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Elevated Ambient PM_{10} Levels Affecting Respiratory Health of Schoolchildren in Chiang Mai, Thailand

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ABSTRACT

The health effects from exposure to particulate air pollution during the dry season (February-March) in northern Thailand, a longstanding problem, have become particularly acute since 2007. Although several studies have reported the effect of ambient PM_{10} levels on airway oxidative stress and pulmonary function among respiratory disease patients, few studies have focused on healthy children, especially in Thailand. We conducted a prospective follow-up study to assess the respiratory health among schoolchildren exposed to different levels of ambient PM_{10} by comparing exhaled malondialdehyde (MDA) concentrations and pulmonary function indices. Exhaled breath condensate (EBC) samples were obtained from individual participants. MDA in EBC has been proposed as a biomarker of airway oxidative stress. The participants were 54 healthy schoolchildren with median (min-max) age of 11 (10-12) years old from a primary school in Chiang Mai City, Thailand. Questionnaires and EBC samples were collected twice, in the rainy season (July 2011, low PM_{10} level) and the dry season (March 2012, high PM_{10} level). It required about 10 minutes to collect EBC samples of 1-3 mL from individual participants, using an EBC-collecting apparatus that was modified specifically for this research from a standard hospital-use apparatus. MDA concentration in EBC was analyzed using HPLC-UV detection. Trained public health personnel performed the pulmonary function tests. The median of exhaled MDA concentrations in the rainy and dry seasons was 0.17 and 0.22 µM, respectively. Mean ± SD of forced expiratory volume in one-second/forced vital capacity ratios (FEV_1/FVC) in the rainy and dry seasons was 94.6 ± 4.4 and 91.3 ± 4.7 percent predicted, respectively. Exhaled MDA concentration significantly increased and the FEV_1/FVC ratio significantly decreased in the dry season ($p<0.05$). This results shows that elevated ambient

PM_{10} levels affect the respiratory health of schoolchildren. Further, exhaled MDA could be used as a biochemical marker of airway oxidative stress due to ambient PM_{10} among healthy schoolchildren.

Keywords: Ambient PM_{10}, Chiang Mai, exhaled breath condensate (EBC), malondialdehyde (MDA), pulmonary function, schoolchildren

INTRODUCTION

Airborne particulate pollution is a longstanding public health problem worldwide. Animal and human studies have reported that exposure to ambient particulate matter was associated with adverse health effects, including increased mortality rates (Lee et al., 1998; Samet et al., 2000) and an increased number of hospital visits due to respiratory and cardiovascular diseases (Ware et al., 1986; Burnett et al., 1997; Peters et al., 1999; Peng et al., 2008). Ambient particulate matter (PM), such as those less than 10 micrometers (μm) in aerodynamic diameter (PM_{10}), contains a complex mixture of pollutants. Many studies have demonstrated that ambient PM_{10} can produce oxidative stress at both the cellular and living systems level, and appear to initiate responses particularly dangerous to children, a population believed to have enhanced vulnerability to ambient PM_{10} (Kelly, 2003; Li et al., 2003; Schwart, 2004). Exhaled breath condensate (EBC) is a new tool for determining pulmonary inflammation and oxidative stress markers implicated in the pathogenesis of various respiratory conditions. EBC collection is relatively simple to perform, inexpensive, non-invasive and safely applied to children and patients with severe diseases; it is also suitable for repeated measurements (Mutlu et al., 2001; Hunt, 2002). Some chemical components in EBC can be used as biomarkers of airway diseases, such as oxidative stress in the lungs (Chan et al., 2009). Oxidative stress can arise for many reasons, including air pollutant exposure. Oxidative stress can be assessed and monitored through the determination of the levels of biomarkers, such as hydrogen peroxide, lipid peroxidation-derived products, namely aldehydes and isoprostanes, and protein carbonyl groups (Taylor, 2011).

Chiang Mai City is located in northern Thailand, approximately 700 km from Bangkok, in the Ping River valley, surrounded by mountains. The Pollution Control Department (PCD), Ministry of Natural Resources and Environment provides information on air quality parameters, including PM_{10}, NO_X, O_3, SO_2, and CO from two air quality monitoring stations in Chiang Mai Province. In recent years, average PM_{10} levels in Chiang Mai City have been elevated in the dry season, especially January to March, exceeding Thailand's standard limit, set at 120 microgram/cubic meter ($\mu g/m^3$).

This study analyzed the effects of exposure to ambient PM_{10} on respiratory health among schoolchildren in Chiang Mai City by comparing pulmonary function indices and exhaled MDA concentration between the rainy and dry seasons.

MATERIALS AND METHODS

Study subjects

The study subjects consisted of 54 primary schoolchildren, 10 to 12 years of age, who resided for at least one year within two kilometers of the air quality monitoring station at Yupparaj Wittayalai School, Chiang Mai City. Subjects diagnosed with asthma, chronic respiratory disease or long-term medication use were excluded from the study. The present study protocol was approved by the Human Experimentation Committee of the Research Institute for Health Sciences (RIHES), Chiang Mai University (HEC approval No. 1/2010). Children and parents gave written informed consent before participating in the study.

Study time period

Data was collected in two periods: 1) the rainy season (July 2011, a month with low PM_{10} levels) and 2) the dry season (March 2012, a month with high PM_{10} levels). These periods were chosen based on the monthly mean concentration of PM_{10} at the air quality monitoring station, Yupparaj Wittayalai School, Chiang Mai City. The PM_{10} concentration varied from 14.1 to 33.8 $\mu g/m^3$ and 40.3 to 195.0 $\mu g/m^3$ in the rainy and dry seasons, respectively. The monthly mean concentration of PM_{10} was 21.3 $\mu g/m^3$ in the rainy season (July 2011) and 106.2 $\mu g/m^3$ in the dry season (March 2012), as shown in Figure 1.

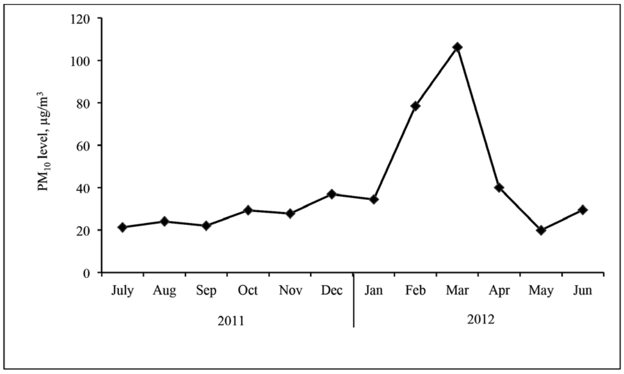

Figure 1. Monthly mean concentration of PM_{10} during the study period at the air monitoring station at Yupparaj Wittayalai School, Chiang Mai City.

Source: The Pollution Control Department, Ministry of Natural Resources and Environment.

Pulmonary function test and exhaled MDA analysis

Pulmonary function test was performed with portable electric mini-spirometer (Datospir, Sibel SA, Barcelona, Spain) administered by a trained technician according to the recommendation of the American Thoracic Society (American Thoracic Society, 1995). The best of three consecutive spirometer recordings was used. The measured parameters were forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and forced expiratory ratio (FEV_1/FVC %).

EBC samples were collected from the schoolchildren using a device we developed (Figure 2). The device consists of a mouthpiece with two way non-rebreathing valve. The mouthpiece was connected to a condensation chamber by flexible plastic tubing and the condensation chamber was immersed in a liquid nitrogen slurry. After rinsing their mouth, subjects were instructed to form a complete seal around the mouthpiece and maintain a dry mouth during collection by periodically swallowing excess saliva. The subjects sat comfortably and wore a nose clip. They were instructed to breathe tidally and very slowly expire through the mouthpiece for 10 minutes to collect approximately 1-3 mL of condensate. The collected EBC samples were frozen immediately in dry ice (–70°C) and stored in the freezer (–20°C) until analysis.

Figure 2. Photograph of a subject using the developed EBC collecting set.

The concentration of MDA in EBC was determined by measuring MDA–thiobarbituric acid (TBA) adducts according to the method previously described by Wong et al. (1987) with slight modifications. Briefly, 50 µL of EBC sample was mixed to 0.44 mol/L of H_3PO_4 (750 µL) and 42 mmol/L of TBA solution (250 µL) and the mixture was heated at 95°C for 60 minutes. Then, the mixture was cooled in ice water for 5 minutes and kept at room temperature for an additional 20 minutes. Three hundred µL of the mixture were transferred into another centrifuge tube with 300 µL of Methanol-NaOH solution and centrifuged at 13000 rpm for 10 minutes. The absorbance was measured at 532 nm after separation with an HPLC system equipped with a mobile phase of 50 mmol/L of KH_2PO_4 (pH 6.8): methanol (60:40, v/v). MDA concentration was calculated from the standard calibration curve and expressed as µmol/L of EBC. The determination of MDA in EBC samples was performed with an eight-point calibration curve based on water blank measurement and seven concentrations (0.08, 0.15, 0.3, 0.6, 1.2 and 2.4 µM). The detection limit was approximately 0.08 µM. For readings that gave results below the method sensitivity, the MDA concentration in EBC was set as LOD/√2, which was 0.28 (Hornung and Reed, 1990).

Data analyses

The Kolmogorov-Smirnov test was used for the fitness of the variables to the normal distribution. Mean or median values were reported according to described central tendencies. The results were expressed as mean ± SD for normally distributed data and median and range for non-normally distributed data. Comparisons of exhaled MDA concentrations between rainy and dry seasons were performed by the Mann-Whitney test and paired t-test for pulmonary function indices. A statistical software package (SPSS Version 17; SPSS Thailand) was used. Statistical significance was defined as $p<0.05$.

RESULTS

General characteristics of the study subjects

Table 1 shows the general characteristics of the schoolchildren participating in the study. Sex distribution of the subjects was homogenous (male: female = 1: 1). Median age of the subjects at the start of the study was 11 years (range 10-12 years). The median height was 144 cm (range 130-164 cm) and median weight was 34 kg (range 25-59 kg).

Table 1. The demographic characteristics and anthropometric measurements of study subjects.

Subject characteristics	Study subjects (n=54)
Male : Female	27 : 27
Age, year *	11 (10-12)
Height, cm*	144 (30-164)
Weight, kg *	34.0 (25-59)

Note: * median (range)

Pulmonary function indices and exhaled MDA concentration

In Table 2, the mean FVC and FEV_1 predicted in the rainy season was 94.4% and 104.5%, respectively, and in the dry season 96.3% and 102.4%, respectively. The means of the predicted FVC and FEV_1 predicted were not significantly different between the rainy and dry seasons. The mean FEV_1/FVC ratios in the rainy and dry seasons were 94.6 and 91.3%, respectively. We found the FEV_1/FVC ratio decreased significantly in the dry season ($p<0.05$). The median concentrations of exhaled MDA in the rainy and dry seasons were 0.17 and 0.22 μM, respectively. The MDA concentrations significantly increased in the dry season ($p<0.05$).

Table 2. The comparison of pulmonary function indices and exhaled MDA concentration of the subjects between the rainy and dry season.

Variables	Mean ± SD		p-value
	Rainy season	Dry season	
FVC, % of predicted	94.4 ± 14.2	96.3 ± 6.0	.272
FEV_1, % of predicted	104.5 ±16.2	102.4 ± 5.8	.321
FEV_1/FVC ratio	94.6 ± 4.4	91.3 ± 4.7	.000**
Exhaled MDA, μM*	0.17 (0.08-0.30)	0.22 (0.08-0.33)	.000**

Note: *median (range); **significant difference between seasons.

DISCUSSION

The results from the present study support our hypothesis that elevated ambient PM_{10} level is positively associated with FEV_1/FVC ratio and exhaled MDA concentration.

In contrast to some earlier results, we did not find differences in the predicted values of FVC and FEV_1 between the rainy and dry seasons. In our study, the FEV_1 predicted value tended to be lower in the dry season than in rainy season; however, the difference was not statistically significant. Other studies have shown that PM_{10} levels adversely affect the pulmonary function of children (Kim et al., 2005; Kasamatsu et al., 2006). Our findings agree with several previous studies of ambient air pollution from Helsinki, Athens, Amsterdam and Burmingham (Hartog et al., 2010), Bangkok (Lungkulsen et al., 2006) and New Zealand (Epton et al., 2008). Researchers of those studies concluded that elevated PM_{10} levels had no relationship with pulmonary function of schoolchildren, but a small effect on respiratory symptoms. Several possible reasons may explain the lack of relationship between PM_{10} and pulmonary function observed in our study. Firstly, in the rainy season, respiratory infections are common and may confound the relationship with PM_{10}, making effects difficult to detect. Secondly, the present study had a rather small sample size of 54 participants; therefore, we did not find a statistically significant adverse change in pulmonary function between the two seasons. We might need a larger sample to detect significant changes in pulmonary function. Thirdly, the present study collected data in March 2012 (dry season), when the PM_{10} level exceeded the national standard of 120 μg/m^3 for only 10 days. Moreover, based on their anthropometric measurements, the subjects in our study were rather healthy schoolchildren; therefore, the effects of PM_{10} may not be detected by pulmonary function testing. The finding may also be confounded by some other environmental factor, such as environmental tobacco smoke and/or cooking smoke at home. In addition, other potential confounders may play a role, including socio-economic factors of household crowding, number of smokers in the family, type of fuel used for cooking, air-conditioned bedroom, indoor pets, traffic levels in the neighborhood or diets. Eroshina et al. (2004) showed that environmental and social factors affected the health, respiratory dysfunction and impaired lung function of schoolchildren in Moscow. To decrease the influence of these confounders, this study was designed to follow up the health outcomes of the same group of schoolchildren in different seasons.

In our study, exhaled MDA concentration in the dry season was significantly higher than in the rainy season. This suggests that PM_{10} contained oxidative activity properties and induced oxidation-dependent alterations in inflammatory cells that were involved in the production of reactive oxygen species (Gonzalez, 2004; Ohyama et al., 2007). Oxidative stress occurs when there is an imbalance between oxidant and antioxidant on a cellular or individual level. Oxidative stress can arise for many reasons, including consumption of alcohol, medications, trauma, toxins, radiation and air pollution (Kelly, 2003; Romieu et al., 2008). Exposure to PM_{10} pollutant gives rise to oxidative stress within the lung, and this initiates responses that are harmful to susceptible populations. The generation of reactive oxygen species can cause oxidative damage to DNA, proteins or lipids in the body. MDA is one of the major final products of lipid peroxidation. The respiratory tract presents a large surface area in contact with air pollution, and is the first target of PM_{10} pollutants, where they evoke an inflammatory response; PM_{10} pollution sis an important trigger of lung inflammation (Flavia et al., 2010). Our findings suggested that MDA concentration in EBC provided an early biological marker for PM_{10} exposure, before clinical symptoms appeared.

CONCLUSION

Elevated ambient PM_{10} levels increased exhaled MDA concentrations and decreased the FEV_1/FVC ratio in schoolchildren, affecting their respiratory health, although the sample size was small. Ambient PM_{10} levels should be better controlled to protect the community.

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