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# Secondhand Smoke Exposure and Serum Cytokine Levels in Healthy Children

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## Abstract

**Background—**Exposure to secondhand smoke (SHS) is associated morbidity in children. Alterations in immune responses may explain this relationship, but have not been well-studied in children. Our objective was to determine the association between SHS exposure and serum cytokine levels in healthy children.

**Methods—**We recruited 1–6 year old patients undergoing routine procedures. A parent interview assessed medical history and SHS exposure. Children with asthma were excluded. Blood was collected under anesthesia. We used Luminex to test for a panel of cytokines; cotinine was determined using an enzyme-linked immunosorbent assay. Children were categorized as no, intermediate, or high exposure. A mixed-effects model was fit to determine differences in cytokines by exposure level.

**Results—**Of the 40 children recruited, 65% (N=26) had SHS exposure; 16 intermediate, and 10 high. There were no differences by demographics. In bivariate analyses, children exposed to SHS had lower concentrations of IL-1β, IL-4, IL-5, and IFN- γ than those with no exposure. In the mixed-effects model, children with any SHS exposure had significantly lower concentrations of IL-1β (0.554 pg/mL vs. 0.249 pg/mL) and IFN- γ (4.193 pg/mL vs. 0.816 pg/mL), and children with high exposure had significantly lower mean concentrations of IL-4 (8.141 pg/mL vs. 0.135 pg/mL) than children with no exposure.

**Conclusions—**This study suggests that SHS exposure decreases expression of some pro-inflammatory cytokines in SHS exposed children, including IFN-γ. Further research to describe the acute and chronic effects of SHS on the immune systems of children is needed.

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**Author Disclosure Statement**

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## Introduction

Secondhand smoke (SHS) exposure has been shown to be a significant source of morbidity within the pediatric population. A variety of diseases are associated with SHS exposure, including lower respiratory infections,[1] asthma,[2,3] sudden infant death syndrome,[3] inflammatory bowel disease,[4] otitis media,[5] metabolic syndrome ,[6] and leukemia.[7] It is estimated that each year in the US, 202,300 new cases of asthma, 300,000 cases of lower respiratory illnesses, and 789,700 ear infections develop due to SHS exposure in children.[8]

The relationship between SHS exposure and the development of these diseases is not yet fully understood. However, evidence from both human and animal models suggests that SHS exposure may increase both local and systemic inflammatory states, and has effects on a variety of types of cytokines, including both $T_H1$ and $T_H2$ (or atopic) responses. For example, in one animal study it was shown that mice exposed to SHS had increased circulating levels of interleukin-1β (IL-1β), a well described pro-inflammatory cytokine.[9] In humans, exposure to SHS increases the concentration of a variety of inflammatory cytokines associated with the $T_H2$ response, including IL-4, IL-5 and IL-6.[10] Elevated serum levels of the aforementioned cytokines have been described for up to 3 hours after exposure to SHS.[11]

Localized inflammation has been noted in children exposed to SHS as well; children exposed to SHS have elevated airway secretions of IL-13.[12] The elevation of pro-inflammatory, particularly $T_H2$, cytokines at both the systemic and local levels may account for the poor respiratory health of children exposed to SHS and the predisposition of exposed children to develop asthma. Moreover, SHS exposure has been shown to decrease levels of interferon-γ (IFN-γ), a cytokine that is integral to the suppression on $T_H2$ responses involved in immunity, in children.[13] Suppression of immune responses to viral and bacterial pathogens may account for the increased prevalence and severity of illness in SHS- exposed children.

Despite the evidence that SHS exposure results in increased inflammatory states, the mechanism and scope of these changes are not fully understood, especially in children. It is unclear whether the developing immune responses in children have the same pro-inflammatory responses to SHS, or if such responses are the predisposing factor in particular disease processes. In addition, other common biomarkers of inflammation in the pediatric population, such as CXCL8 (IL-8) and IL-10, have not yet been well studied in SHS-exposed children. In order to determine the association between SHS exposure and serum markers of inflammation in a pediatric population, we completed a pilot study of 40 healthy children undergoing routine operative procedures as part of a study demonstrating 1) the feasibility of collected serum samples from a pediatric population, and 2) the levels of a panel of circulating cytokines in the serum of healthy children. As an exploratory study, we hypothesized that cytokine levels would vary between SHS exposed and non-exposed children, and that in particular, levels of IFN-γ would be lower in SHS-exposed children.

## Methods and Materials

### Patients

This study took place at Golisano Children's Hospital at the University of Rochester Medical Center in Rochester, NY from 2009–2010. Patients included healthy children, between the ages of 1 and 6, undergoing routine operative procedures, including dental restoration, gastrointestinal endoscopies, or tonsillectomies. Patients who had illness symptoms, such as cough or rhinorrhea, were excluded from the study. Patients were also excluded from the study if they had active medical conditions, including asthma, allergies,

eczema, inflammatory bowel disease, and prematurity or if azithromycin, NSAIDs, COX-2 inhibitor, oral corticosteroids, inhaled corticosteroids or albuterol were taken in the two weeks preceding the study. Patients included those who were SHS-exposed and who were not SHS-exposed. A total of 40 children were recruited for the study.

### Study Design

A parental interview was conducted to determine if the patient had any pre-existing medical conditions, was currently taking medications, and the extent of their SHS exposure. A blood sample (10 mL) was collected from each patient during the operative procedure. The blood samples were tested for cotinine using an enzyme-linked immunosorbent assay (ELISA),[14] and a variety of interleukins (IL-1β, IL-4, IL-5, IL-6, TNF-α, IFN- γ, CXCL8, IL-10, and IL-13) using a Luminex cytokine kit.[15] All laboratory testing was conducted at the Rochester Clinical Translational Science Institute Core Laboratories. The study was approved by the University of Rochester Research Subjects Review Board.

### Statistical Analysis

Based on parent surveys and cotinine measurements, patients were classified in one of the following categories: no SHS exposure, intermediate SHS exposure, or high SHS exposure. Analysis was first conducted by comparing 20 patients with no SHS exposure to 20 children with some SHS exposure. Those classified as having some SHS exposure had either a detectable level or cotinine or noted smoke exposure of any kind in the parent report. A second analysis was performed with 20 patients with no SHS exposure and 11 with high SHS exposure. A high level of SHS exposure indicates both evidence of smoke exposure in the parent report and a detectable level of cotinine. Chi-square and t-tests were used to assess differences between the SHS-exposed and non-exposed groups in demographics and medical care factors.

To assess differences in cytokine expression by SHS-exposure, a model was fit including child-specific and SHS effects. It was assumed that log (cytokine expression) was normally distributed with a possibly different mean for exposed children. There were substantial numbers of data not observed because the cytokine levels were below the limit of detection. These values were imputed using the assumption of log-normality in each case. By imputing these values, values that were below the limit of detection could be estimated, thus propagating the uncertainty to the tests for differences. This method is more conservative because of the uncertainty of non-measured values and has been found to have less bias that using the traditional method of substituting half the limit of detection.[16]

The results in Table 2 present sample means and t-tests for the traditional method of substituting limit of detection/2 for unobserved values. The results in Table 3 present the estimated average cytokine expression levels and test for SHS effects using a mixed effects model with imputed values for measurements below the limit of detection. We fit the 40 children and 9 cytokines in one model, $\log(Yij) = \mu j + \beta j\, xi + ri + \varepsilon ij$ for i = 1,…, 40 and j = 1,…, 9 where μj gives the unexposed (log-)mean expression level of the jth cytokine, βj gives the exposure effect, ri are random effects for the children, and εij are random errors. The ri and εij are all independent and normally distributed with mean 0 and variances View the MathML sourceσr2 and View the MathML sourceσj2, respectively, estimated from the data. All analyses were done using WinBUGS.

## Results

A total of 40 children were included in the study. The mean age was 3.55 years (range 1–6 years); 50% were males, 87.5% white, 5% African-American, 5% Hispanic, and 2.5%

multiracial. The operatative procedures included 70% dental restorations, 17.5% endoscopies, and 12.5% otolarygologic procedures. Two (5%) children did indicate having a history of eczema; however it was not active at the time of the study and they were on no medications. Other medical conditions noted included autism, celiac disease, congenital heart defect, epilepsy, and Type 1 diabetes.

A total of 65% (N=26) of the children were classified as SHS-exposed, as determined from the parent questionnaire and/or cotinine results. Specifically, 16 children had intermediate SHS exposure and 10 children had high SHS exposure. This exposure occurred from family members in the child's primary residence (35%), in a car (30%), a relative's house (20%), a public place (15%), from visitors to the child's primary residence (12.5%), a friend's house (7.5%), daycare (2.5%), or a social event (2.5%). In the primary residence, the main source of exposure was the mother (27.5%) and father (20%) followed by the grandmother (7.5%), the grandfather (2.5%), siblings (2.5%), or other family members (e.g. stepfathers) (7.5%). There were 8 children (20%) identified as SHS exposed by cotinine level that were not identified by parent report. Table 1 demonstrates the relationship between SHS-exposure, and the demographic and medical care variables.

Cytokine levels of children with some SHS exposure (intermediate and high) were compared to cytokine levels of children with no SHS exposure. The mean concentration of IFN-γ (10.368 vs. 3.295, $p<0.05$) was significantly lower for the any SHS exposed children than the non-SHS exposed children (Table 2). The cytokine levels for children with high SHS exposure were also compared to children with no SHS exposure. The mean concentrations of IL-1b (0.599 vs. 0.426, $p<0.05$), IL-4 (16.483 vs. 9.57, $p<0.05$), IL-5 (0.823 vs. 0.43, $p < 0.05$) and IFN- γ (10.368 vs. 3.209, $p<0.05$) were significantly lower for the SHS exposed children.

Using the more conservative mixed effects model, the cytokine levels were compared between groups (Table 3). Those children with some SHS exposure had significantly lower mean concentrations of IL-1b (0.554 vs. 0.249) and IFN- γ (4.193 vs. 0.816). Children with high exposure had significantly lower mean concentrations of IL-4 (8.141 vs. 0.135) than children with no SHS exposure.

## Discussion

Understanding the effect of tobacco smoke exposure on the expression of a broad variety of cytokines in children will help us better capture the complex interaction between tobacco smoke and the immune system, and develop interventions for children who continue to be exposed. Our study has several important implications. First, since few studies have examined serum expression of cytokines in children, and fewer have compared the levels between smoke-exposed and non smoke-exposed children, we think it is important to know that a broad range of cytokines can be measured in children's serum, and that we can measure significant differences in some of these based on tobacco smoke exposure levels.

Overall, we found generally lower cytokine levels in children exposed to SHS. This is consistent with other studies that have suggested that children exposed to SHS have suppressed immune responses.[18–20] Even using the more conservative statistical model, our study found a significant decrease in IFN-γ amongst SHS- exposed children; this is consistent with the prior finding of IFN-γ suppression in children exposed to SHS.[13]

Our sample of children deliberately excluded those with a history of asthma or severe allergy as we were interested in describing differences among healthy children with smoke exposure. Therefore it was interesting to note the relative decrease in IL-4, and non-expression of IL-13, among children exposed to tobacco smoke. While further research is

needed to clarify this relationship, we speculate that children who are predisposed to atopy and therefore expression of IL-4 and IL-13 will have developed asthma by this age, and were thus excluded from this study. This is supported by the fact that none of the children with high levels of SHS exposure expressed IL-4 or IL-13.

Exposure to SHS is not at a constant level; rather children are exposed intermittently in large doses, and may have lower background rates of exposure from what has been called "thirdhand smoke" (THS) or the off-gassing of chemicals and particulates that occurs after the cigarette has been extinguished.[24] Since all of our subjects were coming in for routine operative procedures and were checked in prior to recruitment, none could have been exposed to direct SHS for at least an hour prior to serum sampling; thus we captured their cytokine profiles in a more chronic state, rather than observing the acute effects of exposure.

While modest in scope, our findings do have significant implications. Further research is necessary to better examine these complex relationships, and to clarify both the acute and chronic immunologic consequences that SHS exposure has on children. Parents who smoke should be aware that SHS exposure has a measurable impact on their child's expression of immune function, and that the suppression that occurs has been associated with an increased risk of infection. While we can't define the consequences of these changes based on this study, the overall increase in respiratory illness in children exposed to SHS supports this. Parents are more likely to quit smoking or reduce exposure when counseling includes biological marker feedback[25]; incorporating information about cytokines and the risks of SHS may increase parents' motivation to protect their children from SHS.

### Limitations

There are significant limitations to our study. Our sample is small, and may not capture the full variability of cytokine expression in children. While we selected children who were previously generally healthy, the conditions (dental caries, abdominal pain, and tonsillar hypertrophy) that lead to their operative procedures, may themselves lead to differential cytokine expression. Since we had limited sensitivity in our cotinine analysis, we based our SHS exposure measure primarily on parent report. While this has been shown to have good validity compared to cotinine testing,[26–28] it still raises the question of social desirability bias.

### Conclusion

Healthy children who are exposed to SHS have decreased IFN-γ expression compared to those who are not SHS-exposed. Pediatricians and other providers of care to children should understand the biochemical risks of exposure, and how this may affect children's immune function, so that they may properly counsel parents about the benefits of smoking cessation and smoke exposure reduction. Further research to describe the acute and chronic effects of SHS on the immune systems of children is needed. Children are vulnerable to the effects of SHS, and are most often incapable of removing themselves from the exposure; and thus should be protected from any exposure to tobacco smoke.

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## Abbreviations

| | |
|---|---|
| **SHS** | Secondhand tobacco smoke |
| **IL** | Interleukin |

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**Table 1**

Demographic and Medical Care Variables by Smoke Exposure

| Variable | Overall | SHS-exposed | Not SHS-Exposed | *p*-value |
|---|---|---|---|---|
| **Gender** | | | | 0.51 |
| Male | 50% | 46.2% | 57.1% | |
| Female | 50% | 53.8% | 42.9% | |
| **Age** | | | | 0.10 |
| Mean | 3.55 | 3.77 | 3.14 | |
| **Race** | | | | 0.25 |
| White | 87.5% | 84.6% | 92.9% | |
| African-American | 5% | 7.7% | 0% | |
| Hispanic | 5% | 7.7% | 0% | |
| Multiracial | 2.5% | 0% | 7.1% | |
| **Procedure** | | | | 0.002 |
| Dental | 70% | 88.5% | 35.7% | |
| Endoscopies | 17.50% | 7.7% | 35.7% | |
| Otolarygologic | 12.50% | 3.8% | 28.6% | |

**Table 2**

Cytokine Levels by SHS Exposure

| | Level of SHS Exposure | N>limit of detection | Mean[a] (pg/mL) | *p*-value |
|---|---|---|---|---|
| IL-1β | *None* | 2 | 0.599 | |
| | *Intermediate* | 1 | 0.470 | 0.052[1] |
| | *High* | 1 | 0.423 | **0.038**[2] |
| IL-4 | *None* | 2 | 16.483 | |
| | *Intermediate* | 2 | 12.273 | 0.083[1] |
| | *High* | 0 | 9.565 | **0.038**[2] |
| IL-6 | *None* | 8 | 9.544 | |
| | *Intermediate* | 8 | 11.912 | 0.529[1] |
| | *High* | 3 | 22.675 | 0.552[2] |
| CXCL8 | *None* | 13 | 6.345 | |
| | *Intermediate* | 15 | 5.840 | 0.436[1] |
| | *High* | 9 | 5.134 | 0.380[2] |
| IL-10 | *None* | 12 | 19.484 | |
| | *Intermediate* | 14 | 22.783 | 0.960[1] |
| | *High* | 7 | 13.584 | 0.222[2] |
| TNF-α | *None* | 14 | 9.722 | |
| | *Intermediate* | 16 | 10.428 | 0.470[1] |
| | *High* | 10 | 10.47 | 0.516[2] |
| IL-5 | *None* | 5 | 0.823 | |
| | *Intermediate* | 5 | 0.573 | 0.080[1] |
| | *High* | 1 | 0.425 | **0.034**[2] |
| IFN-γ | *None* | 5 | 10.368 | |
| | *Intermediate* | 2 | 3.350 | **0.036**[1] |
| | *High* | 2 | 3.209 | **0.036**[2] |
| IL-13 | *None* | 1 | 6.867 | |
| | *Intermediate* | 0 | 5.05 | 0.445[1] |
| | *High* | 0 | 5.275 | 0.484[2] |

[a] Mean is using limit of detection/2 for missing values

[1] t-test none vs. intermediate/high

[2] t-test none vs. high

**Table 3**

Comparison of Cytokine Levels Using a Mixed Effects Model

| | Level of SHS Exposure | | | Level of SHS Exposure | | |
|---|---|---|---|---|---|---|
| | **None** | **Any** | **Significantly Different?** | **None** | **High** | **Significantly Different?** |
| IL-1β | **0.554** | **0.249** | **Yes** | 0.465 | 0.183 | |
| IL-4 | 4.247 | 1.242 | | **8.141** | **0.135** | **Yes** |
| IL-6 | 3.518 | 1.896 | | 3.267 | 1.267 | |
| IL-8 | 5.787 | 5.204 | | 5.782 | 4.601 | |
| IL-10 | 16.070 | 15.680 | | 16.090 | 11.850 | |
| TNF-α | 9.282 | 10.210 | | 9.269 | 10.320 | |
| IL-5 | 0.640 | 0.340 | | 0.518 | 0.152 | |
| IFN-γ | **4.193** | **0.816** | **Yes** | 3.610 | 0.769 | |
| IL-13 | 0.420 | 0.016 | | 0.340 | 0.031 | |

[a]95% CI effect of smoking coefficient excludes no effect

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