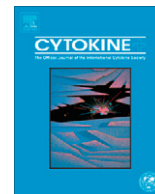


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Short Communication

Secondhand smoke exposure and serum cytokine levels in healthy children

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ABSTRACT

Introduction: Exposure to secondhand smoke (SHS) is associated with 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 Multiplex Assays 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-1 β , 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-1 β (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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1. Introduction

Secondhand smoke (SHS) exposure is 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], and otitis media [3]. 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 [4].

Abbreviations: SHS, secondhand tobacco smoke; IL, interleukin.

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The relationship between SHS exposure and the development of these diseases is not yet fully understood. Evidence from both human and animal models suggests that SHS exposure may increase 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. In one animal study, mice exposed to SHS had increased circulating levels of interleukin-1 β (IL-1 β), a pro-inflammatory cytokine [5]. In humans, exposure to SHS increases expression of inflammatory cytokines associated with the T_H2 response, including IL-4, IL-5 and IL-6 [6]. Elevated serum levels of these cytokines have been described for up to 3 h after exposure [6].

Children exposed to SHS have elevated upper airway secretions of IL-13 [7]. The elevation of pro-inflammatory 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. In older children, SHS exposure has also been shown to decrease levels of interferon- γ (IFN- γ) as detected from mitogen stimulated mononuclear cells; INF- γ is

involved in protection against viral and bacterial pathogens [8]. Suppression of immune responses may account for the increased prevalence and severity of illness in SHS-exposed children.

Despite the evidence that SHS exposure results in altered inflammatory states, the mechanism and scope of these changes are not fully understood, especially in children. Few studies have measured circulating cytokines in this population. 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 to determine (1) whether we could measure cytokine levels in the serum of healthy children, and (2) if there were differences between the cytokine levels of SHS exposed and non-exposed children.

2. Methods

2.1. 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 ages 1–6 years, undergoing routine same-day operative procedures. Patients who had illness symptoms, such as cough or rhinorrhea, were excluded from the study, as were patients with active medical conditions, including asthma, allergies, and eczema, or if anti-inflammatory medications were taken in the 2 weeks preceding the study. A total of 40 children were recruited for the study.

2.2. 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 after anesthesia induction. The blood samples were tested for cotinine using an enzyme-linked immunosorbent assay (ELISA), and cytokines (IL-1 β , IL-4, IL-5, IL-6, TNF- α , IFN- γ , CXCL8, IL-10, and IL-13) using a Luminex Multiplex Assay. Since this was an exploratory study, we chose the panel of cytokines based prior studies, or the potential for physiologic relationship. 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.

2.3. 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 patients with no SHS exposure to 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 patients with no SHS exposure and 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.

To assess differences in cytokine expression by SHS-exposure, a model was constructed 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

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%	
Otolaryngologic	12.50%	3.8%	28.6%	

Table 2a

Mean^a cytokine levels by SHS exposure.

	Level of SHS exposure			Level of SHS exposure		
	None	Any	p-value	None	High	p-value
IL-1 β	0.599	0.470	0.052	0.599	0.423	0.038
IL-4	16.483	12.273	0.083	16.483	9.565	0.038
IL-6	9.544	11.912	0.529	9.511	22.675	0.552
CXCL8	6.345	5.840	0.436	6.345	5.134	0.380
IL-10	19.484	22.783	0.960	19.484	13.584	0.222
TNF- α	9.722	10.428	0.470	9.722	10.470	0.516
IL-5	0.823	0.573	0.080	0.823	0.425	0.034
IFN- γ	10.368	3.350	0.036	10.368	3.209	0.036
IL-13	6.867	5.050	0.445	6.867	5.275	0.484

The bolded values are statistically significant at $p < .05$.

^a Mean is using limit of detection/2 for missing values.

Table 2b

Comparison of cytokine levels using a mixed effects model^b.

	Level of SHS exposure			Level of SHS exposure		
	None	Any	Significantly different?	None	High	Significantly different?
IL-1 β	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	
CXCL8	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	

^b 95% CI effect of smoking coefficient excludes no effect.

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 than that using the traditional method of substituting half the limit of detection [9].

The results in Table 2a present sample means and t-tests for the traditional method of substituting limit of detection/2 for unobserved values. The results in Table 2b 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, $\log(Y_{ij}) = \mu_j + \beta_j x_i + r_i + \varepsilon_{ij}$ for $i = 1, \dots, 40$ and $j = 1, \dots, 9$ where μ_j gives the unexposed (log-)mean expression level of the j^{th} cytokine, β_j gives the exposure effect, r_i are random effects for the children, and ε_{ij} are random errors. The r_i and ε_{ij} are all inde-

pendent and normally distributed with mean 0 and variances σ_r^2 and σ_j^2 , respectively, estimated from the data. All analyses were done using WinBUGS.

3. 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 operative procedures included 70% dental restorations, 17.5% endoscopies, and 12.5% otolaryngologic 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 children with any SHS exposure than the non-SHS exposed children (Table 2a). 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 2b). 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.

4. 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. 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, it is important to recognize 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 [10–12]. 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 older children exposed to SHS [8], and replicates this finding using an ELISA.

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 would thus have been 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.

While modest in scope, our findings hint at 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 may occur 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 [13]; incorporating information about cytokines and the risks of SHS may increase parents' motivation to protect their children from SHS.

4.1. Limitations

There are significant limitations to our study. Our sample is very 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 [14], it still raises the question of social desirability bias. Finally, many of the cytokine levels were below the limit of detection; while we used a conservative method for estimating the true distribution, we can't know for sure that we were able to do this accurately.

5. Conclusions

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 often incapable of removing themselves from the exposure; and thus should be protected from any exposure to tobacco smoke.

Authors' Contributions

KW participated in the study conception, design, analysis, interpretation of results, and manuscript revision. SP and SW participated in the acquisition and preliminary analysis of the data, as well as drafting and revising the manuscript. EW participated in

study conception and design, interpretation of results, as well as revision of the manuscript. TL and KE played a substantial role in data analysis and interpretation of the findings, as well as manuscript revision. AC was involved in the study conception and design.

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