehicular traffic, rapid urbanization and industrialization in the developed and developing countries (Brunekreef and Holgate, 2002) have contributes towards the increase in air pollution and concerns about adverse health effects due to air pollution (WHO, 2000). Air pollutants are divided into particulate matter (PM), volatile organic compounds (VOC) and halogen compounds. Atmospheric particles are generally described according to their morphology and composition. The PM with a diameter of less than 10 micrometers (μm) but greater than 2.5 μm is known as Particulate Matter 10 micron fraction (PM_{10}). Those having a diameter less than 2.5 but greater than 0.1 μm is designated as Particulate Matter 2.5 micron fraction ($PM_{2.5}$) while particles with a diameter of less than 0.1 μm are considered as the ultrafine particles (UFP). The size distinction is important as the particle size reflects in part, the penetration potential into the respiratory tract (Ibald-Mulli and Wichmann, 2002; Bai *et al.*, 2007).

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Recently, studies have shown that air pollution highly affected human respiratory system especially the lungs. During inhalation, the particulate matters (PMs) are brought deeply into the lungs and are deposited in the alveolar sacs. The deposition of these particles provokes inflammatory responses which cause alveolar macrophage activation and acute inflammation (Oberdorster *et al.*, 2000). Moreover, it also triggers the production of biomarkers. The activation of the biomarkers may cause inflammation which is a response of a tissue to injuries (Oberdorster *et al.*, 2000). Interleukin-6 (IL-6) and Tumor necrosis factor-alpha (TNF-α) are the main cytokines that involve in the inflammatory process in the lung (Mary, 1996; Carswell *et al.*, 1975).

Workers involved in transportation industry are highly exposed to the traffic air pollutants. Thus, they are at high risk in getting respiratory and lung diseases due to the exposure to traffic air pollutant and would seem to deserve particular attention for risk assessment (Jenkins *et al.* 1992). This present study aims to determine the relationship between PMs and traffic air pollutant's biomarkers (IL-6 and TNF-α, in this study) among the public bus drivers in Klang Valley, Malaysia. Due to the nature of the job, bus drivers are among the risk group being exposed to highly polluted air consisting of a mixture of air pollutants for about eight hours without any personal protective equipment.

Methodology

Study Background

This cross-sectional comparative study was performed among 62 bus drivers from a public bus company and 62 administrative staff. They were all male, nonsmokers, age between 20 to 55 years, Malaysian nationality and with no history of chronic lung and respiratory diseases. The respondents were participated in PM (PM_{10}, $PM_{2.5}$ and UFP) exposure measurement and analysis of sputum biomarkers (IL-6 and TNF-α) concentration. Questionnaires adapted from American Thoracic Society were used to obtain background information about the respondents.

PM Data

Exposures to PMs were sampled using DustTrak Aerosol Monitor, SidePak™ AM510 Personal Aerosol Monitor and P-Trak Ultrafine Particle Counter. For each respondent, the exposure level of PM was measured for eight hours. During the exposure measurement, the instruments were placed within the driving zone of the bus drivers and working zone of the administrative staff. The instruments were also placed away from the direct sources of PM (eg. photocopy machine, printer and vacuum cleaner) to avoid higher particles exposure from direct sources.

Measurement of Human Sputum IL-6 and TNF-α

The concentrations of the biomarkers were analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) according to the manufacturer's instruction. Sputum induction was performed by inhalation of isotonic saline solution (NaCl 0.9%) (Cataldo *et al.,* 2001) and the aerosols were produced by ultrasonic nebulizer (Cianchetti *et al.,* 2004). Sputum samples collected from the respondents were ultracentrifuged for 90 min at 25100 rpm at 4°C (Out *et al.,* 2001). The IL-6 and TNF-α concentration of the respondents were determined from a standard curve for IL-6 and TNF-α. All sputum biomarkers analysis were conducted at Chemical Pathological Laboratory, Department of Pathology, Universiti Putra Malaysia.

Statistical Analysis

Data were analyzed using Statistical Packages for Social Sciences (SPSS, version 13). Normality test used was Kolmogorov Smirnov with a Lilliefors Significance level ($p \leq 0.05$) for normal distribution.

Results and Discussions

Socio-demographic Information

TABLE 1 shows the socio-demographic information for both exposed and comparative groups. Basically, no significant differences were observed between the bus drivers and comparative group in terms of age, height, weight and duration of work. The mean age for the study and comparative groups were 38.30 $\pm$ 6.08 years and 36.64 $\pm$ 5.41 years, respectively. Aging has been associated with various lung diseases, mainly caused by the participation of thymus in human immunological function. In order to control the aging effects, the present study was restricted to the respondents aged 20-55 years. The mean height of the study group was 167.49 $\pm$ 6.50 centimeter (cm) and mean height of the comparative group was 169.05 $\pm$ 6.42 cm. On the other hand, the mean weight was 72.72 $\pm$ 11.57 kilogram (kg) and 70.37 $\pm$ 7.39 kg for the study group and the comparative group, respectively.

Comparison of Personal Exposure Level of PM

One of the major finding of this study was the significant difference in personal exposure level to PM between the bus drivers and the comparative group. The mean of personal exposure level of bus drivers for PM was significantly different between the groups (PM_{10}, $t = 8.137$; $p < 0.001$; $PM_{2.5}$, $t = 9.945$; $p < 0.001$ and UFP, $t = 19.608$; $p<0.01$) (Table 1). The data shows that the personal exposure level to PM among bus drivers was higher than the administrative staff.

Atmospheric PM in the urban areas was mainly generated by vehicular combustion. The rapid urbanization in the cities such as Kuala Lumpur requires an increased need for transportation (Sydbom *et al.,* 2001). Hence, more vehicles were found in the urban areas compared to the rural areas. The increasing numbers of vehicles especially diesel vehicles increase the atmospheric PM level (Donaldson *et al.,* 2005; Harrison, 1999; Aarnio *et al.,* 2008; Fang *et al.,* 2008; Hussein *et al.,* 2005). Ambient air at the road sides was polluted by the combustion, non-combustion and suspension emission produced by vehicles (Sydbom *et al.,* 2001).

Adverse health effects have been associated with the increase ambient PM10 gobally (USEPA, 2005; Becker *et al.,* 2003; Schwartz *et al.,* 1995). According to USEPA (2005), exposure to the indoor air pollutants inside buses will lead to adverse health effect including acute and chronic effects. The large fraction of combustion from the diesel engines ended up inside buses are particulate

matters ($PM_{10,}$ $PM_{2.5}$ and UFP), nitrogen dioxide, carbon monoxide and volatile organic compounds (VOCs).

It was estimated that over 600 million people in urban areas worldwide are exposed to dangerous levels of traffic generated air pollutants. In addition, the mean exposure concentration of $PM_{2.5}$ among bus drivers was higher compared to other traffic related exposure reported by a research conducted among 58 traffic related workers such as bus drivers, vendors, traffic police, and gas station attendants and 10 office workers as controls group (Cacciola *et al.*, 2002). Our findings are in aggreement as reported by Cacciola *et al.* (2002) that the bus drivers were the group with highest exposure concentration of $PM_{2.5}$.

UFP are particles with low mass concentration which are always present in the urban atmosphere in large numbers of quantity. These particles are highly reactive and not very stable but are always freshly-generated from sources such as combustion processes, tailpipe emissions and gas to particle conversions (Oberdorster, 1996). Donaldson *et al.*, (2001) demonstrated that UFP are generally produced by combustion processes and their particle sizes were extremely smaller than the particles in dusty places.

TABLE 1- Comparison of age, anthropometrical measurements and exposure level of PM among the respondent

Variables	Study group (n = 62) Mean ± SD	Comparative group (n = 62) Mean ± SD	t-value	p-value
Age (Years)	38.30 ± 6.08	36.64 ± 5.41	1.59	0.115
Height (cm)	167.49 ± 6.50	169.05 ± 6.42	-1.35	0.180
Weight (kg)	72.72 ± 11.57	70.37 ± 7.39	1.35	0.180
PM_{10} (mg/m^3)	0.094 ± 0.063	0.027 ± 0.014	8.137	0.000**
$PM_{2.5}$ (mg/m^3)	0.072 ± 0.032	0.029 ± 0.011	9.945	0.000**
UFP (pt/cc)	$1.31 \times 10^8 \pm 5.04 \times 10^7$	$5.40 \times 10^6 \pm 3.00 \times 10^6$	19.608	0.000**

Comparison of Concentration of Biomarkers

Our results show that the mean level of biomarkers were significantly lower among the comparative group as compared to the bus drivers ($p<0.05$). This indicates that the probability for the comparative group to get lung diseases is less than the bus drivers. In the present study, IL-6 was used as a biological indicator of inflammation in the lung. The median and interquartile range for IL-6 among the study and the comparative groups were 6.36 ± 3.88 pg/mL and 5.28 ± 4.14 pg/mL, respectively. Moreover, the median and interquartile range for TNF-α among the study and comparative groups were 24.67 ± 14.41 pg/mL and 13.68 ± 9.10 pg/mL, respectively. A significant difference for the biomarkers concentration between exposed group and comparative group (IL-6, $z = -2.43$, $p<0.05$; TNF-α, $z = -5.88$, $p<0.01$) is observed (**TABLE 2**).

TABLE 2- Comparison of biomarkers concentration

Variables	Study group (n = 62) Median ± IQR	Comparative group (n = 62) Median ± IQR	z-value	p-value
IL-6 Level (pg/mL)	6.36 ± 3.88	5.28 ± 4.14	-2.43	0.015*
TNF-α Level (pg/mL)	24.67 ± 14.41	13.68 ± 9.10	-5.88	0.000**

Generally, biomarkers such as IL-6 increase due to the exposure particles (Veranth *et al.*, 2008; Rosas *et al.*, 2007). Particulate exposures lead to the activation of alveolar macrophages (AM) for clearance mechanism followed by inflammation. Moreover, accumulation of PM in the human lung may lead to chronic inflammation. Mary (1996) demonstrates the role of proinflammatory cytokine IL-6 in pulmonary inflammation induced by exposure to environmental air pollutants. Similar results were also found in a study conducted by Veranth *et al.*, (2008) who found an increased cytokine binding with increasing particles concentration. In line with this, Li *et al.*, (2002) reported a positive relationship between DEP and inflammatory effect with data showing that the increased bronchoalveolar lavage fluid concentration of TNF-α was resulted from the exposure to carbon core particles of diesel exhaust particles. DEP consists of a complex mixture of petrochemical-derived organics adsorbed into particles and causes inflammatory effects on lungs.

TNF-α is released when there is an interaction of AM with atmospheric particles and in the same time increased their phagocytic activity and oxidant production (Goldsmith *et al.*, 1998; Hiramatsu *et al.*, 2003).

Correlation between Exposure Level to PM (PM_{10}, $PM_{2.5}$ and UFP) and IL-6 Concentration

Spearman Correlation test shows that there is a significant correlation between exposure level of PM and concentration of IL-6 for PM_{10} [study group ($r = 0.273$, $p<0.05$) and comparative group ($r = 0.268$, $p<0.05$)], $PM_{2.5}$ [study group ($r = 0.502$, $p<0.01$) and comparative group ($r = 0.443$, $p<0.01$)] and UFP, (**TABLE 3**). Also, $PM_{2.5}$ and UFP among bus drivers ($r = 0.673$, $p<0.01$) and among comparative group ($r = 0.538$, $p<0.01$). This clearly shows that the level of PM influences the concentration of IL-6 among the respondents.

TABLE 3- Correlations between exposure level to PM (PM_{10}, $PM_{2.5}$, and UFP) and IL-6 among the respondent

Biomarker	PM exposure	Study group (n=62)		Comparative group (n=62)	
		r	p	r	p
IL-6 (pg/mL)	PM_{10}	0.273	0.032*	0.268	0.035*
	$PM_{2.5}$	0.502	0.000**	0.443	0.000**
	UFP	0.673	0.000**	0.538	0.000**
TNF-α (pg/mL)	PM_{10}	0.249	0.051	0.287	0.024*
	$PM_{2.5}$	0.457	0.000**	0.335	0.008**
	UFP	0.438	0.000**	0.387	0.002**

Note: * Significant at $p < 0.05$
** Significant at $p < 0.01$

Stephan *et al.*, (2001) observed circulating levels of IL-6 elevated in subjects exposed to high levels of PM_{10} during an episode of acute air pollution. The cytokine induced a systemic response that has an important role in the pathogenesis of the cardiopulmonary adverse health effects associated with atmospheric pollution (Stephan *et al.*, 2001). Becker *et al*, (2005) found that PM_{10} interactes with alveolar macrophages (AM) and airway epithelial cells in vitro. The interactions produce variety of biomarkers including IL-6 as a defense mechanism to fight against the foreign particles that entered the body. The inhaled particles, with a mass median aerodynamic diameter < 10 μm could provoke more inflammatory effects compared to larger particles. PM_{10} could reach the lower respiratory tract where they are actively phagocytized by AM and produced proinflammatory cytokines (Veranth *et al.*, 2008; Monn and Becker *et al.*, 1999).

$PM_{2.5}$ which is smaller in size inhaled deeply into the lung and exerts their toxic effects on alveolar cells including macrophages, neutrophils and epithelial cells (Salvi and Holgate, 1999; Pozzi *et al.*, 2003). During the interaction between the $PM_{2.5}$ and alveolar cells, inflammatory mediators are secreted and phagocytosis takes place in order to protect the organism from the negative effects provoked by the foreign particles (Kreyling *et al*,. 2002). IL-6 is one of the important cytokine involve in the cleaning mechanism against $PM_{2.5}$ (Auger *et al.*, 2006; Lei *et al.*, 2005).

The ultrafine fraction of the urban ambient air particles was slightly, but significantly, more potent to induce IL-6 release than the total urban ambient air (WHO, 2003). It is less than 0.1 μm in size and has a large surface area (Ibald-Mulli and Wichmann, 2002) and can be brought deeply into the alveolar region and may also penetrate into the interstitial spaces (Oberdorster *et al.*, 1992). The deposition of inhaled UFP in the alveolar region of the lung are not efficiently phagocytized by alveolar macrophages, rather, they penetrate into and interact with alveolar epithelial, interstitial, and endothelial cells thereby inducing the release of proinflammatory and anti-inflammatory mediators (Oberdorster *et al.*, 1992; Totlandsdal *et al.*, 2008).

When compared to fine particles, the UFP have a higher deposition probability particularly in small airways and the alveolar region of the lungs, greater access to interstitial spaces and are less well phagocytized by AM. All these characteristic lead the UFP to a high access to the blood circulation, induce more oxidative stress and more pro-inflammatory responses than larger particles (Wilson *et al.*, 2002; Kreyling *et al.*, 2002).

Correlation between Exposure Level to PM (PM_{10}, $PM_{2.5}$ and UFP) and TNF-α Concentration

Spearman Correlation test shows a correlation between exposure level of PM and concentration of TNF-α for PM_{10} (exposed group, $r = 0.249$, $p=0.051$; comparative group, $r = 0.287$, $p<0.01$), $PM_{2.5}$ (study group, $r = 0.457$, $p<0.01$; comparative group, $r = 0.335$, $p<0.01$) and UFP (study group, $r = 0.438$, $p<0.01$; comparative group, $r = 0.387$, $p<0.01$) (**TABLE 3**).

Jimenez *et al.,* (2000) reported that the macrophage exposed to PM_{10} stimulates a pro-inflammatory response in lung epithelial cells. The findings showed that PM_{10} may trigger an epithelial cell inflammatory response via macrophage mediators TNF-α if exposed directly to pollutants. TNF-α increases in a dose-dependent manner when AM is exposed to ambient particles and the particles with different composition and size produce a similar response, as also noted by Eeden *et al.* (2001). Cytokines involvement increases in the systemic inflammatory response induced by PM_{10} (Eeden *et al.*, 2001). The results of Eeden's study clearly revealed that the productions of TNF-α were elevated by the exposure to $PM_{10.}$ The interaction of AM with atmospheric particles increases their phagocytic activity, oxidant production, and the release of inflammatory mediators such as TNF-α (Eeden *et al.*, 2001).

Jalava *et al.,* (2006) found a positive relationship between $PM_{2.5}$ and TNF-α production. The PMs were sampled in four size ranges, i.e. coarse (PM_{10} – $PM_{2.5}$), intermodal size range ($PM_{2.5}$ – PM_1), PM_1– $PM_{0.2}$ and ultrafine ($PM_{0.2}$) particles. The particle size range of $PM_{2.5}$ highly provoked the production of inflammatory cytokine of TNF-α compared to other size of particles (Jalava *et al.*, 2006).

UFP are particles with less than 0.1 µm (Ibald-Mulli and Wichmann, 2002) and primarily produced by combustion in diesel engines. These highly reactive particles are present in large number in the urban air. Due to the smaller size, they are able to penetrate epithelium and vascular walls to enter into the bloodstream. UFP are found to provoke carcinogenicity (Cammer *et al.*, 1988), autoimmune disorder (Yashino and Sagai, 1999) and increase cardiovascular disorders (Schwarts *et al.*, 1995; Seaton *et al.*, 1995). Thus, the present study selected TNF-α as a biomarker to measure the probability of TNF-α production due to the UFP exposures. Our results show that there is a significant correlation between the exposure level to UFP and TNF- α concentration.

Conclusion

The study shows that diesel exhaust particles form an important source of pulmonary inflammation among the bus drivers. The diesel exhaust particles include PM_{10}, $PM_{2.5}$ and UFP. The exposure levels of the PM_{10}, $PM_{2.5}$ and UFP were significantly higher ($p<0.01$) among bus drivers and associated with elevated concentration of IL-6 and TNF-α ($p<0.05$). The prevalence of respiratory symptoms (cough and phlegm) were also found significantly higher among the bus drivers compared to the comparative group. The concentration of IL-6 was mainly contributed by the exposure to PM_{10}, $PM_{2.5}$ and UFP while TNF-α concentration was mainly contributed by $PM_{2.5}$ and UFP.

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