UNIVERSITI PUTRA MALAYSIA

INDOOR AIR POLLUTANTS IN SCHOOLS AND THEIR RELATIONSHIP WITH AIRWAY INFLAMMATION, RESPIRATORY AND ALLERGIC SYMPTOMS AMONG SCHOOL CHILDREN IN HULU LANGAT, MALAYSIA

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By

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Thesis Submitted to School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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October 2021

Chair : Professor Juliana Jalaludin, PhD
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Many recent reviews and research have found that interaction exposure to indoor pollutants and a spectrum of allergens can cause allergic reactions, raise the likelihood of developing asthma and exacerbate existing asthma. This study aimed to investigate the relationships between indoor air pollutants, the diversity of fungal relative abundance in the settled dust samples, respiratory and allergic symptoms, and airway inflammation levels (FeNO levels and expression of adhesion molecules) among school children in urban and suburban areas. This comparative cross-sectional study was conducted in the Hulu Langat District, Selangor, Malaysia and involved eight secondary schools located in urban and suburban areas respectively. The indoor air concentration of NO_2, CO_2, PM_{10}, $PM_{2.5}$, formaldehyde, as well as temperature and relative humidity were measured using active and passive sampling techniques from 4 classrooms in each school. The settled dust samples were collected inside the classrooms using a vacuum cleaner and further analysed using metagenomic techniques to characterise the fungi composition. The personal and health information were collected among 470 school children aged of 14 years old, who were randomly selected from the schools and classrooms aforementioned, using a questionnaire adapted from the International Study of Asthma and Allergies in Childhood (ISAAC) and the European Community Respiratory Heath Survey (ECRHS). Then, FeNO levels were measured, induced sputum samples were collected and IgE-mediated allergy (allergic skin tests) were conducted concurrently with environmental monitoring. Sputum samples were further analysed to investigate the expression of CD11b, CD35, CD63 and CD66b on both eosinophil and neutrophil using flow cytometry techniques. The 2-level logistic regression (school and children) analysis, general linear regression, and logistic regression with complex sampling were used determining the influencing factors.

The results showed that temperature, NO_2, PM_{10}, $PM_{2.5}$ and formaldehyde concentrations in schools located in the urban areas were significantly higher than in the suburban areas. However, all indoor parameters for both areas were below the local and international recommendation guidelines, except for formaldehyde. In the profile analysis, the fungal diversity levels were significantly abundant in suburban samples than the urban, in which *Aspergillus clavatus* (37.8%) *and Hyphoderma multicystidium* (13.6%) were dominant. Moreover, regression analysis revealed that the manifestations of wheezing, eczema, rhinitis and nasal infection were associated with PM_{10} (OR = 1.08, 95% CI = 1.01 - 1.17), CO_2 (OR = 1.03, 95% CI = 1.01 - 1.05), formaldehyde (OR = 1.10, 95% CI = 1.03 - 1.17), the relative abundance of *Xenasmatella ardosiaca* (OR = 1.40, 95% CI = 1.09 - 1.81) and *Cladosporium halotolerans* (OR = 27.43, 95% CI = 2.21 - 340.87), and a few indoor home environment factors. This study also found that the expression profile of CD11b, CD35, CD63 and CD66b on eosinophil and neutrophil cells were influenced by indoor pollutants and several fungal taxa (*Trichosporon asahii* (OR = 4.01, 95% CI = 1.02 - 16.45), *Hannaella pagnoccae* (OR = 3.33, 95% CI = 1.05 - 10.55), *Hazslinszkyomyces aloes* (OR = 7.58, 95% CI = 9.80 - 58.76), *Papiliotrema bandonii* (OR = 3.78, 95% CI = 1.77 - 8.06), *Candida parapsilosis* (OR = 1.94, 95% CI = 2.12-18.31)) after controlling for confounders. Overall, this study demonstrated that many rare and unculturable fungi taxa together with exposure to indoor air pollutants were potentially attributable to an increase in the airway inflammation reactions and the manifestation of asthma development among school children. The novelty of this study lies in the inclusion of rare and unculturable fungi taxa data characterised from metagenomics protocols, and multi-dimensional expression data of adhesion molecules (CD11b, CD35, CD63 and CD66b) analysed using flow cytometry, whilst simultaneously evaluated with indoor pollutant parameters using a regression for complex samples to enhance health risk assessment of these school children. In this context, future endeavors to determine the prolonged effects and patterns of inflammatory biomarkers in relation to indoor air pollutants are warranted.

Abstrak tesis yang dikemukakan kepada Senat of Universiti Putra Malaysia sebagai memenuhi keperluan untuk Doktor Falsafah

BAHAN PENCEMARAN DI SEKOLAH DAN HUBUNGAN ANTARA INFLAMASI SALUR PERNAFASAN, SIMPTOM RESPIRATORI DAN ALERGI DI KALANGAN PELAJAR SEKOLAH DI HULU LANGAT, MALAYSIA

Oleh

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Terdapat banyak kajian saintifik menunjukkan terdapat interaksi di antara pendedahan terhadap bahan pencemaran dan alergen yang menyumbang kepada tindak balas alergi, peningkatan risiko asma serta membuat keadaan asma bertambah teruk. Tujuan utama kajian ini adalah untuk mengkaji hubungan antara bahan pencemaran, fungus di dalam habuk yang terendap, simptom respiratori dan alergi serta tahap inflamasi salur pernafasan (FeNO, ekspresi biopenanda molekul rekatan) di kalangan pelajar sekolah di kawasan bandar dan pinggir bandar. Ini merupakan kajian perbandingan keratan lintang yang dijalankan di daerah Hulu Langat, Selangor, Malaysia dan melibatkan lapan buah sekolah menengah yang terletak di kawasan bandar dan pinggir bandar. Kaedah aktif dan pasif telah digunakan untuk menilai tahap NO_2, CO_2, PM_{10}, $PM_{2.5}$, formaldehid, serta suhu dan kelembapan relatif di dalam 4 bilik darjah di setiap sekolah. Habuk yang terenap di dalam kelas telah dikumpulkan menggunakan penyedut hampagas dan komposisi fungus telah diuji menggunakan kaedah *metagenomics*. Data peribadi dan kesihatan telah dikumpulkan dari 470 pelajar sekolah yang berumur 14 tahun, yang dipilih secara rawak dari kelas dan sekolah yang sama menggunakan borang soalselidik yang diadaptasi daripada International Study of Asthma and Allergies in Childhood (ISAAC) dan European Community Respiratory Heath Survey (ECRHS). Setelah itu, pengukuran tahap FeNO, aruhan sampel kahak dan ujian alergi telah dijalankan bersama dengan pemantauan kualiti udara. Analisa makmal dijalankan untuk sampel kahak bagi menilai ekspresi CD11b, CD35, CD63 dan CD66b pada eosinofil dan neutrofil menggunakan teknik *flow cytometry*. Analisa 2-level logistik regresi (sekolah dan pelajar), regresi linear dan logistik regresi untuk sampel kompleks digunakan untuk mengkaji faktor yang mempengaruhi.

Hasil kajian menunjukkan suhu, kepekatan NO_2, PM_{10}, $PM_{2.5}$ dan formaldehid yang diukur di sekolah bandar lebih tinggi dan ketara berbanding sekolah pinggir bandar. Tetapi, semua bahan pencemar yang diukur untuk kedua-dua kawasan masih dibawah paras yang disyorkan di peringkat nasional dan antarabangsa, kecuali formaldehid. Tahap kepelbagaian fungus yang diekstrak dari sampel pinggir bandar lebih ketara berbanding dari kawasan bandar, didominasi oleh *Aspergillus clavatus* (37.8%) dan *Hyphoderma multicystidium* (13.6%). Seterusnya, analisa menggunakan 2-level logistik regresi mendedahkan manifestasi nafas berdehit, ezema, rhinitis dan jangkitan salur pernafasan mempunyai perkaitan signifikan dengan PM_{10} (OR = 1.08, 95% CI = 1.01 - 1.17), CO_2 (OR = 1.03, 95% CI = 1.01 - 1.05), formaldehid (OR = 1.10, 95% CI = 1.03 - 1.17), fungus *Xenasmatella ardosiaca* (OR = 1.40, 95% CI = 1.09 - 1.81) dan *Cladosporium halotolerans* (OR = 27.43, 95% CI = 2.21 - 340.87) serta faktor persekitaran di rumah. Hasil dapatan kajian ini juga menunjukkan profil expresi CD11b, CD35, CD63 dan CD66b pada eosinofil dan neutrofil dipengaruhi oleh bahan pencemar dan beberapa spesies fungus (*Trichosporon asahii* (OR = 4.01, 95% CI = 1.02 - 16.45), *Hannaella pagnoccae* (OR = 3.33, 95% CI = 1.05 - 10.55), *Hazslinszkyomyces aloes* (OR = 7.58, 95% CI = 9.80 - 58.76), *Papiliotrema bandonii* (OR = 3.78, 95% = CI 1.77 - 8.06), *Candida parapsilosis* (OR = 1.94, 95% CI = 2.12 - 18.31)) apabila kawalan faktor perancu dilakukan. Kajian ini membuktikan banyak fungus yang jarang ditemui dan sukar untuk dibiakkan serta pendedahan kepada bahan pencemaran berpotensi meningkatkan inflamasi salur pernafasan dan manifestasi asma di kalangan pelajar sekolah ini. Keaslian kajian ini merangkumi penggunaan data fungus yang jarang ditemui dan sukar dibiakkan menggunakan kaedah *metagenomics*, data kepelbagaian ekspresi biopenanda molekul rekatan (CD11b, CD35, CD63 dan CD66b) yang dianalisa menggunakan kaedah *flow cytometry*, serta penilaiannya bersama bahan pencemaran menggunakan regresi untuk sampel kompleks bagi mempertingkatkan penilaian risiko kesihatan di kalangan pelajar sekolah ini. Dalam konteks ini, kajian yang mendalam amat diperlukan untuk memastikan kesan jangkamasa panjang dan corak expresi biopenanda inflamasi yang berkaitan dengan bahan pencemaran.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABREVIATIONS

α	Alpha
β	Beta
°C	Degree celsius
%	Percentage
A_{260nm}	Optical density at wavelength 260 nanometer
A_{280nm}	Optical density at wavelength 280 nanometer
µL	Microliter
mL	Mililiter
µm	Micrometer
mm	milimeter
g	Gram
g	Relative centrifugal force
ng	Nanogram
±	Plus-minus sign
ppm	Part per million
ppb	Part per billion
nm	Nanometer
m^2	Square Meters
bp	Base pair
CD	Cluster of differentiation
IL	Interleukine
FEV_1	Forced expiratory volume in the first second
IQR	Interquartile range

OR	Odd ratio
CI	Confidence interval
R^2	Coefficient of determination
x	Interaction term
RH	Relative humidity
PM_{10}	Particle with aerodynamic diameters of less than 10 µm
$PM_{2.5}$	Particle with aerodynamic diameters of less than 2.5 µm
NO_2	Nitrogen dioxide
CO_2	Carbon dioxide
SO_2	Sulphur dioxide
O_3	Ozone
VOC	Volatile organic carbon
ETS	Environmental tobacco smoke
NO	Nitric oxide
FeNO	Fractional exhaled nitric oxide
Eos	Eosinophil
Neu	Neutrophil
Derp1	*Dermatophagoides pteronyssinus*
Derf1	*Dermatophagoides farina*
OTU	Operational taxonomic unit
ISAAC	International Study of Asthma and Allergies in Childhood
ECRHS	European Community Respiratory Heath Survey

CHAPTER 1

INTRODUCTION

1.1 Background

Air pollution represents the biggest environmental health risk. Exposure to air pollutants from both indoor and outdoor sources has been linked to induction of systemic inflammation, oxidative stress generation and development of respiratory and cardiovascular diseases among all ranges of vulnerable groups. These effects have been well documented in many studies and reports. About 1.1 million deaths were attributable to ambient air pollution reported in Western Pacific countries with Malaysia encountering the number of years of life loss of 160,693 in the year 2012 (WHO, 2018). Moreover, in Malaysia, the prevalence of asthma increased markedly from 5.8% to 8.9% for children aged of 6 - 7 and 13 - 14 years old, respectively (Manan, Jaafar, & Hod, 2017; Sharif et al., 2019).

Asthma is the leading cause of illness among children and has a significant impact on their quality of life. The Third National and Health Morbidity Survey reported that 32.1% of children had visited emergency departments in Malaysian hospitals due to asthma exacerbation (Ministry of Health Malaysia, 2008). The sources of air pollution from outdoors such as benzene and organic compounds released by car emission, as well as particles and irritants gases including nitrogen dioxide (NO_2), sulphur dioxide (SO_2), ozone (O_3) and allergen, contribute to the aggregating of asthma (Carrillo et al., 2018). The complex mixture of exposure to those pollutants at low and high concentrations may create respond adversely to the high risk individual. This condition gets worse in the school environment with the norm setting of schools being nearby high traffic density areas, especially in urban areas (Khreis & Nieuwenhuijsen, 2017). Indeed, the links between urban air pollutants and asthma morbidities are well established (Naja, Permaul, & Phipatanakul, 2018; Vardoulakis & Osborne, 2018). Several previous studies conducted in the district of Hulu Langat, Selangor, which has experienced massive urban sprawl over the last two decades, have indicated that indoor air pollutants were partially responsible for the observed higher asthma prevalence and morbidity among pre-school children (Aziz, Jalaludin, & Bakar, 2014; Kamaruddin, Jalaludin, & Choo, 2015). However, there are insufficient data from this district about the trends of prevalence of asthma associated with indoor air pollutants in the 14 years old age group.

Therefore, the need to conduct asthma related screening and research in schools will help to identify the school children with high risk while findings from the research are really helpful for the policymakers to direct attention towards asthma care management.

1.2 Problem Statement

Until recently, there have been several reports on the interrelationship between asthma development among children and the school micro-environment. It has been suggested that the most potential risk factors are environmental factors, which include indoor air pollutants, and microorganisms such as bacterial and fungal (Annesi-Maesano et al., 2013; Esty et al., 2019; Rivas et al., 2018). These environmental factors vary geographically and are highly associated with traffic emission, industrial activities, climate and urbanisation. Such key factors released the main components of air pollutants, including NO_2, CO, O_3, SO_2, VOCs, and particulate matters, consequently affecting the life quality and respiratory system (Annesi-Maesano et al., 2013; Bo et al., 2017). Evidence shows that these pollutants are associated positively with asthma development, especially among children (Bo et al., 2017; Schultz, Litonjua, & Melén, 2017; Verdier et al., 2014).

Besides, several studies have shown an association between indoor pollutants and respiratory outcomes, including respiratory symptoms, allergies symptoms, pulmonary function, airway inflammations and genetic instability (Agache et al., 2020; Bellanti & Settipane, 2019; Ki-hyun Kim, Ara, & Kabir, 2013). For examples, a manifestation of wheezing and nasal symptoms among school children was associated with PM_{10}, $PM_{2.5}$, formaldehyde, O_3, SO_2 and NO_2 (Hasunuma et al., 2018; Holst et al., 2020; Madureira et al., 2015). Exposure to a high concentration of $PM_{2.5}$, NO_2, and SO_2 was also found to have a strong association with airway inflammation (Kamaruddin et al., 2019; Olaniyan et al., 2020; Zainudin, Jalaludin, & Sopian, 2019). One prospective cohort study reported that exposure to NO_2 and $PM_{2.5}$ at an early age is significantly associated with the risk of incident asthma up to age 14 - 16 years old (Gehring et al., 2015).

Therefore, understanding the profile of indoor air quality in the school environment is critical for control and mitigation measures. Indeed, most school buildings in Malaysia are designed with natural ventilation systems and often situated nearby heavy traffic roads, which increases the risk of exposure to air pollution. Furthermore, indoor air quality in classrooms is much affected by the indoor sources and infiltration of outdoor sources, such as traffic emission, industrial and construction activities, urbanisation and natural sources (Bennett et al., 2018). Thus, the detrimental health impacts are more prominent to the school children in urban and suburban areas compared to rural (Chandra et al., 2018; Del-Rio-Navarro et al., 2020; Paciência & Rufo, 2020). Additionally, there is little literature establishing the differences between exposure in urban and suburban school environments.

In the past decades, a number of literature reviews concluded that exposure to indoor air pollutants has detrimental effects on children's health including morbidity from respiratory diseases (Chen et al., 2020; Lee, Lee, & Bae, 2014; Liu et al., 2018a; Manisalidis et al., 2020). Asthma is the most common illness of childhood, affecting 339 million and resulting in 13,909 children deaths globally in 2016 (WHO, 2018). In Malaysia, approximately 44,155 people (135.52 per 100,000 population) were hospitalised due to asthma and 91 deaths (0.28 per 100,000 population) were recorded in year 2019 (Ministry of Health Malaysia, 2020). Hence, the risks of asthma in children have received incessant attention. In fact, children are more vulnerable to the adverse effects of air pollutants due to their higher breath volume relative to body weights compared to adults. Moreover, they breathe air nearer to the ground and are directly exposed to the higher concentration of settled air pollutants (Goldizen, Sly, & Knibbs, 2016).

Moreover, a growing body of literature has reported that fungal, bacterial and their components identified from the indoor environment were also positively associated with respiratory outcomes among school children (Annesi-Maesano et al., 2013; Cai et al., 2011; Oliveira et al., 2019). Studies conducted by Norbäck et al. (2017a, 2014) and Olaniyan et al. (2019) found that *Aspergillus versicolor* DNA, Alternaria, Cladosporium spores, and 3-hydroxy fatty acids, were significantly associated with daytime breathlessness, respiratory infections and impaired the respiratory performance among school children. However, unculturable or fastidious fungal had less attention in research literature although they have potential health risks to humans (Okten et al., 2020). One epidemiological study has shown that a rare and difficult to culture fungal genus Volutella and Cryptococcus were associated with asthma severity (Dannemiller et al., 2016).

In recent years, the effects of indoor air pollutants on the respiratory system were determined using noninvasive approaches and measurable indicators such as biomarkers and bioindicators, which allow for characterising precisely the asthma endotypes / phenotypes, monitoring and predicting the responses (Tiotiu, 2018). Of all the biomarkers available, the most studied biomarker is fractional exhaled nitric oxide (FeNO). Nevertheless, FeNO assessment requires complementary assessments in order to provide additional clinical information (James & Hedlin, 2016). Therefore, assessment of FeNO with a combination of activation and degranulation of eosinophil and neutrophil in sputum samples would be able to identify airway inflammation to a greater extent. Furthermore, previous studies have described the important roles of adhesion molecules expression in mediating the leukocytes trafficking and recruitment to the inflamed airway (Rao, Ge, & Sriramarao, 2017). One study found that the upregulation of CD11b, CD35, and CD16 in the blood was associated with elevation of PM_{10} and $PM_{2.5}$ (Banerjee et al., 2012; Karottki et al., 2015). However, there is scarcity of evidence-based data on the application of these biomarkers in diagnostic testing of asthma.

Additionally, several factors can regulate the development of asthma among school children at the early adolescence stage. Review has described that the pattern of asthma at this age group was much influenced by extrinsic factors (exposure to pollutants, physical activities), smoking, fluctuations of sexual hormones, obesity, late onset of atopy, and intolerance to aspirin (Benedictis & Bush, 2007). The mechanisms of the association with these factors are not fully clarified, especially in the context of Malaysian school children who are from urban and suburban areas. Hence, further epidemiological studies with a large number of samples, different geographical factors, and environmental parameters need to be undertaken.

Furthermore, there are limited or insufficient data about the prevalence of asthma and asthma-associated risk factors among school children, especially for the early adolescent group.

Therefore, this research intends to evaluate the relationship of indoor air pollutants in the schools' environment setting with airway inflammation, respiratory and allergic symptoms among school children aged 14 years old in both urban and suburban areas. The findings from this research not only contribute to the present knowledge of the detrimental effects of indoor air pollutants, but also provide new direction for diagnostic strategies and treatment options of asthma.

1.3 Importance of the Study

The outcomes of this study through in-depth analysis can be used and translated into a shared effort of exposure prevention and mitigation by governmental agencies, professional bodies and communities. Furthermore, the information may assist the Ministry of Education and Ministry of Works to draw out policies or guidelines for the optimisation of safe school environment and ultimately minimise the exposure risks. This starting point can make a vital contribution in the children's environmental health initiatives as well as the National Environmental Health Action Plan (NEHAP) 2019 in Malaysia.

Furthermore, the findings from this preliminary study can be further extended for the development of risk thresholds precisely for adhesion molecules (CD11b, CD35, CD63 and CD66b) and FeNO levels that can be used in research and clinical settings. This also can lead to the development of new therapeutic approaches for immune-related diseases includes allergic and asthma that targeting specific immune cells and adhesion molecules in the inflammatory cascade. In fact, the majority of potential therapeutic agents for asthma are focused on this pathway (Zhu, Ciaccio, & Casale, 2018).

Given the complex pathogenesis of airway inflammation, a combined analysis of biomarkers between FeNO levels and expression of adhesion molecules is particularly significant. This approach can yield an informative data to address the gaps in the literature regarding the pathophysiologic mechanisms that linked air pollutants in the school environment with airway inflammation.

1.4 Research Questions

This study attempts to answer the following research questions.

1.4.1 General Research Question

What are the factors that influence airway inflammation, respiratory and allergic symptoms among school children aged 14 years old in Hulu Langat district, Selangor?

1.4.2 Specific Research Questions

1. What are the concentration levels of specific indoor air pollutants in the school micro-environment in the Hulu Langat district, Selangor, and how they are different between urban and suburban areas?
2. What are the percentages of the relative abundance of fungal in the school micro-environment in the Hulu Langat district, Selangor, and how they are different between urban and suburban areas?
3. Does school children aged 14 years old in the eight secondary schools in urban areas of the Hulu Langat district, Selangor have a higher prevalence of respiratory and allergic symptoms than a group from suburban areas?
4. Is there a difference in the proportions of IgE-mediated allergy in school children aged 14 years old from the urban areas compared with suburban areas?
5. Is there a difference in the levels of respiratory inflammation in school children aged 14 years old from the urban areas compared with suburban areas?
6. What are the factors that influence the respiratory and allergic symptoms among school children aged 14 years old in Hulu Langat district, Selangor?
7. What are the factors that influence the level of FeNO in school children aged 14 years old in Hulu Langat district, Selangor?
8. What are the factors that influence the expression of adhesion molecules on the leukocytes in the sputum samples collected from school children aged 14 years old in Hulu Langat district, Selangor?

1.5 Study Objectives

1.5.1 General Objective

The general objective of this study was to evaluate the relationship of indoor air pollutants in the schools' environment setting with airway inflammation, respiratory and allergic symptoms among school children aged 14 years old in both urban and suburban areas of the Hulu Langat district, Selangor.

1.5.2 Specific Objectives

1. To measure and compare the key indoor air pollutants (temperature, relative humidity, carbon dioxide, nitrogen dioxide, particulate matters and formaldehyde) from schools in urban and suburban areas.
2. To measure and compare the diversity of fungal relative abundance in settled dust collected from schools in urban and suburban areas.
3. To determine and compare the prevalence of respiratory and allergic symptoms among school children from urban and suburban school areas.
4. To determine and compare the proportions of IgE-mediated allergy and airway inflammation levels based on FeNO levels and expression of adhesion molecules on eosinophil and neutrophil in sputum samples among school children aged 14 years old from urban and suburban school areas.
5. To determine the selected factors (personal risk factors, indoor pollutant parameters, relative abundance of fungi, indoor home environmental factors) that influence the respiratory and allergic symptoms among school children aged 14 years old.
6. To determine the selected factors (personal risk factors, indoor pollutant parameters, relative abundance of fungi, indoor home environmental factors) that influence the level of FeNO among school children aged 14 years old.
7. To determine the selected factors (personal risk factors, indoor pollutant parameters, relative abundance of fungi, indoor home environmental factors) that influence the expression of adhesion molecules in the sputum samples.

1.6 Study Hypotheses

1.6.1 General Hypotheses

Personal risk factors, indoor pollutant parameters, the relative abundance of fungi and indoor home environmental factors significantly influence airway inflammation, respiratory and allergic symptoms among school children aged 14 years old in both urban and suburban areas of the Hulu Langat district, Selangor.

1.6.2 Specific Hypotheses

1. The levels of temperature, relative humidity, nitrogen dioxide, carbon dioxide, particulate matters and formaldehyde collected from indoor classrooms are significantly higher in schools in urban areas compared to suburban areas.
2. The diversity of fungal relative abundance is significantly higher in settled dust samples from suburban compared to urban school areas.
3. The prevalence of respiratory and allergic symptoms are significantly higher among school children aged 14 years old from urban than suburban school areas.
4. The proportions of IgE-mediated allergy and levels of airway inflammation (FeNO levels and expression of adhesion molecules) are significantly higher among school children aged 14 years old from urban than suburban school areas.
5. Personal risk factors, indoor pollutant parameters, relative abundance of fungi and indoor home environmental factors significantly influence the respiratory and allergic symptoms among school children aged 14 years old.
6. Personal risk factors, indoor pollutant parameters, relative abundance of fungi and indoor home environmental factors significantly influence the level of FeNO among school children aged 14 years old.
7. Personal risk factors, indoor pollutant parameters, relative abundance of fungi and indoor home environmental factors significantly influence the expression of adhesion molecules in the sputum samples.

1.7 Definition of Variables

1.7.1 Conceptual Definitions

Airway Inflammation

Airway inflammation is defined as an interaction of inflammatory cells and multiple mediators with structural cells in response to the stimulus, such as allergens, bacteria, virus, fungi and protozoa which lead to airway hyperresponsiveness, bronchial inflammation, mucus secretion and airflow limitation (Turkalj, Erceg, & Dubravčić, 2019). Clinically, airway inflammation is defined by the measurement of airway cell count and differential cellularity, for example granulocytes in sputum, metabolites, transcriptomics and nitric oxide (Goyal & Chang, 2016; Lim & Nair, 2018).

Respiratory Symptom

Respiratory symptoms are defined as the manifestation of one or more symptoms of cough, production of phlegm, wheezing, shortness of breath and chest tightness that vary over time and in intensity (Reddel et al., 2015).

Allergic Symptoms

Manifestations of allergic symptoms depend on the type of allergen exposure, through skin, ingestion or inhalation, or eyes and characterised by Th2 inflammatory responses. The common allergen includes pollen, foods, medication, house dust mites, pet dander, mould or fungi and venom in insects (InformedHealth.org, 2020). The examples of main allergic symptoms include sneeze, stuffy, itchy and runny nose, postnasal drip and itchy nose which are common for allergic rhinitis. Meanwhile, itchy, red and watering of eyes are associated with rhinoconjuctivitis, whereas raised welts on the skin, itchy and red rash are the results of skin reactions (Kusunoki et al., 2017; Seidman et al., 2015).

Doctor-Diagnosed Asthma

Initial diagnosis is done by a physician to determine the variability of respiratory symptoms and airflow limitation characteristic over time and intensity using physical examination, history taking, lung function tests, bronchial provocation tests, and allergy tests (GINA, 2020).

Atopy

Atopy refers to the clinical hypersensitivity state or allergy due to exaggerated production of immunoglobulin E (IgE) immune response from stimuli exposures and can lead to sensitivity reactions. Atopy status can be identified by allergy skin test or measuring the level of specific IgE in serum (Andiappan et al., 2014; Patel & Saltoun, 2019).

Urban

The Department of Statistics Malaysia has defined urban as the gazetted areas with their adjoining built-up areas, with a combined population of 10,000 or more at the time of Census 2010 or the special development areas that can be identified, which had a population of 10,000 or more with at least 60% of the population (aged 15 years and above) involved in non-agricultural activities (Department of Statistics Malaysia, 2020).

Suburban

Suburban refers to the development area or township located adjacent to the urban core and existing either as part of the urban area or on the outskirts of an urban area (Cox, 2017). The Department of Statistic Malaysia has characterised that the rural area has the total population of less than 10,000, which is at strata 3 and 4. Therefore, the suburban area is at strata 2 with a total population of 10,000 or more (Department of Statistics Malaysia, 2011, 2020).

1.7.2 Operational Definitions

Airway Inflammation

Airway inflammation is the excess level of fractional exhaled nitric oxide (FeNO) produced from epithelial cells in response to the inflammation reaction with the levels of 25 ppb and more (25 - 50 ppb = intermediate; > 50 ppb = high) (Dweik et al., 2011). The expression of adhesion molecules (CD11b, CD35, CD63, CD66b) on eosinophil and / or neutrophil are measured using flow cytometry technique. Increased expression of these adhesion molecules can be correlated with airway inflammation reactions (Konrad et al., 2019).

Respiratory Symptoms

Respiratory symptoms of wheeze, wheeze with breathlessness, wheeze without a cold, nocturnal attack of chest tightness, daytime attack of breathlessness, breathlessness after strenuous activity, and nocturnal attack of breathlessness were considered as present when school children and their guardians gave positive response to the respective questions, “Have you ever had wheezing or whistling in the chest at any time in the last 12 months?”, “Have you been at all breathless when the wheezing noise was present?”, “Have you had this wheezing or whistling when you did not have a cold?”, “Have you woken up with the feeling of tightness in your chest at any time in the last 12 months?”, “Have you had an attack of shortness of breath during the day time when you were at rest at any time in the last 12 months?”, “Have you had an attack of shortness of breath after strenuous physical activity in the last 12 months?”, and “Have you been woken up by an attack of shortness of breath at any time in the last 12 months?” (Simoni et al., 2010).

Allergic Symptoms

Allergy symptoms of rhinitis in the past 12 months and skin allergy in the past 12 months were considered present when the respondents gave a positive response to the following questions adopted from the International Study of Asthma and Allergies in Childhood (ISAAC), in accordance with previous studies (Govaere et al., 2009; Kusunoki et al., 2017), “In the past 12 months, have you had a problem with sneezing, or a runny, or a blocked nose when you did not have a cold or the flu?” , “Have you ever had an itchy rash which was coming and going for at least 6 months?”, “Have you had this itchy rash at any time in the last 12 months?” and “Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?”.

Doctor-Diagnosed Asthma

Doctor diagnosed asthma was defined as positive responses to all three following questions adopted from the European Community Respiratory Health Survey (ECRHS), “Have you ever had asthma?”, “Did a physician or health professional diagnosed you of asthma?” and “Have you had any asthma attack in the last 12 months?”. This identification of doctor diagnosed asthma was based on self-report and verified during face-to-face interviews and telephone calls with the participant’s respective guardians (Sá-Sousa et al., 2014, 2019; Sistek et al., 2006).

Atopy

Atopy was defined as a significant positive allergy skin test reaction to at least one of the applied allergens. Positive reaction was determined from the allergen's wheal diameter of 3 mm and more (ASCIA, 2016).

Urban

The Federal Department of Town and Country Planning, Malaysia has updated the urban hierarchy based on number of population (more than 80,000), the centrality of the center, functionally services (economic, border towns, tourism, special industry, special national interest, future transportation/communication nodes), level of health and educational services, and the ability of the centre to stimulate economic growth. From this report, Kajang, Ampang Jaya (Ulu Langat) and Batu 9 Cheras are classified as urban (major towns) with the total population of 307,200, 342,700 and 232,100 for year 2010, respectively (Jabatan Perancangan Bandar & Desa, 2014).

Suburban

Suburban was defined as a township located on the outskirts of the urban area of the Territory of Kuala Lumpur, Kajang, Ampang Jaya (Ulu Langat) and Batu 9 Cheras with residential areas and less commercial activities than urban areas (Aida et al., 2016).

1.8 Conceptual Framework

The conceptual framework is illustrated in Figure 1.1. This study aims to determine the prevalence of respiratory and allergic symptoms and airway inflammation levels among school children aged 14 years old and determine their relationship with indoor air pollutants in the schools' environment setting. The indoor air quality in the school environment can be affected by various factors such as traffic emission, industrial activities, climate and urbanisation.

Indoor air quality is influenced by the combined effects of physical, chemical and biological parameters. However, only relative humidity, temperature, concentrations of particulate (PM_{10} and $PM_{2.5}$), CO_2, NO_2, formaldehyde and the relative abundance of fungi represent the key factors of interest in this study. The route of exposure to air pollutants is mainly through inhalation, ingestion and direct contact with skin or eyes (Manan, Jaafar, & Hod, 2017). Evidence shows that these indoor pollutants are associated positively with asthma development that can be characterised by airway inflammation, airway hyperresponsiveness and reversible airway obstruction. These complex pathophysiologic mechanisms lead to the manifestation of any combination of respiratory symptoms include wheezing, shortness of breath, chest tightness and cough (Licari et al., 2018; Schultz et al., 2017).

The primary end points of this study were respiratory and allergic symptoms, and airway inflammation. The airway inflammation was characterised by FeNO levels and expression of adhesion molecules (CD11b, CD35, CD63 and CD66b) on both eosinophil and neutrophil cells measured from exhaled airway and sputum samples, respectively. Previous study has described the important roles of adhesion molecules expression in mediating the leukocytes trafficking and recruitment to the inflamed airway (Rao et al., 2017). Therefore, the adhesion molecules of CD11b, CD35, CD63 and CD66b on both leukocytes became the targets of research interest in this current study. In particular, CD11b (Mac-1, αM integrin, CR3, C3biR) is one of the subunits for *β*2 integrins that possess a large array of functions in exerting and modulating the immune cells (Bednarczyk et al., 2020). Besides, CD35 (CR1) is a multifunctional polymorphic glycoprotein that presents on plasma membranes of granulocytes, monocytes, erythrocytes, B-lymphocytes, T cells and Langerhan cells. The biological function of CD35 varies with the cell type. The expression of CD35 on eosinophil and neutrophil promotes their activation and phagocytosis of C3-bound targets (Dallaire et al., 2003; Liu & Niu, 2009). Additionally, a recent review reported that CD35 is stored in secretary vesicles of neutrophil and translocated to the plasma membranes on cell activation (Vandendriessche et al., 2021). CD63 is a member of tetraspanins and expressed in intracellular membranes of specific granules of eosinophil, and secretory granules of neutrophil and basophil (Carmo et al., 2016). Functionally, CD63 is classically used as an activation marker for basophil, neutrophil and eosinophil (Kraft et al., 2013). In addition, CD66b (CEACAM8, CGM6, NCA-95) is a member of the human carcinoembryonic Ag (CEA) family and is exclusively expressed on granulocytes. Indeed, CD66b is recognized as an activation marker for granulocyte (Yoon, Terada, & Kita, 2007).

Moreover, there are several factors can regulate the development of asthma among school children at the early adolescence stage. Review has described that the pattern of asthma at this age group was heavily impacted by extrinsic factors (exposure to pollutants, physical activities), smoking, fluctuations of sexual hormones, obesity, late onset of atopy, and intolerance to aspirin (Benedictis & Bush, 2007).

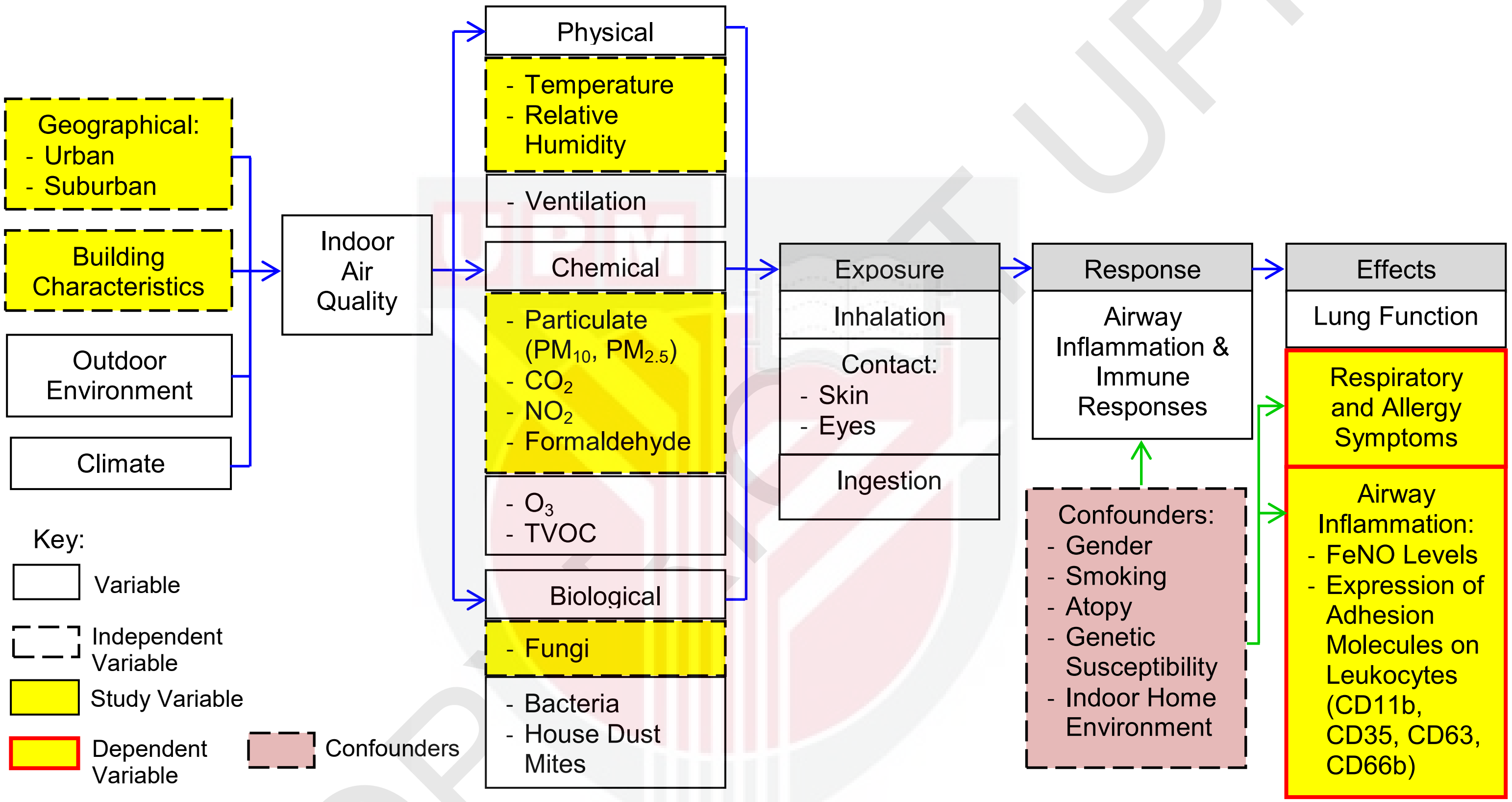

Figure 1.1 : Conceptual framework of indoor air quality in school environment and its relationship with respiratory and allergy symptoms, and airway inflammation among school children

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