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# Salivary cotinine levels in children with otolaryngological disorders

Christine M. Clark [a], Jillian N. Printz [a], Lauren E. Stahl [b], Brett E. Phillips [b], Michele M. Carr [c, *]

[a] The Pennsylvania State University, College of Medicine, 500 University Drive, Hershey, PA 17033-0850, USA
[b] Department of Surgery, The Pennsylvania State University, College of Medicine, 500 University Drive, Hershey, PA 17033-0850, USA
[c] Department of Otolaryngology-Head and Neck Surgery, West Virginia University, 1 Medical Center Drive, Morgantown, WV 26501, USA

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ABSTRACT

*Objective:* To determine if salivary cotinine, a biomarker for tobacco smoke exposure, is elevated more often or to a higher degree in children meeting criteria for tonsillectomy or tympanostomy tube insertion.
*Methods:* Saliva samples were obtained from 3 groups of children for salivary cotinine measurement. Group 1 served as healthy controls. Group 2 consisted of subjects meeting tympanostomy tube criteria. Group 3 consisted of patients meeting tonsillectomy criteria. Environmental tobacco smoke (ETS) exposure was defined as a salivary cotinine concentration ≥1.0 ng/mL. Demographic data, smoke exposure history, and co-morbidities were also determined.
*Results:* 331 patients were included, with 112 in Group 1, 111 in Group 2, and 108 in Group 3. No differences were encountered for smoke exposure by history or smoker's identity, salivary cotinine level, or frequency of positive cotinine results. 42.6% of Group 1 had positive salivary cotinine compared to 51.8% of Group 2 and 47.7% of Group 3. Group 1 had a mean salivary cotinine level of 2.42 ng/mL compared to 2.54 ng/mL in Group 2 and 2.60 ng/mL in Group 3. The frequency of positive cotinine levels was higher than expected based on parental history. Among subjects with positive cotinine levels, 93 had no ETS exposure, and 64 had ETS exposure by history.
*Conclusion:* Approximately 50% of children who undergo tonsillectomy and tympanostomy tube insertion have objective evidence of ETS exposure. Parental history underestimates passive smoke exposure, which can impact perioperative care.



## 1. Introduction

The deleterious health effects associated with secondhand smoke exposure in pediatric patients have been well described in the literature [1,2]. Much attention has been paid to the pulmonary complications stemming from this health risk; however, there are also detrimental otolaryngological consequences.

It has long been suspected that exposure to cigarette smoke is correlated with recurrent otitis media (OM) in children [3,4]. Secondhand smoke exposure has been correlated with a higher incidence of new episodes of OM during the first three years of life [5]. Those with passive smoke exposure who undergo tympanostomy tube placement for OM experience higher rates of postoperative complications [6].

The risks of secondhand smoke are not limited to otologic problems. Prior studies have demonstrated an association between environmental tobacco smoke (ETS) and conditions warranting tonsillectomy. Straight et al. showed that children who underwent tonsillectomy for recurrent tonsillitis were more likely to have been exposed to ETS than those who had hernia repair surgery [7]. Other studies have shown that parental smoking is linked to recurrent tonsillitis and tonsillectomy [8–10]. ETS exposure has also been shown to be associated with snoring, sleep disturbances, and obstructive sleep apnea (OSA) severity in children [11–13].

Passive exposure to smoke can be determined via subjective or objective methods. Patient and parental reporting of smoke exposure history have been found to be unreliable [14,15]. Thus, quantifiable measurement techniques, such as the measurement of salivary, serum, and urinary cotinine concentrations, have been established in order to provide a more accurate assessment of secondhand smoke exposure. Cotinine, the predominant

* Corresponding author. Department of Otolaryngology-Head and Neck Surgery, West Virginia University, 1 Medical Center Drive, Morgantown, WV 26501, USA.
*E-mail address:* mmcarr2001@gmail.com (M.M. Carr).

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metabolite of nicotine, is the biomarker of choice for both passive and active cigarette smoke exposure.

The goal of this study is to determine if elevated salivary cotinine levels are correlated with a higher incidence of recurrent tonsillitis or sleep-disordered breathing (SDB) requiring tonsillectomy. We also aim to compare the cotinine levels among these patients to those in healthy controls and children meeting criteria for tympanostomy tubes. Additionally, we hypothesize that parental reports likely under-represent children's actual second- or third-hand smoke exposure.

## 2. Material and methods

### 2.1. Subjects

Children less than 14 years of age were enrolled in this study in our outpatient academic otolaryngology clinic as well as a general outpatient academic pediatric clinic from June through September of 2016. Subjects were divided among three groups. Group 1 served as healthy controls, and these subjects had no history of recurrent OM, persistent OM with effusion, recurrent tonsillitis, or SDB and were not acutely ill at the time of sample collection. Group 2 was comprised of patients with a history of recurrent OM or persistent OM with effusion meeting criteria for tympanostomy tube insertion. Group 3 consisted of patients with recurrent tonsillitis or SDB meeting criteria for tonsillectomy. Criteria for surgery was consistent with American Academy of Otolaryngology-Head and Neck Surgery Clinical Practice Guidelines [16,17]. Children with a personal history of smoking or craniofacial anomalies and syndromes, including Trisomy 21, were excluded from this study. We determined that a sample size of 110 children per group would be sufficient to detect a difference of 25% in incidence between the control group and each of the study groups with a power of 85% and alpha of 0.05.

### 2.2. Data collection

The following demographic data were collected during each subject's outpatient clinic visit: gender, age, major medical comorbidities, history of secondhand smoke exposure, and smoker's identity. Salivary cotinine was used as a measure of ETS because it is interchangeable with plasma cotinine levels but noninvasive and easy to collect, making it more suitable for our pediatric cohort [18]. Saliva samples were obtained using SalivaBio Swabs (Salimetrics, PA). Following collection, samples were stored in sterile tubes that were labeled with each subject's corresponding study number. The tubes were subsequently placed in ice and transported to the lab, where they were stored at −20 °C until analyzed. Salivary cotinine levels were then measured in the lab by a qualified laboratory technician using a commercially available quantitative enzyme immunoassay per manufacturer's protocol (Salimetrics, PA). The assay's lower limit of sensitivity was 0.15 ng/mL, and all values below this cutoff were recorded as 0.0 ng/mL. Secondhand smoke exposure was defined as a salivary cotinine concentration of 1.0 ng/mL or higher as specified by the manufacturer. Those with cotinine concentrations below this cut-off were considered to have minor secondhand smoke exposure, and concentrations above it were consistent with significant exposure (Salimetrics, PA).

### 2.3. Statistical analyses

Data analysis was performed in SPSS 22 (IBM, Armonk, NY). Non-parametric Kruskal-Wallis tests were used to compare the frequencies and skewed continuous data among the three groups. Mann-Whitney U testing was performed in order to make pairwise comparisons between each group. Linear regression was also performed.

### 2.4. Ethical approval

The study design was approved by the Institutional Review Board at the Penn State Milton S. Hershey Medical Center. The purpose of the study and risks of involvement were explained to potential participants and their guardians, and signed informed consent was obtained from participants' guardians with signed assent from subjects over the age of 7 years.

## 3. Results

Three hundred thirty-four patients had saliva samples adequate for testing. Three were excluded because of overlapping diagnoses (1 in each group). Results are depicted in Table 1 and Fig. 1. Among the subjects in Group 2, 76.8% had recurrent OM, and 24.1% had persistent OM with effusion. Among Group 3 subjects, 69.4% had SDB, and 28.8% had recurrent tonsillitis. There were significant differences in patient age and otolaryngological diagnoses between the groups, as expected. There were significant differences in comorbidities between the groups. More children in Group 3 had asthma compared to Groups 1 and 2. Additionally, a higher proportion of subjects in Group 2 had developmental delay compared to the other two groups, and children in Groups 2 and 3 were more likely to be taking allergy medications than those in the healthy control group. There were no significant differences for gender. There were no differences in ETS exposure by history, smoker's identity, salivary cotinine level, or frequency of positive cotinine results. The frequency of positive cotinine tests was greater than expected from the history provided by parents.

Among the subjects with positive cotinine levels, 93 had no reported ETS exposure, and 64 had ETS exposure by history alone. Data are listed in Table 2. The only significant difference between these groups was that the cotinine level was lower in children with no ETS exposure by history.

Linear regression showed that the major determinant of salivary cotinine level was history of smoke exposure.

## 4. Discussion

Cotinine is the predominant metabolite of nicotine, and it provides an objective measurement of ETS exposure. It is present in the saliva, plasma, and urine. Salivary cotinine is preferred over nicotine as a measure of ETS exposure due to its long half-life of 15–19 h. It reflects recent ETS exposure of up to 4 days preceding sample collection. Salivary cotinine concentration increases with secondhand cigarette exposure in non-smokers, and the sample can be collected non-invasively [19,20]. Nicotine is present in various human foods; consequently, nicotine concentrations remain a relatively unreliable measure of ETS exposure [21]. Prior data have demonstrated that the mean salivary cotinine concentration from dietary sources alone is 0.022 ng/mL, which falls significantly below the limit of detection for analytical methods and makes salivary cotinine a more accurate representation of ETS exposure [22].

ETS exposure is a widely accepted risk factor for recurrent OM and OM with effusion. Previous studies have linked elevated serum and salivary cotinine with these entities. Strachan *et* al. showed that salivary cotinine trended to higher levels with increasing abnormality of tympanograms, suggesting that ETS exposure was related to Eustachian tube dysfunction. This study featured a group of 872 students, with only 69 of them having at least one flat