ORIGINAL ARTICLE

Association of Exposure to $PM_{2.5}$ and PM_{10} with DNA Damage in Exfoliated Buccal Mucosa Cells among Primary School Children Living Nearby Palm Oil Activity at Semenyih, Selangor

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ABSTRACT

Introduction: Exposure of $PM_{2.5}$ and PM_{10} released from combustion of biomass activity caused respiratory health among children. Objective: This study aims to determine the association between exposure of $PM_{2.5}$ and PM_{10} with DNA damage in primary school children living nearby palm oil combustion activity at Semenyih. **Methods:** A cross sectional comparative study were conducted among Malay primary school children in school A located 2.7km from palm oil activity (N=82) and school B located about 40km away from the palm oil area (N=85). A standardized questionnaire were distributed to respondent's parents. Concentrations of $PM_{2.5}$ and PM_{10} were measured by using Dust Trak DRX Aerosol Monitor Model 8534 and Escort LC Personal Sampling Pump. Measurement of indoor and outdoor air pollutants were conducted in schools and home. Buccal cells were collected, which then followed by micronucleus assay. **Results:** Concentration of PM_{10} and $PM_{2.5}$ at home of studied group were significantly higher compared to comparative group with p value (p=0.007) and (p=0.018) respectively. PM_{10} and $PM_{2.5}$ of studied schools were significantly higher compared to comparative schools with p value (p=0.014) and (p=0.04) respectively. MN frequencies of studied group were significantly higher compared to comparative group (p=0.001). Significant difference of respiratory symptoms were found between two groups which are cough, phlegm, wheezing and chest tightness (p=0.001). There were significant correlation between PM_{10} with MN frequency of studied group and comparative group with r= 0.562; p=0.001. **Conclusion:** This study indicated that the exposure of PM_{10} and $PM_{2.5}$ would increase the risk of having respiratory health symptoms and might induce the micronuclei formation among children who lived near palm oil activity area.

Keywords: Children, Particulate matters, Respiratory health, Micronuclei Frequency (MN)

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INTRODUCTION

Air pollution has become the most significant concern as the pollutants in the ambient air can threaten human health. It is crucial to combat pollutant emissions such as the evaluation on the characteristics of particulate matters (PM) at different sources to protect both human and the environment (1). World Health Organization stated that the polluted environment can cause an estimated of 12.6 million deaths globally (2). From the numerous deaths, young children contribute 23% of total global mortality and 26% of deaths (3).

Oil palm is one of the major crops that are planted in Malaysia. The plantation of oil palm was aggressively growing from early 1875. Malaysia has become the second largest producer and importer of palm oil in the world (4). Since Malaysia needs to produce the oils and fats due to world demand, every factory needs to increase its production whereby it operate during the day and night. Unfortunately, palm oil activities generate air pollutants besides liquid effluent and solid waste. Each palm oil will have one boiler to carry out combustion process (5).

A susceptible group especially elderly, pregnant women, the person who is suffering from any respiratory health problem and children are prone to get any respiratory problems when inhaling those particulate matters. The person who had a history of suffering respiratory problem faced difficulty in breathing and even worsening their health condition. Exposure to air pollutant at early age can cause impair of lung development, reduce lung function and increase risk of chronic lung disease in adulthood (2). These are due to immune system and underdeveloped organs of children. Children inhale more air relative to their weight and lung surface compared to adult (6).

Previous study reported that exposure to air particulate matters associated with prevalence of respiratory health symptoms among children living in high polluted area (7). Therefore, children become an interest group in this study as they were the most affected.

The exposure of particulate matter such as PM_{10} and $PM_{2.5}$ can be assessed by micronuclei (MN) frequency as the biomarkers of genetic damage (8). MN assay is extensively used to assess various environmental exposure such as pollution, industrial toxicants, radiation and numerous medical treatments for public health beneficial especially in children (9). The formation of extranuclear bodies from breakage of chromosome or the whole chromosome does not reach the spindle poles during cell division are called as micronuclei. After failure of mitotic spindle, the micronuclei will form, thus it will lead to misattachments of microtubules (10). The micronuclei can be identified by its round or oval shape with the size of 1/3- 1/16 diameter of main nucleus and found in the cellular cytoplasm (11).

This study aim to determine the association between exposure of $PM_{2.5}$ and PM_{10} with DNA damage in primary school children living nearby palm oil combustion activity at Semenyih. We investigated the potential genotoxicity of PM_{10} and $PM_{2.5}$ by determining the MN frequency among children. Apart from that, we investigated the respiratory health symptoms of children which may be affected by the inhalation of particulate matters.

MATERIALS AND METHODS

Study background
This study was a cross sectional comparative study conducted among Malay primary school children who lived near palm oil activity and far from palm oil activity in Selangor, Malaysia. A total of 167 primary school children with aged between 9 to 11 years old were recruited from two selected primary schools. The exposed group (N=82) was primary school children who studied at Sekolah Kebangsaan A located 2.7km from palm oil activity. Meanwhile, the comparative group (N=85) was among primary school children who studied at Sekolah Kebangsaan B located about 40km away from the palm oil area. Parents answered the given questionnaire that comprised of eight section including sociodemographic background, health status, respiratory health history, eating habits, indoor and outdoor environment information, history health of parents and permission on collecting dust samples in homes. The structured questionnaire was adapted from American Thoracic Society (Questionnaire ATS –DCD-78C for children) and questionnaire from American Academy of Paediatrics. Children who involved were given an approval from their parents or guardian before taking parts in this study. This study was conducted from February 2019 until June 2019.

School and home monitoring
Measurement of the PM_{10} and $PM_{2.5}$ were sampled at two selected schools from 7.20 a.m until 2.00 p.m of 6 hours during school period. The measurement was taken at the back of classroom by using Dust Trak Aerosol Monitor Model 8534. Apart from that; personal sampling pump by Zefon was placed inside the children's bedroom or living room and operated for 24-hours to measure PM_{10} and $PM_{2.5}$ at respondent's house. The equipment was calibrated before used and placed at the same level of children's breathing zone, where 1 meter above from ground and 1.5 meter away from windows and door to avoid obstruction.

Collection of exfoliated buccal mucosa and MN assay
The children need to rinse their mouth twice with natural mineral water before sample collection. No eating was allowed before the buccal cell collection (12). The sample of buccal mucosa cells was gently scraped in the inner surface of cheek for 10 times in circular motion by using cytobrush. The samples was immediately dipped into microcentrifuge tubes containing 0.1M phosphate buffer solution (PBS) (pH7.5) and stored at -20 C. The cells were centrifuged and washed with 0.1M PBS, then smeared on the clear glass slides and leaved overnight. Next, the cells were fixed with 1% glutaraldehyde in cold 0.1M PBS for 20 minutes and pre-treated with 5N HCl for 30 minutes. The glass slides were stained with Schiff reagent for 90 minutes and counterstained with 0.1% Fast Green for 20 seconds (13). The slides then were examined at 40X magnification by using light microscope. Scoring of micronuclei frequency was performed by using formula (14);

where, X = Micronuclei score (MN)
2000 is referring to 2000 binucleated cells analysed for presence of MN

$$\frac{\text{Number of X}}{2000} \times 1000$$

Statistical analysis
Obtained data were analysed by using Statistical Package for Social Sciences (SPSS) version 23. The normality test was performed first in order to determine the distribution of data among studied and comparative group. The parametric or non-parametric test were determined for the subsequent statistical analysis. The significant level of this study was set at $p<0.05$. Mean, standard deviations, minimum and maximum were determined by performing univariate analysis. In the other hand, bivariate analysis was conducted in order to find the significant difference between variables in this study.

RESULTS

Characteristics of respondent's house
Based on Table I, there were higher number of respondents 44(53.7%) who lived more than 1000

meters from main road in studied group while, in comparative group only 3(3.5%) of the respondent lived more than 1000 meters from main road. Only 5 (6.1%) of respondent among studied group who lived less than 500 metres from palm oil factory and most of them live more than 3 km from it. The respondents were asked about the perception of their surrounding area whether they were dusty or not and the results showed moderate dusty with 59 (72%) and 52 (61.2%) from studied and comparative group respectively.

Table I: Characteristics of respondent's house

Variables	Studied group n=82 Total (%)	Compara-tive group n= 85 Total (%)	χ^2	p- value
Distance from main road				
<100 meters from house	5 (6.1)	3 (3.5)	1.652	0.648
>100-500 meters from house	5 (6.1)	9 (10.6)		
>500-1000 meters from house	28 (50.9)	27 (31.8)		
> 1000 meters from house	44 (53.7)	46 (54.1)		
Distance from palm oil factory				
<500 meters	5 (6.1)	-		
>1-1.5 km	18 (22.0)	-		
>1.5-3 km	18 (22.0)	6 (7.1)	40.993	**0.001***
>3 km	41 (50.0)	79 (92.9)		
Distance from power station				
<500 meters	-	-		
>1-1.5 km	-	-	0.953	0.329
>1.5-3 km	6 (7.3)	10 (11.8)		
>3 km	76 (92.7)	75 (88.2)		
Perception on surrounding area				
Very dusty	5 (6.1)	2 (2.4)	5.124	0.077
Moderate dusty	59 (72.0)	52 (61.2)		
Less dusty	18 (22.0)	31 (36.5)		

Concentration of air pollutants

Table II showed that the concentrations of PM_{10} and $PM_{2.5}$ of studied schools were higher (105.21±46.11); (68.83±13.03) compared to comparative schools (53.92 ±12.37); (50.80 ±17.45). Meanwhile for measurement in studied respondents' house, the concentration of PM_{10} and $PM_{2.5}$ were higher in studied group (104.29±58.70), (74.29±42.57) compared to comparative group (29.29±16.69); (28.57±32.11). The data showed that the p value of PM_{10} and $PM_{2.5}$ between studied and comparative group were less than 0.05. It can be concluded that there were a significant difference of mean between studied and comparative schools and respondents' house.

Comparison of MN Frequency among respondents for studied and comparative group

Based on Table III, MN frequency of studied group was higher (Median±IQR) (4.00±3.00) compared to comparative group (0.50±1.00). The results showed that there were significant difference of mean between studied and comparative group (p=0.001)

Prevalence of respiratory health symptoms

Based on Table IV, only coughing showed the most

Table II: PM_{10} and $PM_{2.5}$ concentration level in schools and respondents' house between studied and comparative group.

Variable	Studied group Mean±SD	Comparative group Mean±SD	t-value	p-value
PM_{10} at school ($\mu g/m^3$)	105.21±46.11 (n=3)	53.92 ±12.37 (n=3)	2.843	**0.014***
$PM_{2.5}$ at school ($\mu g/m^3$)	68.83±13.03 (n=3)	50.80 ±17.45 (n=3)	2.287	**0.040***
PM_{10} at respondents' house ($\mu g/m^3$)	104.29±58.70 (n=30)	29.29±16.69 (n=32)	3.25	**0.007***
$PM_{2.5}$ at respondents' house ($\mu g/m^3$)	74.29±42.57 (n=30)	28.57±32.11 (n=32)	-2.37	**0.018***

*significant at p<0.05

Table III: MN Frequency among studied and comparative group

Variables	Studied group N=82 Median (IQR)	Comparative group N=85 Median (IQR)	z-value	p-value
MN Frequency	4.00(3.00)	0.50(1.00)	-10.978	**0.001***

N= 167, Man - Whitney U Test, *significant at p<0.05

Table IV: High and low PM_{10} and $PM_{2.5}$ concentration level with respiratory symptoms

	High PM_{10} n=30	Low PM_{10} n=32	χ^2	P-value	PR	95% CI
	Total (%)					
Cough[a]						
Yes	9(30)	1(3.1)	8.267	**0.005***	13.286	1.565-112.803
No	21 (70)	31 (96.9)				
	High $PM_{2.5}$ n=32	Low $PM_{2.5}$ n=32	χ^2	P-value	PR	95% CI
	Total (%)					
Cough[a]						
Yes	9 (30)	2 (6.3)	5.984	**0.020***	6.429	1.259-32.827
No	21 (70)	30(93.8)				

*Significant at p<0.05
[a]Fisher exact test

reported respiratory health symptoms as the respondents were exposed to high level of PM_{10} with 9 (30%) respondents. There was only 1(3.1%) respondent who exposed to low level of PM_{10} was having cough, others were not. There were significant difference between exposure of two category PM_{10} with reported respiratory health symptoms (p<0.05). Respondents who exposed to high level of PM_{10} were 13 times likely to get cough, (PR= 8.267, 95%CI 1.565-112.803).

Furthermore, based on Table IV there were 9 (30%) out of 32 respondents reported on cough with high exposure of $PM_{2.5}$. There were significant difference between two categories of exposure level and cough (p<0.05). Respondents exposed to high level of $PM_{2.5}$ were 6 times as likely to get cough, (PR=6.429, 95%CI 1.259-32.827).

Correlation between PM_{10} and $PM_{2.5}$ (Home) with MN Frequency of studied group and comparative group

Based on Table V, there was a significant association between PM_{10} with MN frequency of studied and

Table V: Correlation between PM_{10} and $PM_{2.5}$ (Home) with MN Frequency of studied group and comparative group

Variables	MN Frequency (N=62)	
	r-value	**p-value**
PM_{10}	0.562	**0.001***
$PM_{2.5}$	-0.041	0.830

Spearman Correlation*significant at p<0.05

comparative group (p= 0.001). Therefore, the correlation result of PM_{10} shows that there was a moderate positive correlation between PM_{10} and MN frequency (r= 0.562).

DISCUSSION

Based on the findings, the concentrations of PM_{10} and $PM_{2.5}$ of studied schools were significantly higher 105.21µg/m³ compared to comparative schools 53.92 µg/m³. Previous study found that the concentration of indoor air pollutants (PM_{10}; 68.52 µg/m³ and $PM_{2.5}$; 41.33µg/m3) are higher in school which located near the palm oil factory (15). Vulnerable groups such as elderly, pregnant women and children who were residing near palm oil were exposed to high indoor and outdoor air pollutants by the emission of air pollutants released from the factory. One of the activities in palm oil factory was the combustion of biomass material such as fibre and shell from fresh fruit brunch as the source of energy for the factory. The burning of waste as a boiler fuel produced a harmful emission in the industry, releasing a massive amount of particles in the form of fly ash to the air (16-17).

Meanwhile, the mean concentration of PM_{10} at home within 24-hours was 104.29µg/m³ for the studied group indicating a higher concentration compared to the comparative group which is 29.29µg/m³. In another study in urban location found that mean in 24 hours of PM_{10} was 85.3 µg/m³ (18). Local study revealed that the mean concentration of PM_{10} in 24 hours nearby palm oil mill was 72.45 µg/m³ (15). The concentration of PM_{10} (104.29µg/m³) was lower compared to 150.0µg/m3 in 24-hours measurement. Moreover, for the concentration of $PM_{2.5}$ at home, the studied group recorded higher 74.29 µg/m³ compared to the comparative group 28.57µg/m³. However, 74.29µg/m³ was lower when comparing with Ambient Air Quality Standard Malaysia, 75.0µg/m³ within the 24-hours period. Previous local study revealed that the mean concentration of $PM_{2.5}$ in 24-hours located near palm oil mill was 42.26 µg/m³ among studied group compared to comparative group, 23.54 µg/m³ (15). The distance of houses from factory can be the factors due to high PM_{10} and $PM_{2.5}$ at home, where half (50%) of the studied group lived less than 3km from the factory as the house were located within zone of impact. The zone of impact taken an area of 5 km radius from the site and it depends on the source strength and the pollutant dilution effect (19). Air pollutants such as $PM_{2.5}$ can persist at the atmosphere for a few days and even for a few weeks as well as the

radius dispersion of particulate matter can travel up to hundreds to thousand kilometres. Meanwhile, PM_{10} can travel in the atmosphere for less than one kilometre to hundreds of kilometres (20).

There were a few factors which contribute to the production of indoor air pollutants such as ventilation and the source of fuel when cooking at home. The ventilation status in houses was one of the factors that contribute to the production of indoor particulate matters by which the particles are coming from the outdoor (21). Particulate emission from palm oil refinery also catching the attention of local authorities due to the combustion process as it cause air pollution (22). According to the World Health Organisation, $PM_{2.5}$ produced from incomplete combustion was identified as a contributing factor on air quality to detrimental effects on health and also climate (23).

The result of the study found that MN frequency of the studied group was higher compared to comparative group where it increases by eight folds from median MN frequency of comparative group. However, the mean MN frequency of this study (4.00%) was slightly lower compared to baseline mean MN frequency (5.7%) from pooled analysis by previous study (24) with range age of 0- 18 years old. MN frequency of this study also lower when compared to MN frequency (6.6%) of children with aged 5 - 11 years old who exposed to air pollutant resulted from study by Pedersen et. al (25). Another study among Brazilian school children found a significant evidence in which increased exposure to $PM_{2.5}$ increase micronuclei frequency in their subjects (26). From this study, it can be concluded that the MN frequency was still does not exceed the baseline mean MN frequency from the pooled analysis.

Most reported respiratory health symptoms were coughing 29.3% from respondents of the studied group followed by wheezing, phlegm and also chest tightness. Meanwhile, for the comparative group, only 1.2% of the respondents experienced cough, phlegm, wheezing and 2.4% of the respondents had reported having chest tightness. Respondents in studied group were 34 times likely to get cough, 15 times likely to get phlegm, 30 times likely to get wheezing and 7 times likely to get chest tightness, (PR= 34.76, 95%CI 4.57-264.17); (PR= 15.83, 95%CI 2.02-124.01); (PR= 30.800, 95%CI 4.040-234.799); (PR= 7.819, 95%CI 1.706-35.841). These outcomes were parallel with previous study whereby the reported respondents who were having a cough, phlegm, wheezing has a significant difference (p<0.01) between studied and comparative group (15). The initial deposition for the particles which is larger than 5 micrometres are likely to deposited at upper respiratory tracts to the entrance of the trachea (27). Particles inhaled into our respiratory tract can penetrate deeply into our lung and even into our bloodstream. The penetration of particulate matter into the lower respiratory tracts

or thoracic regions can cause respiratory morbidity and asthma (28). Previous study has found that that there was a significant association between exposures to PM_{10} at home with the prevalence of coughing (29). The exposure to particulate matters was associated with increased respiratory symptoms (30).

Previous study found that micronuclei frequency was associated with PM_{10}, NO_2 and CO where it comes from an automotive emission (31). Another previous study found that exposure to $PM_{2.5}$ and PM_{10} caused DNA damage in children nearby busy traffic road (32). PM_{10} was associated with mutagenic activities (33) and it gave a significant health impact as the chemical deposited in an organ that probably cause in vivo somatic cell mutation and development of cancer (31). There were increased level of DNA damage found in subjects who are working or living near an oil refinery compared with people living nearby to industries and restricted traffic (33).

CONCLUSION

In summary, this research indicated that the exposure of concentration PM_{10} and $PM_{2.5}$ would increase the risk of having respiratory health symptoms among primary school children who lived near palm oil activity area. The exposure of particulate matters might induce the micronuclei formation among affected children. This study would be the preliminary data of air quality at school and housing area located near palm oil factory. As for recommendation, the management of palm oil factory and authorities need to monitor the emission released from the palm oil activities frequently, meanwhile the community residing near palm oil factory area need to increase their awareness about the quality of air surrounding them. Parent should seek for doctor's consultation regarding their children health. Moreover, there is a need to conduct a more comprehensive study to investigate the component found in particulate matters that could induced the formation of micronucleus. The management of palm oil should revise the effectiveness of existing control measure on the emission of air pollutants. For instance, the use of bag filters was suggested before the polluted air release to the environment. However, there are also some limitation in this study as the number of respondents for sampling of dust particles at home is insufficient to represent the whole area that are affected by the palm oil activities.

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