The Impact of Exposure to Indoor Pollutants on Allergy and Lung Inflammation among School Children in Selangor, Malaysia: An Evaluation Using Factor Analysis

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ABSTRACT

A cross-sectional study of 470, 14-year-old students from 8 secondary schools located in sub-urban and urban areas in Hulu Langat district, Selangor, Malaysia was undertaken to determine the impact of atmospheric indoor air pollutants on atopy, asthma, respiratory symptoms and lung inflammation among school children. The students were surveyed using ISAAC and ECRHS questionnaires, their FeNO levels were measured and allergic skin prick tests were conducted. Active and passive sampling was used to measure the classroom indoor air concentration of NO₂, CO₂, formaldehyde, PM₁₀, PM_{2.5}, temperature and relative humidity. Linear mixed model, two-levels multiple logistic regression, PCA and SPC were applied to determine the complex relationship between respiratory symptoms, personal factors, FeNO levels and atmospheric indoor pollutants. 20.6% of students reported daytime breathlessness and 55.5% reported having rhinitis in the last 12 months. Atopy was prevalent in 57.7% of students, with predominant sensitization to Derp1 (51.9%) and Derf1 (47.9%) among doctor's diagnosed asthmatic students. Indoor air pollutants in urban area schools were significantly higher than those in sub-urban areas ($p < 0.001$). There was a significant association between exposure to PM₁₀ (OR = 2.66, 95% CI: 1.33–5.30) with skin allergy symptoms in the past 12 months. The PCA suggested that the most prominent factor associated with increasing FeNO levels was PM₁₀, with 73.5% of the variation. SPC predicted the pattern of FeNO at an upper confidence limit (UCL) of 104.21 ppb with increasing PM₁₀ concentration in the classroom (UCL = 40.23 $\mu\text{g m}^{-3}$). Exposure to PM₁₀ and PM_{2.5} significantly influenced the inflammation of the school children's lungs. Moreover, there were associations between self-reported wheezing, daytime and nocturnal attack of breathlessness with doctor's diagnosed asthma among school children.

Keywords: Allergy; Asthma; Indoor pollutants; Principle component analysis.

INTRODUCTION

The indoor environment plays a significant role in human health. There is much literature describing how indoor air pollutants contribute significantly to the triggering of allergic

responses and the development of asthma through complex pathophysiological changes among children (Douglas and Elward, 2011). As children nowadays spend much of their time in an indoor environment, either at home or inside the classroom at school, the quality of indoor air they are exposed to becomes relevant. A number of studies have demonstrated that schools in the urban and sub-urban area are affected with poor air quality originating from heavy traffic, construction and industrial activities (Chithra and Nagendra, 2018). Other factors affecting the indoor air quality include

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the ventilation system, occupancy, type of furniture and activities conducted inside the classroom (Gao *et al.*, 2014).

In assessing the effects of indoor air pollutants, the use of fractional exhaled nitric oxide (FeNO) measurement to monitor airway inflammation is highly recommended and acceptable. FeNO levels determination is a non-invasive, fast and cheap technique, and an acceptable surrogate biomarker of airway inflammation (Kim *et al.*, 2016; Zissler *et al.*, 2016). Previous epidemiological studies have found association between FeNO levels with the air pollutants concentrations in the classrooms (Grutta *et al.*, 2012; Acat *et al.*, 2017). This relationship is commonly analysed using robust regression analysis. However, a more advanced data analytical method to better identify and distinguished significant patterns of the data is supportive in this field. As such, the application of chemometric techniques such as Principle Component Analysis (PCA) and Statistical Process Control (SPC) are highly recommended. Presently, there remains a paucity of evidence on the application of PCA in determining the association of FeNO levels in relation to allergy and air pollutants concentrations indoor, especially in tropical countries.

This study aims to, firstly, compare indoor CO₂, NO₂, PM₁₀, PM_{2.5}, formaldehyde concentrations, temperature and relative humidity in urban and sub-urban area schools in Hulu Langat, Selangor. Second, to identify the association between personal factors, atopy, parental asthma/allergy, doctor's diagnosed asthma, and school location with respiratory and allergic symptoms among the secondary students. The third aim was to determine the association between measured indoor air pollutants with lung inflammation and respiratory symptoms. Finally, to identify the sources of classroom indoor pollutants contributing to the variation of lung inflammation among children.

METHODOLOGY

Study Population

This study included eight randomly selected secondary schools in Hulu Langat, Selangor, Malaysia. We targeted school children at age of 14 years old (Form Two) and randomly selected from four classes in each school. Briefly, the total sample of students recruited and received guardian's consent was 470. Students with the history of smoking in the last 12 months and students who received antibiotic treatments in the past four weeks were excluded from this study. The school areas were classified as urban and sub-urban by the Ministry of Education, Malaysia based on the locale classification of ecological measures. The data collection for health and clinical assessments were conducted on the same week as technical monitoring in August until November 2018 and February 2019. The study protocol was approved by the Ethics Committee for Research Involving Human Subjects Universiti Putra Malaysia (JKEUPM) (JKEUPM-2018-189) and each of the students was given a written consent form for guardian's approval before this study has started.

Assessment of Health Data

A standardized questionnaire was adopted from the

European Community Respiratory Health Survey (ECRHS) and the International Study of Asthma and Allergies in Childhood (ISAAC) (Norbäck *et al.*, 2017). The questionnaire was distributed to the selected students and administered with the guidance from the researchers. This questionnaire consisted of the following sections: background information, asthma and allergy information and symptoms for the last 6 months and 1 year (16 questions), health condition (9 questions), childhood information (3 questions), indoor house condition (11 questions) and allergy symptoms for the last 3 months (18 questions).

Assessment of Fractional Exhaled nitric Oxide (FeNO)

FeNO measurement was conducted using a electrochemical hand-held FeNO analyser (NIOX VERO, Circassia, Sweden) as recommended by the American Thoracic Society/European Respiratory Society (ATS/ERS) (Dweik *et al.*, 2011). The students were asked to inhale deeply through the mouthpiece attached to the patient filter and immediately exhale slowly for about six to ten seconds at a constant flow rate (50 mL s⁻¹). This process was repeated at least two times and the average value of reproducible FeNO measurements was used as the main outcome variable. Students were instructed to refrain from eating and drinking for one hour before the FeNO assessment to avoid the effects of non-disease related factors that influence the FeNO values.

Allergy Skin Test

An allergy skin prick test was performed on the volar side of the forearm using *Dermatophagoides pteronyssinus* (Derp1) (house dust mite), *Dermatophagoides farina* (Derf1) (house dust mite), *Cladosporium herbarium* (fungi), *Alternaria alternata* (fungi), and *Felis domesticus* (cat) allergens in liquid form (Prick-Test Diagnostic, ALK-Abelló, Madrid, Spain). Histamine (10 mg mL⁻¹) and normal saline were used as a positive and negative control respectively. The response was measured after 15 minutes by measuring the wheal diameter. Positive sensation to an allergen was considered when the wheal diameter was at least 3 mm. Atopy was defined as a significant positive skin test reaction to at least one of the applied allergens (ASCI, 2016).

Assessment of School Environment

Environmental parameters including temperature (°C), relative humidity (%) and carbon dioxide (ppm) were measured in the classrooms during learning session by using Q-Trak™ IAQ monitor (Model 7565 TSI Incorporated, Minnesota, USA) with the average logging values of over one minute. The accuracy of this device on temperature, relative humidity, and CO₂ are $\pm 0.6^\circ\text{C}$, $\pm 3\%$, and ± 50 ppm, respectively. Sampling for the particles was measured by using Dust-Trak monitor ((Model 8532 TSI Incorporated, Minnesota, USA) at a sampling rate of 1.7 L min⁻¹ with a detection limit of 0.001–150 mg m⁻³. The concentration of formaldehyde (mg m⁻³) was measured by using PPM Formaldemeter™ htV-m (PPM Technology Ltd, Wales, UK) with accuracy of 10.0% at 2 ppm. The instruments used were placed one metre from the ground in the centre of the classrooms. All instruments used were periodically calibrated.

In each school, a total of four hours measurements were taken from four randomly selected classrooms for period of an hour's time each during learning session and has been previously described (Norback *et al.*, 2014; Kamaruddin *et al.*, 2015; Suhaimi *et al.*, 2017). The NO₂ (µg m⁻³) concentration was measured by using diffusion sampler (IVL, Goteborg, Sweden) for a period of a week. The protocols of NO₂ sampling methods are described elsewhere (Langer *et al.*, 2015). The sampler tubes were returned to IVL Swedish Environmental Research Institute Laboratory (Goteborg, Sweden), an accredited laboratory for further analysis. This passive sampling technique provides an average concentration of NO₂ in the air for during a week with the limit of detection (LOD) of 0.5 µg m⁻³ and 10.0% of measurement uncertainty (Foldvary *et al.*, 2017). Details on building information, floor furnish, furniture and type of ventilation system were noted prior technical assessment.

Statistical Analysis

Descriptive test analysis was performed by the Statistical Package for Social Science (SPSS) 25.0. The associations between respiratory and allergic symptoms with personal factors and indoor pollutant concentrations were analysed by 2-Level Hierarchic Multiple Logistic Regression. Subsequently, the association between FeNO levels and environmental parameters were explored by applying Principle Component Analysis (PCA) using statistical package XLSTAT Evaluation 2019.2.3 (Addinsoft, New York, US). PCA was performed separately on doctor's diagnosed asthma and atopy children with Varimax rotation to reduce the dimensionality of data. Finally, the Statistical Process Control (SPC) analysis was performed using JMP data analysis software to estimate FeNO levels and concentrations of PM₁₀ and PM_{2.5} based on the circles of correlation and loading factors examined in PCA process (Al-Zoughool and Al-Mistneer, 2018).

RESULTS

Personal Characteristics and Health Symptoms

Of the total 470 secondary school children who participated, 182 (38.7%) were male and 288 (61.3%) female. Majority

of the children were Malay (86.8%). 57.4% of students that were randomly selected were from schools located in the urban area, while 42.6% were from the sub-urban schools. 271 (57.7%) of the school children were observed to have positive sensitization to at least one of the applied allergens. The prevalence of doctor's diagnosed asthma among the students was 10.6%, while 33.0% reported either their father or mother having asthma or allergies (Table S1 in Supplementary material). Furthermore, the prevalence of atopy among doctor's diagnosed asthmatic children was 14.4% (OR = 2.87, 95% CI: 1.43–5.76). Moreover, 16.2% (OR = 2.56, 95% CI: 1.36–4.82) of school children with atopy reported wheezing symptom in the past 12 months (Table 1).

Skin Prick Test Result

Table 2 lists the skin prick test responses among students with doctor's diagnosed asthma. More than half (51.9%) of students had positive test responses to the *Dermatophagoides pteronyssinus* (Derp1-house dust mite) allergen with 70.0% of them being asthmatic ($p = 0.007$). Similarly, 47.9% of students had responded to *Dermatophagoides farina* (Derf1-house dust mite) with 68.0% of them recorded as asthmatic ($p = 0.003$). The allergic responses to *Felis domesticus* (cat) was 30.0% among students diagnosed with asthma ($p < 0.001$). The least common allergen responses among students were to the fungi *Cladosporium herbarium* (1.1%) and *Alternaria alternata* (1.5%).

FeNO Levels in Relation to Personal Characteristics

We determined the mean of FeNO levels in all students to be 32.32 ppb (± 28.83), ranging from 5 ppb to 187 ppb. The FeNO levels among those with the atopic condition were significantly higher than non-atopic condition ($p < 0.001$). Furthermore, students with doctor's diagnosed asthma had higher FeNO levels than the non-asthmatic students. However, the mean FeNO levels between students in sub-urban and urban areas were not statistically different ($p > 0.05$) (Table 3). Additionally, weight (kg) and BMI (kg m⁻²) parameters did not show any statistical correlation with FeNO levels ($p > 0.05$). However, there was a correlation with height (cm) ($p = 0.013$) (Table S2 in Supplementary material).

Table 1. Prevalence of doctor's diagnosed asthma, parental asthma/allergy, allergic symptoms and respiratory symptoms (N = 470).

Personal factors and symptoms	Overall	Atopic	Non-atopic	p-value	OR	95% CI
Personal factors						
Doctor's diagnosed asthma	10.6	14.4	5.5	0.002*	2.87	1.43–5.76*
Parental asthma/allergy	33.0	31.7	34.7	0.503	0.88	0.59–1.29
School located in urban area	57.5	59.6	40.4	0.315	1.21	0.84–1.75
Allergic symptoms						
Rhinitis in the past 12 months	55.5	61.3	38.7	0.074	1.40	0.97–2.02
Skin allergic in the past 12 months	14.5	61.8	38.2	0.459	1.22	0.72–2.07
Respiratory symptoms						
Wheezing symptom in past 12 months	12.3	16.2	7.0	0.003*	2.56	1.36–4.82*
Daytime breathlessness	20.6	18.8	23.1	0.255	0.77	0.49–1.21
Nocturnal attacks of breathlessness	9.1	55.8	44.2	0.797	0.92	0.49–1.73

OR = Odd ratio.

* $p < 0.05$.

Table 2. Prevalence of allergen test result among doctor's diagnosed asthma (N = 470).

Allergen tested		Doctor's diagnosed asthma			
		Overall (%)	Yes (%)	No (%)	p-value
House dust Mites	Derp1	51.9	70.0	49.8	0.007*
House dust Mites	Derf1	47.9	68.0	45.5	0.003*
Fungi	<i>C.herbarium</i>	1.1	4.0	0.7	0.158
Fungi	<i>A.alternata</i>	1.5	4.0	1.2	0.351
Cat	<i>D.farina</i>	12.6	30.0	10.5	< 0.001**

*p < 0.05; **p < 0.001.

Table 3. Comparison of FeNO levels (ppb) between respondents' personal factors (N = 470).

Characteristic of student	Mean ± SD (ppb)	t	p-value
Gender			
Male	33.10 (24.76)	0.490	0.624
Female	31.83 (31.16)		
Sensitization			
Atopic	40.97 (32.95)	-8.919	< 0.001**
Non-atopic	20.54 (15.70)		
Doctor's diagnosed asthma			
Yes	61.46 (48.24)	-4.715	< 0.001**
No	28.85 (23.34)		
Location of schools			
Sub-urban	31.68 (31.17)	-0.417	0.677
Urban	32.80 (27.01)		

**p < 0.001.

Comparison of the Classroom Indoor Pollutants

All classrooms had natural ventilation and were equipped with ceiling fans (mean = 3 pieces/class). There were bookshelves and soft boards in every classroom and some also had window curtains. NO₂ concentrations were measured in the classroom for a period of one week. Other parameters of temperature, relative humidity, CO₂, particulate and formaldehyde were measured during class sessions. There were significant differences in all the indoor environmental parameters between urban and sub-urban schools, except the concentration of CO₂ (Table 4).

Association between respiratory symptoms and allergies was analyzed by multiple logistic regression while controlling the atopic condition, gender, doctor's diagnosed asthma, parental asthma/allergic and location of schools. The risk factors for wheezing were female, with atopic condition, doctor's diagnosed asthma and parental asthma/allergic. The

group of doctor's diagnosed asthma students reported more wheezing, breathlessness during daytime and also nocturnal attacks. Students with parental asthma/allergy also reported more rhinitis during the past 12 months. There was a protective association between daytime breathlessness and attending urban schools (Table 5).

Next, we investigated the association of respiratory symptoms and allergies against indoor environmental pollutants measured inside the classrooms while controlling atopy, gender, doctor's diagnosed asthma, parental asthma/allergy and school area. As shown in Table 6, there was a positive association between PM₁₀ and wheezing. Furthermore, daytime breathlessness was positively associated with increased NO₂ concentration. There was also a positive association between rhinitis reported in the past 12 months with increased formaldehyde concentration. Reports of symptoms of skin allergies in the past 12 months also had a

Table 4. Comparison of environmental parameters between urban and sub-urban schools (N = 32).

Parameter	Urban Median (IQR)	Sub-urban Median (IQR)	p-value
	n = 16	n = 16	
Temperature (°C)	29.0 (2.0)	27.5 (1.0)	< 0.001**
Relative Humidity (%)	74.7 (9.5)	81.4 (7.5)	< 0.001**
NO ₂ (µg m ⁻³)	32.0 (7.0)	19.0 (22.5)	< 0.001**
CO ₂ (ppm)	453.0 (34.5)	455.5 (25.5)	0.462
PM _{2.5} (µg m ⁻³)	24.6 (2.4)	22.0 (1.9)	< 0.001**
PM ₁₀ (µg m ⁻³)	41.6 (7.5)	37.0 (4.9)	< 0.001**
Formaldehyde (mg m ⁻³)	13.2 (9.3)	3.1 (5.2)	< 0.001**

IQR = Interquartile range.

**p < 0.001.

Table 5. Association (OR, 95% CI) between personal characteristics and respiratory symptoms and allergies (N = 470).

Personal characteristics	Respiratory symptoms		Allergic symptoms		
	Wheezing	Daytime breathlessness	Nocturnal attacks of breathlessness	Rhinitis in the past 12 months	Skin allergic in the past 12 months
Atopy	2.12 (1.05–4.28)*	0.71 (0.43–1.16)	0.91 (0.46–1.80)	1.53 (1.04–2.26)*	1.25 (0.72–2.17)
Female	2.47 (1.20–5.09)*	1.65 (0.98–2.72)	1.67 (0.79–3.54)	1.19 (0.80–1.78)	1.63 (0.89–2.96)
Doctor's diagnosed asthma	7.99 (3.84–16.63)*	3.27 (1.55–6.90)*	3.99 (1.82–8.74)*	1.03 (0.53–1.99)	1.71 (0.74–3.95)
Parental asthma/allergy	1.49 (0.79–2.80)	0.83 (0.49–1.39)	1.29 (0.66–2.52)	1.61 (1.06–2.44)*	1.37 (0.75–2.51)
Urban schools	1.39 (0.26–7.42)	0.65 (0.21–2.03)	0.52 (0.10–2.64)	0.83 (0.29–2.34)	1.86 (0.55–6.31)

*p < 0.05.

OR (OR = Odds ratio) was calculated by 2-Level Hierarchic Multiple Logistic Regression.

Table 6. Association (OR, 95% CI) between respiratory symptoms and allergies among students with the indoor pollutants (N = 470).

Indoor pollutants	Respiratory symptoms		Allergic symptoms		
	Wheezing	Daytime breathlessness	Nocturnal attacks of breathlessness	Rhinitis in the past 12 months	Skin allergic in the past 12 months
NO ₂ (µg m ⁻³)	1.44 (0.46–4.54)	4.63 (1.26–16.95)*	1.35 (0.45–4.01)	2.07 (1.10–3.89)*	3.68 (1.07–12.69)*
CO ₂ (ppm)	1.01 (0.96–1.02)	3.44 (0.36–32.93)	5.52 (0.23–130.8)	1.65 (0.23–11.73)	2.01 (0.16–25.37)
PM ₁₀ (µg m ⁻³)	2.33 (1.12–4.80)*	1.36 (0.57–3.19)	2.01 (0.77–5.23)	1.75 (0.97–3.17)	3.94 (1.50–10.36)*
PM _{2.5} (µg m ⁻³)	1.03 (0.88–1.20)	1.74 (0.37–8.17)	1.05 (0.77–1.31)	1.01 (0.85–1.08)	0.96 (0.82–1.13)
Formaldehyde (mg m ⁻³)	1.55 (0.50–4.80)	0.97 (0.90–1.06)	1.24 (0.48–3.21)	3.32 (1.69–6.51)*	2.41 (0.96–6.07)

*p < 0.05.

OR calculated for 10 µg m⁻³ increase in concentration of NO₂.OR calculated for 100 ppm increase in concentration of CO₂.OR calculated for 10 µg m⁻³ increase in concentration of PM₁₀ and PM_{2.5}.OR calculated for 10 mg m⁻³ increase in concentration of formaldehyde.

OR (OR = Odds ratio) was calculated by 2-Level Hierarchic Multiple Logistic Regression.

positive association between the increased concentrations of CO₂ and PM₁₀, respectively.

Symptoms in Relation to Personal Characteristics and Indoor Classroom Pollutants

In the final model analysis, 2-levels logistic regression model (school and student) was applied to determine the association between respiratory and allergic symptoms against personal factors and environmental pollutants variables, which had a significant association in the previous analysis step. More students with atopic condition experienced wheezing ($p = 0.005$) than those without atopy. More female students had reported with wheezing than male students ($p = 0.024$). The students with diagnosed asthma had reported more wheezing ($p < 0.001$), daytime breathlessness ($p = 0.003$) and nocturnal attack of breathlessness ($p = 0.015$) symptom, compared to those without asthma. There was a protective association of daytime breathlessness reported among students from schools in urban areas compared to students from sub-urban areas ($p = 0.008$). Rhinitis in the past 12 months was observed more among students with parental asthma/allergy ($p = 0.009$). Considering the environmental pollutant exposures, PM₁₀ was positively associated with wheezing ($p = 0.354$) and skin allergic symptom ($p = 0.012$), increased concentration of NO₂ was associated with daytime breathlessness symptom ($p = 0.010$), and increased CO₂ concentration in the classroom was positively associated with allergic skin symptom ($p = 0.072$) (Table 7).

Principle Component Analysis (PCA)

Next, we applied PCA to explore the association between the levels of FeNO and seven environmental parameters

measured inside the classroom, followed by Varimax orthogonal rotation and finally Statistical Process Control (SPC) to establish the contributing baselines. Eigenvalue of 1.0 or greater are considered as significant factors in the PCA process. The analysis was carried out separately for doctor's diagnosed asthma and atopic groups. Based on the Eigenvalues of factors in the analysis, three factor models were selected with a cumulative variability of 73.5% and 68.5% for doctor's diagnosed asthma and atopic conditions, respectively (Table 8).

Together, both axes of PCA explained 51.6% of the variation in the doctor's diagnosed asthma group. The concentration of CO₂ (ppm) and relative humidity (%) were positively correlated with axis one and had a strong loading factor. On the second component separate the concentration of particles, FeNO levels among asthmatic students with the formaldehyde, NO₂, CO₂ and temperature. A strong loading factor for the concentration of NO₂ ($\mu\text{g m}^{-3}$) and formaldehyde (mg m^{-3}) were identified in Factor 2. While Factor 3 had strong loading factor of PM_{2.5} ($\mu\text{g m}^{-3}$) and moderate loading factor of PM₁₀ ($\mu\text{g m}^{-3}$) and FeNO levels (ppb). This shows that the contribution of FeNO levels among doctor's diagnosed asthma group much contributed by the particles, especially PM_{2.5}. The contribution and cosines percentages tabulated in Table 8, confirmed this interpretation with the values of 54.9% and 65.9% for PM₁₀ and PM_{2.5}, respectively.

There was an inconsistency result for the PCA in the group of students with atopic condition. Factor analysis yielded three separated factors that could be explained 68.5% of the total variance in the data set. The concentration of particles, temperature, relative humidity and CO₂ (ppm) were positively correlated in axis one. The concentrations of

Table 7. Final model of the association between respiratory symptoms and allergies among secondary students.

	OR (95% CI)	p-value
Wheezing		
Atopic	2.33 (1.17–4.60)	0.016*
Female	2.62 (1.32–5.18)	0.006*
Doctor's diagnosed asthma	6.55 (3.26–13.15)	< 0.001**
PM ₁₀	1.08 (1.01–1.17)	0.042*
Daytime breathlessness		
Doctor's diagnosed asthma	2.51 (1.32–4.76)	0.005*
NO ₂	1.15 (1.01–1.31)	0.027*
Nocturnal attacks of breathlessness		
Doctor's diagnosed asthma	4.57 (1.49–13.97)	0.015*
Rhinitis in the past 12 months		
Atopy	1.57 (1.06–2.31)	0.023*
Parental asthma/allergy	1.65 (1.10–2.48)	0.015*
NO ₂	1.04 (0.99–1.09)	0.098
Formaldehyde	1.10 (1.03–1.17)	0.003*
Skin allergic in the past 12 months		
NO ₂	5.54 (0.82–37.43)	0.072
PM ₁₀	2.66 (1.33–5.30)	0.012*

OR = Odd ratio.

* $p < 0.05$; ** $p < 0.001$.

OR calculated for 10 $\mu\text{g m}^{-3}$ increase in concentration of NO₂.

OR calculated for 10 $\mu\text{g m}^{-3}$ increase in concentration of PM₁₀.

OR calculated for 10 mg m^{-3} increase in concentration of formaldehyde.

Table 8. PCA FeNO level for doctor's diagnosed asthma (G1) and atopic students (G2) on environmental parameters factor scores after Varimax rotation, contributions of the observations to the components and squared cosines of the observation of the principle components 1, 2 and 3.

Parameter	C1	C2	C3	ctr ₁	ctr ₂	ctr ₃	cos ₁ ²	cos ₂ ²	cos ₃ ²
G1									
FeNO (ppb)	−0.223	−0.392	−0.630	2.284	7.926	22.645	5.0	15.4	39.7
NO ₂ (μg m ^{−3})	0.180	0.933	−0.114	1.482	44.884	0.740	3.2	87.1	1.3
Temperature (°C)	−0.659	0.491	0.332	19.867	12.440	6.279	43.4	24.1	11.0
RH (%)	0.802	−0.084	−0.041	29.378	0.363	0.094	64.2	0.7	0.2
CO ₂ (ppm)	0.820	0.172	−0.053	30.742	1.526	0.159	67.2	3.0	0.3
PM ₁₀ (μg m ^{−3})	−0.417	−0.092	0.741	7.968	0.434	31.333	17.4	0.8	54.9
PM _{2.5} (μg m ^{−3})	−0.059	−0.058	0.812	0.159	0.176	37.559	0.3	0.3	65.9
Formaldehyde (mg m ^{−3})	−0.421	0.791	0.145	8.121	32.250	1.192	17.8	62.6	2.1
Σ	27.3	51.6	73.5						
G2									
FeNO (ppb)	−0.029	0.010	0.961	0.031	0.006	89.368	0.1	0.0	92.4
NO ₂ (μg m ^{−3})	−0.030	0.947	−0.005	0.034	51.418	0.002	0.1	89.7	0.0
Temperature (°C)	0.781	0.341	−0.065	22.545	6.661	0.406	61.0	11.6	0.4
RH (%)	−0.727	0.069	0.081	19.526	0.270	0.632	52.8	00.5	0.7
CO ₂ (ppm)	−0.513	0.443	−0.165	9.723	11.271	2.628	26.3	19.7	2.7
PM ₁₀ (μg m ^{−3})	0.758	0.034	0.058	21.266	0.065	0.322	57.5	0.1	0.3
PM _{2.5} (μg m ^{−3})	0.616	−0.021	−0.253	14.025	0.026	6.211	37.9	0.0	6.4
Formaldehyde (mg m ^{−3})	0.589	0.727	0.067	12.850	30.284	0.431	34.8	52.8	0.4
Σ	33.8	55.6	68.5						

The positive important contributions are in **bold**. For convenience, squared cosines (cos²) and contributions (ctr) have been multiplied by 100. Σ = variability (%).

PM₁₀ (μg m^{−3}) and temperature had a strong loading factor in Factor 1. Component 2 essentially illustrated that the concentration of NO₂ (μg m^{−3}) was positively correlated with formaldehyde in the vertical axis. The contribution and cosines percentages showed that in Component 3 the environmental parameters were not correlated with the FeNO levels among children with allergic sensitization even though it had strong loading factor (Fig. 1).

For the final analysis, we used Statistical Process Control (SPC) aiming to the estimation of FeNO levels due to the contribution of PM₁₀ and PM_{2.5} concentrations inside the classrooms. After determination of possible contributing factors in the PCA with Varimax rotation, the average limit detected for FeNO levels was 32.32 ppb and upper control limit (UCL) was 104.21 ppb. Overall, the levels of FeNO among secondary students were classified as intermediate (25.00–49.99 ppb). The upper control limit of PM₁₀ predicted inside the classroom was 38.58 μg m^{−3} which is considerably lower than the standard set by the new Malaysian Ambient Air Quality Standard 2018 Interim (120 μg m^{−3}). The UCL of PM_{2.5} predicted was 23.99 μg m^{−3} and also below the standard level (50 μg m^{−3}). A comparable output for three graphs revealed that the pattern of FeNO levels among students was consistency with the increasing concentrations of PM₁₀ and PM_{2.5} measured inside the classrooms, especially those who study in urban area. The variation of FeNO levels were unpredictable and could be affected by particle variation. It was determined that many school children in urban area had FeNO levels above 50 ppb and average value, while school children with atopic condition had above 100 ppb. This indicated a high potential of elevated FeNO levels

above the normal range, especially in doctor's diagnosed asthma and atopic school children in relation to PM₁₀ and PM_{2.5} exposure at above the upper control limit (Fig. 2).

DISCUSSION

We found that the prevalence of atopy among school children at the age of 14 years old was slightly higher (57.7%) than the children in a previous study of 487 children (40.3%) conducted in Terengganu, Malaysia (Ma'pol *et al.*, 2019). Literature had also demonstrated that the prevalence of allergy among children was increasing substantially in many countries (Downs *et al.*, 2001). Findings from this study also showed the prevalence reactivity of house dust mite allergen (Derp1 = 51.9%, Derf1 = 47.9%, mix = 55.7%) was lower than the study by Gendeh *et al.* (2004). In that study, they found that in 141 children with asthma, 79.1% responded to mixed mites and 58.1% to cat allergen. Additionally, Yadav and Naidu (2015) showed that 63.3% of Malaysian children were reactive to Derp1. Moreover, according to Nadzrah *et al.* (2015), 82.4% of the Asian population had a positive response toward house dust mites compared to Europeans (41.2%). The high prevalence of sensitization to mixed mites among asthmatic children was expected, and there is always the potential for increased risk of bronchial hyperresponsiveness and asthma symptoms (Nelson, 2000).

Respiratory symptoms were common among female children than male and this result was similar to those found in the previous study conducted in Johor Bahru, Malaysia and Kansai, Japan (Cai *et al.*, 2011; Takaoka *et al.*, 2017).

Fig. 1. Circle of correlation of FeNO levels and environmental parameters for (a) doctor's diagnosed asthma and (b) atopic condition of school students. The measured variables are shown as vectors which signal the combined strength and direction of relationship. The angle between vectors gives the degree of correlation. The angles of FeNO levels, PM₁₀ and PM_{2.5} were in the same quadrant and small with high loading factors indicating that they were strongly correlated with D1 and D2.

Fig. 2. Statistical process control (SPC) analysis to estimate the pattern of (a) FeNO levels, the concentrations of (b) PM₁₀ and (c) PM_{2.5}. The result inside the blue box (left side) represents the students and classrooms in urban area. UCL = upper control limit; LCL = lower control limit.

Some studies suggested that female sex hormones may play a role in the development and outcome of respiratory symptoms (Dharmage *et al.*, 2019; Honkamäki *et al.*, 2019). The mechanism of this hormonal factor worsening the symptoms largely unknown, however literature suggested to include increase serum levels of progesterone, increase the synthesis of prostaglandin and abnormal β_2 -adrenergic receptor regulation (Zillmer *et al.*, 2014).

In line with the literature, we found that the FeNO levels

were significantly higher among doctor's diagnosed asthma and atopic school children. A study by Xu *et al.* (2011) in China showed values of 39.7 ± 1.6 ppb and 27.1 ± 1.9 ppb, respectively for asthmatic and atopic children. Meanwhile, a local study conducted in Terengganu, Malaysia reported that the mean FeNO levels among the asthmatic were 28 ± 3.2 ppm, and the non-asthmatic was 21 ± 1.4 (Ma'pol *et al.*, 2014), which were lower than the current study. The mean FeNO levels in this study was higher than in other reported

studies. This may be due to the high prevalence of atopy among these school children, especially in the urban school area. Previous studies have shown that the prevalence of atopic sensitization, particularly to the house dust mites were positively associated with urbanization and exposure to the tropical urban environment (Andiappan *et al.*, 2014; Song *et al.*, 2015; Lambert *et al.*, 2018). Moreover, the presence of subclinical inflammation of lower respiratory airway and accumulation of eosinophil in upper and lower airway likely increased the levels of FeNO in atopic children (Kumar *et al.*, 2013). The variation of FeNO levels with height in our study was consistent with the findings from previous studies. Increase of height from 120 to 180 cm was associated with the doubling of FeNO levels (Alving and Malinovski, 2010). Positive associations of FeNO levels with weight and BMI were inconsistent and has been described in a few studies but only among healthy subjects (Maestrelli *et al.*, 2008; Yiwa *et al.*, 2017).

The concentration of CO₂ in both school areas were below the recommended limit of 1,000 ppm. Similarly, the PM₁₀ and PM_{2.5} concentrations were below the 24 hours mean of World Health Organization (WHO) guideline (PM₁₀ = 50 µg m⁻³, PM_{2.5} = 25 µg m⁻³), the National Ambient Air Quality Standard by U.S. EPA (PM₁₀ = 150 µg m⁻³, PM_{2.5} = 35 µg m⁻³) and the new Malaysian Ambient Air Quality Standard 2018 Interim Target-2 (PM₁₀ = 120 µg m⁻³, PM_{2.5} = 50 µg m⁻³). This was due to sufficient natural ventilation system, wide window designs on both sides of the classroom as well as being equipped ceiling fans. The classroom were designed with well-balanced ventilation providing for a more comfortable room temperature and reducing particulate and CO₂ concentrations (Schibuola *et al.*, 2016). Additionally, Silvestre *et al.* (2009) reported that by opening 56.0% of classroom's window under natural ventilation conditions, CO₂ concentration was able to be kept at below 1000 ppm.

Malaysia has a low variation in temperature and humidity all year around, therefore with the natural ventilation flow through the windows in the classrooms, indoor and outdoor levels of pollutants would be expected to be constant throughout the year. This is supported by several studies that have determined equal indoor to outdoor (I/O) ratios for PM₁₀, PM_{2.5}, NO₂, CO, and volatile organic compounds (VOCs) measured in schools throughout Peninsular Malaysia (Abidin *et al.*, 2014; Mohamed *et al.*, 2016; Norbäck *et al.*, 2017). In this study, the schools, classrooms and children were randomly selected from all secondary schools in Hulu Langat area, Malaysia. Thus, we concluded that this study was less likely influenced by selection bias. However, the cross-sectional study design applied here limits the possibility to draw conclusion on causality.

Hierarchic multiple logistic regression analysis revealed that there were significant associations between PM₁₀ and NO₂ concentrations with wheezing and daytime breathlessness. A similar finding in the literature showed exposure to traffic pollutants (PM₁₀ and NO_x) were associated with an excess risk of persistent wheezing and sensitization to inhaled allergens (Esposito *et al.*, 2014; Nelson *et al.*, 2018). Asthmatic school children reported wheezing, daytime and nocturnal attack of breathlessness more frequently, symptoms common in

asthmatic children (Platts-Mills *et al.*, 2012). Conversely, in this current study the symptoms of rhinitis, daytime and nocturnal attack of breathlessness was not significantly associated with children from the urban area. This discrepancy trends cannot be explained only by children's characteristics, but by an interaction between genetic susceptibility and urban air pollutants exposure (Liu *et al.*, 2018; Paciência and Rufo, 2020).

In the final regression model, we found that PM₁₀ were positively associated with wheezing and skin allergy symptoms, while the increased concentration of NO₂ was associated with daytime breathlessness symptom. This result was similar to those reported by Dimitrova *et al.* (2012), where they found that every additional 36 mg m⁻³ (daily mean) of PM₁₀ increased the OR of asthma incidence by 12.6% (95% CI: 5.8–19.4%). This association can probably be explained by the fact that PM₁₀ triggers a series of pathological mechanism including innate immunity inflammation, oxidation stress, apoptosis and autophagy and imbalance of T-helper cells. Particles modulate airway macrophages host defences by increasing IL-6 levels and suppressing CD11b macrophages in human alveoli. Particles were also capable of provoking Th2-mediate immune responses by increasing IL-10 secretion in lipopolysaccharide induced antigen-specific T cells through the Nrf2 signalling pathway (Wu *et al.*, 2018).

In this study, the concentration of NO₂ measured inside classrooms was also below the WHO annual mean guideline (40 µg m⁻³) (WHO, 2005). Nitrogen dioxide has been shown to have deleterious effects on the airway resulted from the production of reactive oxygen species (ROS) which mediate the cellular injury in the airway (Gillespie-Bennett *et al.*, 2011; Priscilla *et al.*, 2017). Recent study estimated that 64.0% of pediatric asthma incidents attributable to ambient NO₂ pollution in urban centre globally (Achakulwisut *et al.*, 2019). We found an association between rhinitis symptoms and indoor formaldehyde concentration. The formaldehyde concentration was below the air quality guideline (110 µg m⁻³) set by WHO (WHO, 2005). Previous study conducted in Malaysia reported formaldehyde mean concentration in the classroom was 4.2 µg m⁻³, and there was an association between formaldehyde and sick building syndrome (Norbäck *et al.*, 2017). Review reported that exposure to formaldehyde at a concentration up to 3.0 ppm or greater associated with sensory and throat irritation (Golden, 2011). Factor analysis revealed that indoor pollutants of formaldehyde and NO₂ were in the same factor with a total variance of 51.6%. NO₂ is a secondary pollutant and originate from the process of fossil fuel combustion, biomass burning, agricultural activities (Hua, 2018). This indicated that the school children in sub-urban and urban area face potential health risks as the location of schools very close by the main roads and industrial area, especially in Hulu Langat. Formaldehyde could originate from the furniture which made up of wood and plastics, pressboard, plywood, textile, table laminate and consumer products (Salthammer, 2019). The secondary formation of formaldehyde through photochemical reactivity of NO₂ in the air with volatile organic compounds (VOCs) causing the formation of different aldehydes (Weschler *et al.*, 2006). It

could be speculated that a high concentration of formaldehyde in this current study due to present all these items in the classrooms which commonly used in the schools with natural ventilation system setting in Malaysia. Factor analysis results elucidate that PM₁₀, PM_{2.5} and CO₂ were most likely originated from the same sources. The primary sources of these pollutants were industrial emissions, transportation, aged traffic emissions (Feroz Khan *et al.*, 2019). The indoor particles might either generated from occupant's activities or re-suspension of deposited particles, soils materials from school children's shoes, skin flakes, furniture fragments and less frequent cleaning (Rufo *et al.*, 2016).

Factor analysis has not been widely applied in studying of asthma related to environmental parameters in Malaysia. This present study confirmed previous findings and contributes additional evidence that suggest the concentration of particles were independently associated with FeNO levels, the biomarker of lung inflammation with a variation of 73.5%. Some studies showing short term exposure to the NO₂, O₃ and particles (PM₁₀ and PM_{2.5}) have been associated with high levels of FeNO in children, independent of asthma and allergic status (Grutta *et al.*, 2012; Aziz *et al.*, 2014; Noor Hisyam and Juliana, 2014). These findings implied that particle-mediate injury follows common pathogenic mechanisms. According to the SPC results, the variation of FeNO levels were out of control and comparable with the elevation of PM₁₀ and PM_{2.5} concentrations at above the UCL 40.23 µg m⁻³ and 23.99 µg m⁻³, respectively, especially in urban area. It could be speculated that school children with asthma and atopic condition had a high potential in elevating FeNO levels in relation to PM₁₀ and PM_{2.5} exposure above the UCL inside their classrooms. A similar prediction was reported by Carlsen *et al.* (2016) determined that particles represent the major contributor to elevate the FeNO levels among children. Liu *et al.* (2015) also reported that school children who study in urban area and exposed to a high concentrations of PM₁₀, SO₂, formaldehyde and acetaldehyde showed significantly increased FeNO levels in asthmatic group. One cohort study from Southern California predicted using multiple linear regression analysis that increased in an annual average of PM_{2.5} concentrations over 2.5 µg m⁻³ were significantly associated with 4.94 ppb higher FeNO levels (Berhane *et al.*, 2014). We found one review reported that the increase in the cumulative lagged average of O₃ also had a robust positive association with 14.3% higher FeNO levels. They also said that DNA methylation of NOS2 (inducible nitric oxide synthase) gene at the highest level (> 56.6%) and a higher concentration of PM_{2.5} positively contributed to the elevation of FeNO levels among children. It is probable that genes including GSTM1, EPHX1 and CAT influence the association between air pollutants and respiratory illnesses (Chen *et al.*, 2015).

Nevertheless, we found several longitudinal panel studies have highlighted the association of long-term exposure of PM_{2.5} and its constituents with attenuation of FeNO levels, particularly time lag longer than six hours and more than two days (Murata *et al.*, 2007; Berhane *et al.*, 2011; Shi *et al.*, 2016; Li *et al.*, 2017). The mediating role of NOS2A and ARG2 (arginase2) methylation in the association between

PM_{2.5} and FeNO analysed in longitudinal studies as temporal plausibility at time lags of one and two days, respectively (Chen *et al.*, 2015; Zhang *et al.*, 2019). Further investigations are needed to clarify this potential reaction. This suggests that elevation of FeNO levels among asthmatic and atopic children is associated with particles, O₃ and genetic influence.

CONCLUSION

To conclude, our findings showed that the average concentrations of indoor CO₂, NO₂, PM₁₀, PM_{2.5}, formaldehyde and the level of temperature in classrooms at urban area were significantly higher when compared to the sub-urban area of Hulu Langat. Major sources of variation for particles, NO₂ and CO₂ were automobile exhaust, industrial emissions and agricultural activities as all schools understudied were located nearby the main roads, industrial and agricultural area. We found self-reported wheezing, daytime and nocturnal attacks of breathlessness were associated with doctor's diagnosed asthma. Exposure to NO₂ and formaldehyde were associated with daytime breathless and rhinitis, respectively. This study suggests that exposure to PM₁₀ and PM_{2.5} above the UCL of 40.23 µg m⁻³ and 23.99 µg m⁻³, respectively had a potential risk in elevation of FeNO levels, especially among asthmatic and atopic children. Further study is warranted to examine the relationship of long-term effects of exposure to indoor pollutants and roadway proximity towards FeNO levels.

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SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.4209/aaqr.2020.03.0128>

REFERENCES

- Abidin, E.Z., Semple, S., Rasdi, I., Ismail, S.N.S. and Ayres, J.G. (2014). The relationship between air pollution and asthma in Malaysian schoolchildren. *Air Qual. Atmos. Health* 7: 421–432. <https://doi.org/10.1007/s11869-014-0252-0>
- Acat, M., Aydemir, Y., Yazıcı, O., Turğut, M., Çörtük, M., Çakar, M., Yaşar, Z., Deniz, S. and Çetinkaya, E. (2017). Comparison of the fractional exhaled nitric oxide levels in adolescents at three schools located three different distances from a large steel mill. *Can. Respir. J.* 2017: 6231309 <https://doi.org/10.1155/2017/6231309>
- Achakulwisut, P., Brauer, M., Hystad, P. and Anenberg, S.C. (2019). Global, national, and urban burdens of paediatric asthma incidence attributable to ambient NO₂ pollution: Estimates from global datasets. *Lancet Planetary Health* 3: e166–e178. [https://doi.org/10.1016/S2542-5196\(19\)30046-4](https://doi.org/10.1016/S2542-5196(19)30046-4)

- Alving, K. and Malinovschi, A. (2010). Basic aspects of exhaled nitric oxide. In *European respiratory monograph: Exhaled biomarker*, Horvath, I. and DeJongste, J.C. (Eds.), pp. 1–31. European Respiratory Society. <https://doi.org/10.1183/1025448x.00028509>
- Al-Zoughool, M. and Al-Mistneer, R. (2018). Risk of asthma symptoms among workers in health care settings. *Int. J. Environ. Impacts* 1: 172–182. <https://doi.org/10.2495/EI-V1-N2-172-182>
- Andiappan, A.K., Puan, K.J., Lee, B., Nardin, A., Poidinger, M., Connolly, J., Chew, F.T., Wang, D.Y. and Rotzschke, O. (2014). Allergic airway diseases in a tropical urban environment are driven by dominant mono-specific sensitization against house dust mites. *Allergy* 69: 501–509. <https://doi.org/10.1111/all.12364>
- Australasian Society of Clinical Immunology and Allergy (ASCIA) (2016). Skin prick testing for the diagnosis of allergic disease - A manual for practitioners. https://www.allergy.org.au/images/stories/pospapers/ASCIA_SPT_Manual_March_2016.pdf
- Aziz, N.A., Jalaludin, J. and Bakar, S.A. (2014). Indoor air pollutants exposure and the respiratory inflammation (FeNO) among preschool children in Hulu Langat, Selangor. *Adv. Environ. Biol.* 8: 164–170.
- Berhane, K., Zhang, Y., Linn, W.S., Rappaport, E.B., Bastain, T.M., Salam, M.T., Islam, T., Lurmann, F. and Gilliland, F.D. (2011). The effect of ambient air pollution on exhaled nitric oxide in the Children's Health Study. *Eur. Respir. J.* 37: 1029–1036. <https://doi.org/10.1183/09031936.00081410>
- Berhane, K., Zhang, Y., Salam, M.T., Eckel, S.P., Linn, W.S., Rappaport, E.B., Bastain, T.M., Lurmann, F. and Gilliland, F.D. (2014). Longitudinal effects of air pollution on exhaled nitric oxide: The Children's Health Study. *Occup. Environ. Med.* 71: 507–513. <https://doi.org/10.1136/oemed-2013-101874>
- Cai, G.H., Hashim, J.H., Hashim, Z., Ali, F., Bloom, E., Larsson, L., Lampa, E. and Norbäck, D. (2011). Fungal DNA, allergens, mycotoxins and associations with asthmatic symptoms among pupils in schools from Johor Bahru, Malaysia. *Pediatr. Allergy Immunol.* 22: 290–297. <https://doi.org/10.1111/j.1399-3038.2010.01127.x>
- Carlsen, H.K., Boman, P., Bjor, B., Olin, A.C. and Forsberg, B. (2016). Coarse fraction particle matter and exhaled nitric oxide in non-asthmatic children. *Int. J. Environ. Res. Public Health* 13: 2–11. <https://doi.org/10.3390/ijerph13060621>
- Chen, R., Qiao, L., Li, H., Zhao, Y., Zhang, Y., Xu, W., Wang, C., Wang, H., Zhao, Z., Xu, X., Hu, H. and Kan, H. (2015). Fine particulate matter constituents, nitric oxide synthase DNA methylation and exhaled nitric oxide. *Environ. Sci. Technol.* 49: 11859–11865. <https://doi.org/10.1021/acs.est.5b02527>
- Chen, Z., Salam, M.T., Eckel, S.P., Breton, C.V. and Gilliland, F.D. (2015). Chronic effects of air pollution on respiratory health in Southern California children: Findings from the Southern California Children's Health Study. *J. Thorac. Dis.* 7: 46–58. <https://doi.org/10.3978/j.issn.2072-1439.2014.12.20>
- Chithra, V.S. and Nagendra, S.S.M. (2018). A review of scientific evidence on indoor air of school building: Pollutants, sources, health effects and management. *Asian J. Atmos. Environ.* 12: 87–108. <https://doi.org/10.5572/ajae.2018.12.2.87>
- Dharmage, S.C., Perret, J.L. and Custovic, A. (2019). Epidemiology of asthma in children and adults. *Front. Pediatr.* 7: 246. <https://doi.org/10.3389/fped.2019.00246>
- Dimitrova, R., Lurpunglukana, N., Fernando, H.J.S., Runger, G.C., Hyde, P., Hedquist, B.C. and Anderson, J. (2012). Physics Relationship between particulate matter and childhood asthma – basis of a future warning system for central Phoenix. *Atmos. Chem. Phys.* 12: 2479–2490. <https://doi.org/10.5194/acp-12-2479-2012>
- Douglas, G. and Elward, K.S. (2011). *Asthma: Clinician's desk reference*. Manson Publishing.
- Downs, S.H., Marks, G.B., Sporik, R., Belosouva, E.G., Car, N.G. and Peat, J.K. (2001). Continued increase in the prevalence of asthma and atopy. *Arch. Dis. Child.* 84: 20–23. <https://doi.org/10.1136/adc.84.1.20>
- Dweik, R.A., Boggs, P.B., Erzurum, S.C., Irvin, C.G., Leigh, M.W., Lundberg, J.O., Olin, A.C., Plummer, A.L., Taylor, D.R. and American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. (2011). An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am. J. Respir. Crit. Care Med.* 184: 602–615. <https://doi.org/10.1164/rccm.9120-11ST>
- Esposito, S., Tenconi, R., Lelii, M., Preti, V., Nazzari, E., Consolo, S. and Patria, M.F. (2014). Possible molecular mechanisms linking air pollution and asthma in children. *BMC Pulm. Med.* 14: 31. <https://doi.org/10.1186/1471-2466-14-31>
- Foldvary, V., Beko, G., Langer, S., Arrhenius, K. and Petras, D. (2017). Effect of energy renovation on indoor air quality in multifamily residential buildings in Slovakia. *Build. Environ.* 122: 363–372. <https://doi.org/10.1016/j.buildenv.2017.06.009>
- Gao, J., Wargocki, P. and Wang, Y. (2014). Ventilation system type, classroom environmental quality and pupils' perceptions and symptoms. *Build. Environ.* 75: 46–57. <https://doi.org/10.1016/j.buildenv.2014.01.015>
- Gendeh, B.S., Mujahid, S.H., Murad, S. and Rizal, M. (2004). Atopic sensitization of children with rhinitis in Malaysia. *Med. J. Malaysia* 59: 522–529.
- Gillespie-Bennett, J., Pierse, N., Wickens, K., Crane, J. and Howden-Chapman, P. (2011). The respiratory health effects of nitrogen dioxide in children with asthma. *Eur. Respir. J.* 38: 303–309. <https://doi.org/10.1183/09031936.0011.5409>
- Golden, R. (2011). Identifying an indoor air exposure limit for formaldehyde considering both irritation and cancer hazards. *Crit. Rev. Toxicol.* 41: 672–721. <https://doi.org/10.3109/10408444.2011.573467>
- Grutta, S., La, Ferrante, G., Malizia, V., Cibella, F. and Viegi, G. (2012). Environmental effects on fractional exhaled nitric oxide in allergic children. *J. Allergy* 2012: 916926. <https://doi.org/10.1155/2012/916926>

- Honkamäki, J., Hisinger-Mölkänen, H., Ilmarinen, P., Piirilä, P., Tuomisto, L.E., Andersén, H., Huhtala, H., Sovijärvi, A., Backman, H., Lundbäck, B., Rönmark, E., Lehtimäki, L. and Kankaanranta, H. (2019). Age- and gender-specific incidence of new asthma diagnosis from childhood to late adulthood. *Respir. Med.* 154: 56–62. <https://doi.org/10.1016/j.rmed.2019.06.003>
- Hua, A.K. (2018). Applied chemometric approach in identification sources of air quality pattern in Selangor, Malaysia. *Sains Malaysiana* 47: 471–479. <https://doi.org/10.17576/jsm-2018-4703-06>
- Kamaruddin, A.S., Jalaludin, J. and Choo, C.P. (2015). Indoor air quality and its association with respiratory health among Malay preschool children in Shah Alam and Hulu Langat, Selangor. *Adv. Environ. Biol.* 9: 17–26.
- Khan, M.F., Hamid, A.H., Bari, M.A., Tajudin, A.B.A., Latif, M.T., Nadzir, M.S.M., Sahani, M., Wahab, M.I.A., Yusup, Y., Maulud, K.N.A., Yusoff, M.F., Amin, N., Akhtaruzzaman, M., Kindzierski, W. and Kumar, P. (2019). Airborne particles in the city center of Kuala Lumpur: Origin, potential driving factors, and deposition flux in human respiratory airways. *Sci. Total Environ.* 650: 1195–1206. <https://doi.org/10.1016/j.scitotenv.2018.09.072>
- Kim, H., Eckel, S.P., Kim, J.H. and Gilliland, F.D. (2016). Exhaled NO: Determinants and clinical application in children with allergic airway disease. *Allergy Asthma Immunol. Res.* 8: 12–21. <https://doi.org/10.4168/aaair.2016.8.1.12>
- Kumar, R., Gupta, N. and Goel, N. (2013). Correlation of Atopy and FeNO in allergic rhinitis: An Indian study. *Indian J. Chest Dis. Allied Sci.* 55: 79–83.
- Lambert, K.A., Bowatte, G., Tham, R., Lodge, C.J., Prendergast, L.A., Heinrich, J., Abramson, M.J., Dharmage, S.C. and Erbas, B. (2018). Greenspace and Atopic Sensitization in Children and Adolescents - A Systematic Review. *Int. J. Environ. Res. Public Health* 15: 2539. <https://doi.org/10.3390/ijerph15112539>
- Langer, S., Beko, G., Bloom, E., Widheden, A. and Ekberg, L. (2015). Indoor air quality in passive and conventional new houses in Sweden. *Build. Environ.* 93: 92–100. <https://doi.org/10.1016/j.buildenv.2015.02.004>
- Li, H., Bai, H., Yang, C., Chen, R., Wang, C., Zhao, Z. and Kan, H. (2017). Acute effects of ambient temperature and particulate air pollution on fractional exhaled nitric oxide: A panel study among diabetic patients in Shanghai, China. *J. Epidemiol.* 27: 584–589. <https://doi.org/10.1016/j.je.2017.01.002>
- Liu, D., Huang, Z. and Huang, Y. (2015). Clinical analysis of fractional exhaled and nasal nitric oxide in allergic rhinitis children. *J. Allergy Ther.* 6: 1. <https://doi.org/10.4172/2155-6121.1000204>
- Liu, H., Dunea, D., Iordache, S. and Pohoata, A. (2018). A review of airborne particulate matter effects on young children's respiratory symptoms and diseases. *Atmosphere* 9: 1–18. <https://doi.org/10.3390/atmos9040150>
- Ma'pol, A., Hashim, J.H., Norbäck, D., Weislander, G., Hashim, Z. and Isa, Z.M. (2019). FeNO level and allergy status among school children in terengganu, Malaysia. *J. Asthma* 3: 1–18. <https://doi.org/10.1080/02770903.2019.1614614>
- Ma'pol, A., Hashim, J.H., Norbäck, D., Weislander, G., Hashim, Z. and Isa, Z.M. (2014). Prevalence of asthma and level of fractional exhaled nitrogen oxide among Malaysian school children. *BMC Public Health* 14: O27. <https://doi.org/10.1186/1471-2458-14-S1-O27>
- Maestrelli, P., Caramori, G., Franco, F. and Fabbri, L.M. (2008). Definition, clinical features, investigations and differential diagnosis of asthma. In *Allergy and Allergic Diseases*, Kay, A.B., Kaplan, A.P., Bousquet, J. and Holt, P.G. (Eds.), pp. 1567–1590, Wiley-Blackwell.
- Mohamed, N., Sulaiman, L.H., Zakaria, T.A., Kamarudin, A.S. and Rahim, D.A. (2016). Health risk assessment of PM₁₀ exposure among school children and the proposed API level for closing the school during haze in Malaysia. *Int. J. Public Health Res.* 6: 685–694.
- Murata, A., Kida, K., Hasunuma, H., Kanegae, H., Ishimaru, Y., Motegi, T., Yamada, K., Yoshioka, H., Yamamoto, K. and Kudoh, S. (2007). Environmental influence on the measurement of exhaled nitric oxide concentration in school children: Special reference to methodology. *J. Nippon Med. School* 74: 30–36. <https://doi.org/10.1272/jnms.74.30>
- Nadzrah, Y.S., Zulkiflee, A. and Prepageran, N. (2015). Common aeroallergens by skin prick test among the population in two different regions. *Primary Health Care* 5: 3. <https://doi.org/10.4172/2167-1079.1000206>
- Nelson, H.S. (2000). The importance of allergens in the development of asthma and the persistence of symptoms. *J. Allergy Clin. Immunol.* 105: S628–S632. <https://doi.org/10.1067/mai.2000.106154>
- Nelson, L., Alex, N., Jane, S.M., Atanu, S. and Zhiwei, G. (2018). Association between traffic related air pollution and the development of asthma phenotypes in children: A systematic review. *Int. J. Chronic Dis.* 2018: 4047386. <https://doi.org/10.1155/2018/4047386>
- Noor Hisyam, N.H. and Juliana, J. (2014). Association between indoor PM₁₀, PM_{2.5} and NO₂ with airway inflammation among preschool children at industrial and sub-urban areas. *Adv. Environ. Biol.* 8: 149–159.
- Norbäck, D., Markowicz, P., Cai, G.H., Hashim, Z., Ali, F., Zheng, Y.W., Lai, X.X., Spangfort, M. D., Larsson, L. and Hashim, J.H. (2014). Endotoxin, ergosterol, fungal DNA and allergens in dust from schools in Johor Bahru, Malaysia- associations with asthma and respiratory infections in Pupils. *PLoS One* 9: e88303. <https://doi.org/10.1371/journal.pone.0088303>
- Norbäck, D., Hisham, J., Hashim, Z. and Ali, F. (2017). Volatile organic compounds (VOC), formaldehyde and nitrogen dioxide (NO₂) in schools in Johor Bahru, Malaysia: Associations with rhinitis, ocular, throat and dermal symptoms, headache and fatigue. *Sci. Total Environ.* 592: 153–160. <https://doi.org/10.1016/j.scitotenv.2017.02.215>
- Paciência, I. and Rufo, J.C. (2020). Urban-level environmental factors related to pediatric asthma. *Porto Biomed. J.* 5: 1–12. <https://doi.org/10.1097/j.pbj.0000000000000057>
- Platts-Mills, T.A., Adachi, M., Busse, W.W. and Holgate, S.T. (2012). Asthma. In *Allergy*, Holgate, S., Church, M.,

- Broide, D. and Martinez, F. (Eds.), pp. 181–202. Elsevier Saunders.
- Priscilla, C.P., David, F.H., Gregory, V.B., Lucas, G., Mark, S.S. and Kurt, D.A. (2017). The pathophysiology of nitrogen dioxide during inhaled nitric oxide therapy. *ASAIO J.* 63: 7–13. <https://doi.org/10.1097/mat.00000000000000425>
- Rufo, J.C., Madureira, J., Fernandes, E.O., Moreira, A., Rufo, C. and Roberto, R. (2016). Volatile organic compounds in asthma diagnosis: A systematic review and meta-analysis. *Allergy* 71: 175–188. <https://doi.org/10.1111/all.12793>
- Salthammer, T. (2019). Formaldehyde sources, formaldehyde concentrations and air exchange rates in European housings. *Build. Environ.* 150: 219–232. <https://doi.org/10.1016/j.buildenv.2018.12.042>
- Schibuola, L., Scarpa, M. and Tambani, C. (2016). Natural ventilation level assessment in a school building by CO₂ concentration measures. *Energy Procedia* 101: 257–264. <https://doi.org/10.1016/j.egypro.2016.11.033>
- Shi, J., Chen, R., Yang, C., Lin, Z., Cai, J., Xia, Y., Wang, C., Li, H., Johnson, N., Xu, X., Zhao, Z. and Kan, H. (2016). Association between fine particulate matter chemical constituents and airway inflammation: A panel study among healthy adults in China. *Environ. Res.* 150: 264–268. <https://doi.org/10.1016/j.envres.2016.06.022>
- Silvestre, C.M., André, P. and Michel, T. (2009). Air temperature and CO₂ variations in a naturally ventilated classroom under a Nordic climate. PLEA2009 - 26th Conference on Passive and Low Energy Architecture, pp. 1–6, Quebec City, Canada.
- Song, W.J., Sohn, K.H., Kang, M.G., Park, H.K., Kim, M.Y., Kim, S.H., Lim, M.K., Choi, M.H., Kim, K.W., Cho, S.H., Min, K.U. and Chang, Y.S. (2015). Urban–rural differences in the prevalence of allergen sensitization and self-reported rhinitis in the elderly population. *Ann. Allergy Asthma Immunol.* 114: 455–461. <https://doi.org/10.1016/j.anai.2015.03.008>
- Suhaimi, N.F., Jalaludin, J. and Bakar, S.A. (2017). Cysteinyl leukotrienes as biomarkers of effect in linking exposure to air pollutants and respiratory inflammation among school children. *Ann. Trop. Med. Public Health* 10: 423–431. <http://www.atmph.org/text.asp?2017/10/2/423/208691>
- Takaoka, M., Suzuki, K. and Norbäck, D. (2017). Current asthma, respiratory symptoms and airway infections among students in relation to the school and home environment in Japan. *J. Asthma* 54: 652–661. <https://doi.org/10.1080/02770903.2016.1255957>
- Weschler, C.J., Wells, J.R., Poppendieck, D., Hubbard, H. and Pearce, T.A. (2006). Workgroup report: Indoor chemistry and health. *Environ. Health Perspect.* 114: 442–446. <https://doi.org/10.1289/ehp.8271>
- WHO (2005). *WHO air quality guidelines global update 2005*. World Health Organization, Denmark.
- Wu, J., Ge, D., Zhou, L., Hou, L. and Zhou, Y. (2018). Effects of particulate matter on allergic respiratory diseases. *Chron. Dis. Transl. Med.* 4: 95–102. <https://doi.org/10.1016/j.cdtm.2018.04.001>
- Xu, F., Zou, Z., Yan, S., Li, F., Kan, H., Norback, D., Wieslander, G., Xu, J. and Zhao, Z. (2011). Fractional exhaled nitric oxide in relation to asthma, allergic rhinitis, and atopic dermatitis in Chinese Children. *J. Asthma* 48: 1001–1006. <https://doi.org/10.3109/02770903.2011.627487>
- Yadav, A. and Naidu, R. (2015). Clinical manifestation and sensitization of allergic children from Malaysia. *Asia Pac. Allergy* 5: 78–83. <https://doi.org/10.5415/apallergy.2015.5.2.78>
- Yiwa, S., Punchama, P., Orathai, J. and Nualanong, V. (2017). Determination of fractional exhaled nitric oxide (FENO) reference values in healthy Thai population. *Asian Pac. J. Allergy Immunol.* 35: 127–131. <https://doi.org/10.12932/AP0840>
- Zhang, Q., Wang, W., Niu, Y., Xia, Y., Lei, X. and Huo, J. (2019). The effects of fine particulate matter constituents on exhaled nitric oxide and DNA methylation in the arginase – nitric oxide synthase pathway. *Environ. Int.* 131: 105019. <https://doi.org/10.1016/j.envint.2019.105019>
- Zillmer, L.R., Gazzotti, M.R., Nascimento, O.A., Montealegre, F., Fish, J. and Jardim, J.R. (2014). Gender differences in the perception of asthma and respiratory symptoms in a population sample of asthma patients in four Brazilian cities. *J. Bras. Pneumol.* 40: 591–598. <https://doi.org/10.1590/S1806-37132014000600002>
- Zissler, U.M., Bieren, J.E., Jakwerth, C.A., Chaker, A.M. and Schmidt-Weber, C.B. (2016). Current and future biomarkers in allergic asthma. *Eur. J. Allergy Clin. Immunol.* 71: 475–494. <https://doi.org/10.1111/all.128281>

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