Indoor air pollutant exposure and eosinophil cationic protein as an upper airway inflammatory biomarker among preschool children

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Abstract

The upper and lower airways of the respiratory tract are functionally linked, with inflammation in the former playing a vital role in the pathogenesis of asthma and allergy. Studying the association between indoor air pollutants with upper airway inflammation in children will help improve childhood asthma and allergy management related to poor indoor air quality. A cross-sectional study was conducted among preschool children in industrial (Kelana Jaya and Shah Alam) and suburban (Semenyih and Hulu Langat) areas in Selangor, Malaysia. A questionnaire adapted from the American Thoracic Society and International Study on Asthma and Allergy in Children was distributed to obtain the respondents’ background information, school, and home environment. Eosinophil cationic protein (ECP) concentrations in nasal swab samples were collected and analyzed to determine the prevalence of upper airway inflammation. An indoor air quality (IAQ) assessment was also conducted in seven preschools in both industrial and suburban areas, including parameters such as particulate matter up to 10 μm in size (PM10), volatile organic compounds (VOCs), total mold, total bacteria, relative humidity, and air temperature. Statistical analysis shows significant differences in PM10, total mold, total bacteria and relative humidity between the study areas (p < 0.05). The ECP levels among respondents vary significantly between study areas (t = 8.473, p < 0.001). The VOC concentration and ECP level are significantly correlated (prevalence ratio 6.41; 95% CI, 1.268 to 32.394) after controlling all confounders. This study concludes that exposure to indoor air pollutants increases the risk of respiratory problems and may have an impact on the inflammatory and secretory response of the nasal mucosa.

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1. Introduction

Indoor air quality (IAQ) is a measure of the cleanliness of the air that we breathe indoors, which may have negative implications to human health. High quality indoor air is important because humans spend 80–95% of their lives indoors [1]. Furthermore, the correlation between IAQ and human health has currently become a major concern, with more studies emerging to understand their relationship. However, studies are lacking on the effects of IAQ on preschool-aged children because data on epidemiological studies on pediatric asthma are often based on extrapolation from older children (i.e., 7 years old and above) [2]. Preschool-aged children are more vulnerable to compromised IAQ than adults and older children because of their immature immune systems, greater food intake, inhaled breath per unit mass, and rapid growth [3]. A study of association also found numerous health implications, particularly asthma, from the exposure of this group of children to indoor pollutants. Asthma severity in children can be related to the level of exposure to common indoor allergens, such as dust mites and cat allergens [4].

In some studies, 95% of asthma cases are associated with nasal disease, whereas other studies maintain that the ideal management of asthma cannot be effective without including the control of upper airway disease [5, 6]. Upper airway inflammation is characterized by rhinitis, sinusitis, and rhinosinusitis, which are related to postnasal drip [5]. Previous studies have measured the outcome of upper airway inflammation on the basis of reported symptoms. In the current study, a biomarker was used to determine the prevalence of upper airway inflammation. Eosinophil cationic protein (ECP) is a biomarker that has been well characterized as an inflammation marker [7]. ECP secretion is commonly associated with allergic rhinitis but can also be used as a marker for acute exposure effects in non-allergic rhinitis triggered by irritants or pollutants [8]. Therefore, diagnosed asthmatic and allergic children are excluded from the sample population to study the relation of exposure with the prevalence of upper airway inflammation. The objective of this study is to identify various indoor air pollutants and associate upper airway inflammation among preschool children influenced by different socioeconomic activities in industrial and suburban areas in Selangor, Malaysia.

2. Methodology

A cross-sectional study was conducted among 98 preschool children aged 5 years old to 6 years old who attended preschools in an industrial area (Shah Alam and Kelana Jaya) and a suburban area (Semenyi and Hulu Langat) in Selangor, Malaysia. The sample size was calculated on the basis of a formula using combined standard deviation from a group comparison study, as described in Lemeshow et al. [9]. The preschools were selected within a 5 km radius of the predetermined industrial and suburban settings. Surveys and inspections were conducted to determine the characteristics of the preschools that could influence exposure to indoor air pollutants, such as their distance from the main road, their proximity to factories, and other potential sources of pollutant emission. Parents or guardians were asked to fill out a questionnaire adapted from the American Thoracic Society and International Study on Asthma and Allergy in Children. Those with known medical evidence of asthma, allergy, and other respiratory illnesses were excluded. Out of the 98 study respondents, a subset of 70 children was drawn for nasal sampling prior to the parents’ consent to the method.

The sampling devices were placed at an area approximately 0.6 m to 0.9 m; this area represented the average breathing zones of the children while seated in the classroom during lesson periods; sampling devices were also placed in the middle of the classroom to obtain representative conditions [10]. The real-time readings of particulate matter up to 10 μm in size (PM10) and volatile organic compounds (VOCs) were recorded continuously for 4 hours during normal class activities by using Dust Trak™ DRX Aerosol Monitor 8534 and ppbRAE VOC Monitor Model PGM-7240, respectively. Temperature and relative humidity were measured periodically in various sampling points throughout the preschools by using TSI Q-Trak™ IAQ Monitor 7575 before obtaining the mean reading. These parameters were also measured in the outdoor air by using the same methods as indoor air monitoring. The microbiological pollutants were measured on the basis of the volume of air sampled (500 L) by using a PBI Duos SAS Super 360™ directed on the surface of a contact plate with agar. The bacterial samples were incubated at 37 °C.
for 24 hours before counting the colony forming units (CFU/m³), whereas mold samples were left at room temperature (23–26 °C) for 5 days before colony counting was conducted.

The nasal swab procedure used in this study was adopted and modified from many different previous studies suited to the Malaysian preschool setting to determine the inflammation of nasal mucosa [7, 11, 12]. Sampling was performed with the respondent sitting in a chair with the head hyperextended or flexed approximately 30° forward. A sterile HmbG cotton swab previously immersed in 0.9% NaCl solution was then inserted in the inferior turbinate of the nasal mucosa and gently rotated 90° to 180°. Immediately after the sampling, the swab was placed into a collection tube containing 2 mL of normal saline (0.9% NaCl). All samples were kept at 0–4 °C before transferring to the lab. The samples were centrifuged twice at 500 and 1000 × g. Centrifugation was conducted within 5 hours of collection. The supernatant was then immediately frozen at −70 °C to −80 °C for the analyses of biomarkers. Nasal sample analysis was conducted by using Cloud-Clone Corp. SEB758Hu Enzyme-linked Immuno sorbent Assay Kit for Ribonuclease A3 (RNASE3) for human ECP sample; this procedure was conducted according to the manufacturer’s standard protocols.

3. Results

3.1. Background and assessment of personal exposure

Data from the collected questionnaires indicated that most children in the studied industrial and suburban areas mostly live near the main road, as suggested by the high number of respondents living within 500 m from the main road (N = 35, 66.0% and N = 27, 65.9%). A high number of respondents in the industrial area live more than 500 m from the main road (N = 14, 26.4%) compared with respondents in the suburban area (N = 7, 17.1%). This finding shows a significant difference in the population pattern, (χ² (3, N = 98) = 21.98, p < 0.001) compared with respondents in the suburban area. No significant differences in proximity to factories were detected between the study areas (p > 0.05).

3.2. Concentration of indoor air pollutants

Analysis of indoor air pollutant level was conducted on the basis of IAQ measurement from all participating preschools in this study. The findings are tabulated in Table 1. The results from the study areas were then compared by using the Mann–Whitney U statistical analysis because the data were not normally distributed. The median of indoor PM10, total mold, and relative humidity measurements in preschools in the industrial area were higher than those in preschools in the suburban area. Moreover, statistical analysis showed a significant difference between the abovementioned parameters and total bacteria in both study areas (p < 0.05). However, no significant differences were observed for VOC levels (ppm) and air temperature levels (°C) in both study areas. Additional IAQ assessment on microbiological contaminants revealed significant differences in mold and bacteria levels between the study areas, with medians of 361 and 344, respectively (p < 0.05).

3.3. ECP levels and association with indoor air pollutants

Nasal samples showed a 100% positive detection of ECP for all 70 respondents in both study areas. An independent samples t-test was used to compare ECP levels among preschool children in the industrial area to the ECP levels among suburban respondents, and the results are presented in Table 2. Statistical analysis shows that the ECP concentrations among preschool children in the industrial area are significantly higher than those of preschool children in the suburban area (p < 0.001). The relationship between ECP levels in the nasal sample with the measured pollutant levels is shown in Table 3. For the purposes of our statistical analysis, ECP levels were categorized into high and low levels on the basis of the median level for both study areas (0.87 ng/mL). Chi-square test indicated that ECP levels are significantly correlated with PM10 and bacteria levels and that ECP levels are insignificantly correlated with VOCs and mold levels.
### Table 1 Indoor air parameters distribution and comparison of PM\(_{10}\), VOCs, microbiological contaminants, relative humidity, and air temperature between study areas

<table>
<thead>
<tr>
<th>Variables</th>
<th>Industrial ((n = 53))</th>
<th>Suburban ((n = 45))</th>
<th>(z) value</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM(_{10}) ((\mu g/m^3))</td>
<td>126.00 (36.00)</td>
<td>85.00 (224.00)</td>
<td>−1.98</td>
<td>0.05*</td>
</tr>
<tr>
<td>VOCs (ppm)</td>
<td>0.02 (0.00)</td>
<td>0.02 (0.02)</td>
<td>−0.93</td>
<td>0.36</td>
</tr>
<tr>
<td>Mold (CFU/m(^3))</td>
<td>361.00 (0.00)</td>
<td>344.00 (95.00)</td>
<td>−2.42</td>
<td>0.02*</td>
</tr>
<tr>
<td>Bacteria (CFU/m(^3))</td>
<td>275.00 (19.00)</td>
<td>286.00 (65.00)</td>
<td>−1.98</td>
<td>0.05*</td>
</tr>
<tr>
<td>Relative Humidity</td>
<td>64.50 (8.20)</td>
<td>64.00 (8.40)</td>
<td>−5.59</td>
<td>0.001**</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>30.10 (0.00)</td>
<td>30.20 (1.20)</td>
<td>−1.42</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*Significant at \(p < 0.05\); **Significant at \(p < 0.001\)

### Table 2 ECP levels among preschool children and comparison between study areas

<table>
<thead>
<tr>
<th>Variables</th>
<th>Industrial ((n = 37))</th>
<th>Suburban ((n = 33))</th>
<th>(t) value</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECP level (ng/mL)</td>
<td>1.37 (0.45)</td>
<td>0.66 (0.22)</td>
<td>8.47</td>
<td>0.001**</td>
</tr>
</tbody>
</table>

**Significant at \(p < 0.001\)

### Table 3 Correlation between ECP concentrations in nasal samples from preschool children with indoor air pollutant levels

<table>
<thead>
<tr>
<th>Variables</th>
<th>High ECP Level (&gt;0.86 ng/mL)</th>
<th>Low ECP Level (&lt;0.86 ng/mL)</th>
<th>(\chi^2) value</th>
<th>(p) value</th>
<th>Prevalence Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM(_{10}) ((\mu g/m^3))</td>
<td>32 (91.4)</td>
<td>23 (65.7)</td>
<td>6.87</td>
<td>0.01*</td>
<td>5.57 (1.41–21.99)</td>
</tr>
<tr>
<td>VOC (ppm)</td>
<td>12 (34.3)</td>
<td>7 (20.0)</td>
<td>1.81</td>
<td>0.18</td>
<td>2.09 (0.71–6.17)</td>
</tr>
<tr>
<td>Mold (CFU/m(^3))</td>
<td>22 (62.9)</td>
<td>17 (48.6)</td>
<td>1.45</td>
<td>0.23</td>
<td>1.79 (0.69–4.65)</td>
</tr>
<tr>
<td>Bacteria (CFU/m(^3))</td>
<td>32 (91.4)</td>
<td>17 (48.6)</td>
<td>15.31</td>
<td>0.001**</td>
<td>11.29 (2.91–43.85)</td>
</tr>
</tbody>
</table>

*Significant at \(p < 0.05\); **Significant at \(p < 0.001\)

### 3.4. Logistic regression for the correlation between indoor PM\(_{10}\), VOC, mold, and bacteria levels with ECP level after controlling confounders

Logistic regression was performed to determine the factors that are significantly correlated with ECP level after controlling the confounders. Table 4 shows the main variables, namely, PM\(_{10}\), VOC, mold, bacteria levels (with confounder of indoor smoking), mosquito coil usage, carpet usage, distance of house from the main road, and factory proximity. From the statistical analysis, VOC is the only statistically significant parameter associated with ECP level (\(p < 0.05\)). On the basis of the prevalence ratio, respondents who are exposed to VOC are six times more likely to experience upper airway inflammation, determined in this case by the presence of ECP in the nasal sample (PR 6.41;95%CI, 1.27 to 32.39).

### Table 4 Logistic regression for association between indoor PM\(_{10}\), VOC, mold, and bacteria levels with ECP concentration after controlling the confounders

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>B</th>
<th>S.E.</th>
<th>(p) value</th>
<th>PR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>−6.22</td>
<td>3.43</td>
<td>0.07</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>PM(_{10})</td>
<td>−19.65</td>
<td>19865.09</td>
<td>0.99</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>
VOC & 1.86 & 0.83 & 0.03* & 6.41 & 1.27–32.39 \\
Mold & $-19.87$ & 12562.01 & 0.99 & 0.00 & $-\$ \\
Bacteria & $42.60$ & 23503.74 & 0.99 & $3.15E+18$ & $-\$ \\
Distance of house from main road & 0.45 & 0.49 & 0.35 & 1.57 & 0.60–4.09 \\
Proximity to factory & 0.06 & 0.42 & 0.89 & 1.06 & 0.45–2.52 \\
Indoor smoking & 0.18 & 0.76 & 0.81 & 1.20 & 0.27–5.36 \\
Mosquito coil usage & $-0.48$ & 0.85 & 0.57 & 0.62 & 0.12–3.25 \\
Carpet usage & 0.09 & 0.75 & 0.90 & 1.90 & 0.25–4.76 \\
House painting within 12 months prior to & $-0.69$ & 0.84 & 0.41 & 0.50 & 0.69–2.59 \\
the study & & & & & \\

*Significant at $p < 0.05$; 95% CI= 95% Confidence Interval; B = Regression Coefficient; SE = Standard Error

4. Discussion

The results from this study indicate that indoor air pollutants may affect respiratory health and biomarkers in the nasal mucosa. This effect can be seen with high concentrations of indoor pollutants, such as high PM$_{10}$, total mold content, and total bacteria content in the indoor air of preschools. However, the PM$_{10}$ level in preschools is within the permissible limit (150 $\mu$g/m$^3$), as suggested by the Recommended Malaysia Ambient Air Quality Guidelines on 24-hour exposure [13] and the Environmental Protection Agency 24-hour PM$_{10}$ guidelines [14]. High levels of indoor pollutants in preschools may be caused by surrounding factors, such as the distance of the preschool to the main road or to factories and the use of carpets inside the preschool. Indoor air pollutants, such as PM$_{10}$ and VOCs, may originate from sources such as carpets, house paint, and outdoor sources such as combustion activities from the road[10]. Furthermore, local environmental factors may influence pollutant readings and contribute to the significant differences between study areas [15, 16].

In the current study, the health outcome is interpreted by the measurement of ECP as a representative of upper airway inflammation among preschool children. The ECP level in nasal mucosa was detected in all respondents; the levels are higher among preschool children living in the industrial area than in respondents in the suburban area. This finding may be due to the higher level of indoor air pollutants in the industrial area than in the suburban area. Moreover, statistical analysis demonstrated that PM$_{10}$ and bacteria levels are significantly correlated with ECP levels in the nasal mucosa. The respondents exposed to high levels of PM$_{10}$ are five times more likely to have higher ECP levels than those exposed to low PM$_{10}$ levels. Furthermore, respondents were 11 times more likely to have higher ECP levels if they were exposed to higher levels of indoor bacteria than those exposed to lower levels of bacteria. This finding is supported by a previous study by Norback et al. [12], who found that particulate pollutants and indoor concentrations of various types of gaseous pollutants have significant correlations with nasal disease. Another study also found that exposure to high levels of formaldehyde (e.g., a VOC), caused concentration of eosinophilic granulocytes and increased albumin in nasal lavage [17]. This finding also supported the result of our logistic regression model, which found that VOCs are the main contributing factor in high ECP levels after controlling all confounders. These results suggest a possible inflammatory and allergic mechanism wherein exposure to VOCs increases the risk of upper airway inflammation six fold among preschool children.

5. Conclusion

The results of this study suggest that indoor air pollutants, particularly VOCs, in preschools are most likely to affect the inflammatory and secretory response of the nasal mucosa. However, the definite causative agent cannot be elucidated because the study was conducted with mixed exposure without disregarding the effects of other pollutants, such as PM$_{10}$ and microbiological contaminants. Moreover, the results suggest that ECP is useful in evaluating upper airway inflammation in healthy persons and is suitable for application in small children. Distinguishing between study areas in terms of exposure to pollutants proved to be difficult because ECP was detected in all respondents’ nasal samples, thus suggesting that suburban children are also at risk of upper airway inflammation.
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References