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The ubiquity of asymptomatic respiratory viral infections in the tonsils and adenoids of children and their impact on airway obstruction

Howard Faden [a, b, *], Vincent Callanan [b], Michael Pizzuto [b], Mark Nagy [b], Mark Wilby [b], Daryl Lamson [c], Brian Wrotniak [b, d], Stefan Juretschko [e], Kirsten St George [c]

[a] State University of New York, School of Medicine, Buffalo, NY, USA
[b] Women & Children's Hospital of Buffalo/Kaleida Health, 219 Bryant St., Buffalo, NY 14222, USA
[c] Laboratory of Viral Diseases, Wadsworth Center, New York State Department of Health, 120 New Scotland Avenue, Albany, NY 12208, USA
[d] D'Youville College, 320 Porter Ave., Buffalo, NY 14201, USA
[e] North Shore-LIJ Health Systems and Hofstra University School of Medicine, 500 Hofstra University, Hempstead, NY 11549, USA

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We would like to dedicate the article to Dr. Linda Brodsky (1952–2014) who spent her entire professional life at the Women and Children's Hospital of Buffalo working to improve the lives of children with airway disease. We would also like to acknowledge Dr. Brodsky's contribution to the standardization of tonsil size in airway obstruction.

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ABSTRACT

Background: Airway obstruction due to enlargement of tonsils and adenoids is a common pediatric problem resulting in sleep disordered breathing. The cause for the relatively abnormal growth of tonsils and adenoids is poorly understood.

Methods: Non-acutely ill children undergoing tonsillectomy and adenoidectomy (T&A) for various reasons were enrolled prospectively in a study to determine the frequency of asymptomatic respiratory viral infections in each lymphoid tissue and to relate the number and types of virus to the degree of airway obstruction. Molecular techniques were used to detect 9 respiratory viruses while Brodsky scores and measurements of percentages airway obstruction were used to estimate the degree of airway compromise due to the tonsil and adenoid, respectively.

Results: Viruses were detected in 70.9% of tonsils and 94.7% of adenoids, $p < 0.001$. Adenovirus was the most common virus detected at 71.1%. Adenoids had an average of 2.4 viruses compared to 0.92 for tonsils, $p < 0.001$. Higher Brodsky scores were only associated with EBV in tonsils, $p = 0.03$, while greater percentages of airway obstruction in the adenoids were associated with adenovirus, EBV, corona virus, parainfluenza virus and rhinovirus, $p \leq 0.005$.

Conclusions: Asymptomatic viral infections are common and directly related to the degree of airway obstruction significantly more often in adenoids than tonsils.

1. Background

Tonsils and adenoids are lymphoid organs located at the entrance way to the respiratory and alimentary tracts. Their location makes them ideal as the first line of defense against inhaled and/or ingested viruses and bacteria. The list of respiratory viruses known to cause upper airway infections is long and includes such common agents as adenoviruses (AdV), Boca virus, coronaviruses (CoV), enteroviruses (EV), Epstein Barr virus (EBV), human metapneumovirus (hMPV), influenza viruses (InfV), parainfluenza viruses (PIV), respiratory syncytial virus (RSV), and rhinoviruses (RV). Each of these viruses along with cytomegalovirus (CMV), human herpes viruses (HHV) 6–8, herpes simplex virus (HSV), human papilloma viruses (HPV), human parvovirus B19 and polyomaviruses (PoV) have been detected in tonsillar and adenoidal tissues of asymptomatic individuals [1–21]. As many as 97% of tonsils and adenoids reportedly contain a detectable virus

* Corresponding author. Division of Infectious Disease, Children's Hospital of Buffalo, 219 Bryant Street, Buffalo, NY 14222, USA.
E-mail addresses: hfaden@upa.chob.edu (H. Faden), vcallanan@kaleidahealth.org (V. Callanan), mikepizzuto@roadrunner.com (M. Pizzuto), nagys6@verizon.net (M. Nagy), mwilby@kaleidahealth.org (M. Wilby), daryl.lamson@health.ny.gov (D. Lamson), wrotniak@dyc.edu (B. Wrotniak), sjuretschko@nshs.edu (S. Juretschko), kirsten.st.george@health.ny.gov (K. St George).

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[7,8,13,18]. Co-infections with multiple types of virus have been observed. Approximately 80% of adenoidal tissues may contain multiple viruses while lower rates have been reported in tonsillar tissue ranging between 59% and 68% [7,8,13,18]. The rate of detection of viruses varies dependent on the diagnostic technique utilized, age of patient, virus type as well as season of year in which specimens were collected. It is almost certain that the number of microbes detected will continue to increase over time with improvements in the diagnostic tools employed. The effects of asymptomatic infections on tonsils and adenoids remain largely unknown.

The present study was designed to compare the frequency of asymptomatic infection with nine common respiratory viruses in the tonsil and adenoid and to determine the effect of asymptomatic infection on airway obstruction in children undergoing elective excision of the tonsil and adenoid.

2. Methods and materials

The study was prospective and approved by the Institutional Review Board. Parents and children above the age of 7 years were fully informed, and they signed consent and assent forms, respectively.

The study began 2/6/13 and was completed 1/21/14. Children between the ages of 1 and 16 years were enrolled by the authors at the Women and Children's Hospital of Buffalo and in their private office outside of the hospital. All surgeries were performed at the Women and Children's Hospital Buffalo. The study was open to children undergoing surgical removal of adenoids and/or tonsils for various reasons, regardless of gender, race or ethnicity. The tonsils were removed by monopolar cautery by two the surgeons and by the microbipolar technique by the third surgeon. In contrast all three surgeons used curettage to remove the adenoids. Children with craniofacial abnormalities and bleeding disorders were excluded. Data recorded included date of birth, gender, height, weight, BMI, weights of tonsils and adenoids and reason for surgery. The diagnosis of obstructive sleep apnea or sleep disordered breathing was a clinical diagnosis; however, the degree of airway obstruction was estimated by the Brodsky scores for the tonsils and percentages of airway obstruction for adenoids. None of the children exhibited symptoms consistent with an acute respiratory illness at the time of surgery.

Palatine tonsils or adenoids were obtained by the surgeons and forwarded to the Pathology Lab where they were weighted and stored at 2–8 °C before being processed by the Virology Lab; all samples were processed in the Virology lab within 24 h of collection. Detection of adenovirus and EBV was initially performed in the Virology Laboratory at the Women and Children's Hospital of Buffalo.

PCR was performed on DNA extracts from the tonsil and adenoid samples using the ABI 7500 Fast Real-Time Taqman PCR System with adenovirus-specific primers (Life Technologies) and probes (eurofins MWG Operon). Primer-BLAST software was utilized to assure primer specificity. Concurrent testing for RNaseP confirmed extraction efficiency and the absence of PCR inhibitors. A previous adenovirus positive respiratory sample, confirmed by DFA (Diagnostic Hybrids D3 Ultra) was extracted and used as a positive control. A nasopharyngeal swab negative for adenovirus was extracted and used as a positive control for RNaseP activity and a negative control for virus assay.

Samples were run in duplicate for both adenovirus and RNaseP. Samples that showed amplification for the target in only one well, or that had a Ct value for the target exceeding 40 cycles were repeated in duplicate. Repeated samples with target amplification curves in at least one well (regardless of Ct value) were considered positive. Repeated samples with no target amplification curves in both wells, and a valid RNaseP amplification were considered negative. There were no samples lacking RNaseP amplification and therefore no samples reported as inhibited. Further molecular studies were performed in the Laboratory of Viral Diseases at the Wadsworth Center, New York State Department of Health, Albany, New York, with conventional PCR and sequence analysis. The hexon and fiber genes were amplified, bi-directionally Sanger sequenced on an ABI3700 and analyzed using NCBI Blast analysis.

All other respiratory viruses were detected at the Wadsworth laboratory using the eSensor® respiratory viral panel kit (GenMark Inc., Freemont, CA.) which detects Influenza A H1 and H3, influenza A H1pdm09, influenza B, respiratory syncytial virus A and B, parainfluenza virus 1, 2 3, and 4, human metapneumovirus, human rhinovirus, adenovirus B/E and C, and coronavirus 229E, NL63, HKU1 and OC43 [22]. All results from the viral panel kit were confirmed by real-time PCR. In cases where rhinovirus could not be distinguished from enterovirus, it was classified as rhinovirus/enterovirus indeterminate.

Descriptive characteristics for patients were computed. Weights, Brodsky scores and percentages of airway obstruction were used as indicators of organ size. Categorical variables were reported as proportions in percentage and continuous level variables as means. Independent t tests were used to assess the relationship of virus frequency to Brodsky scores and percent of airway obstruction. The Brodsky scores were arbitrarily grouped into low scores of 1 and 2 or high scores of 3 or 4. Percent of airway obstruction was similarly grouped into low scores of 50% or less and high scores of greater than 50%. The independent *t*-test was used to assess the association between low and high Brodsky scores and nine virus types. The independent *t*-test was used to assess the association between low and high percentages of airway obstruction and the same nine viruses. The chi-square test was used to assess the association between the number of virus types and the Brodsky scores and the percentages of airway obstruction. Paired *t*-test was used to assess differences between the number of virus types between tonsils or adenoids. Pearson correlation was used to assess the association between age and total virus number types present. The association between age and total number of virus types was assessed by Pearson correlation. Analysis of variance was used to assess the association between Brodsky score and percent airway obstruction with the weights of tonsils and adenoids, respectively. All statistical tests were assessed assuming two-tailed hypotheses and with alpha of 0.05. All analyses were conducted with SYSTAT 13 (SYSTAT Software, 2004).

3. Results

Fifty-nine children were enrolled in the study ranging in age between one year and 16 years with a mean of 6.2 years. There were 35 females (59.3%). The BMI ranged from 12.9 to 49.4 kg/square meter with a mean of 17.4; 14 (23.7%) children were classified as either obese (eight) or overweight (six). Thirty-eight (62.7%) children underwent surgery for sleep disordered breathing (SDB), seven of whom developed complications of recurrent or chronic (ROC) otitis media and one with ROC tonsillitis. Twelve children underwent surgery for ROC tonsillitis alone. Four children underwent surgery for ROC adenoiditis with tonsillitis or ROC otitis media. A single patient underwent surgery for chronic adenoiditis. A total of 59 tonsils and 57 adenoids were excised. The mean weights of tonsils and adenoids were 6.7 and 2.0 g, respectively. Fifty-five tonsils and 57 adenoids were available for nucleic acid extraction.

Viruses were detected in 39 of 55 tonsils (70.9%) compared to 54 of 57 (94.7%) adenoids, $p < 0.001$. Multiple viruses were detected in