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Detection of high-risk human papillomavirus infection in tonsillar specimens using 2 commercially available assays☆,☆☆,★,★★

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ABSTRACT

The objective of the study is to determine the prevalence of high-risk human papillomavirus (hrHPV) infection in tonsillar swabs and tissue: Patients undergoing tonsillectomy for nonmalignant causes were enrolled. A flocked swab and fresh tissue were collected from the left and right tonsil of each patient. Specimens were tested for hrHPV DNA using the Roche cobas test and for the presence of E6/E7 messenger RNA using the Hologic Aptima hrHPV test.

Of the 193 patients enrolled, 129 were in the pediatric group (ages 1–12 years; median, 5 years), and 64 were in the adult group (ages 13–55; median, 22 years). All swab and tissue specimens were negative for hrHPV by both methods. Positive, negative, and internal controls performed as expected.

We found a 0% rate of infection indicating that detectable hrHPV infection in tonsillar tissue appears to be uncommon in the children and adults in the population sampled.

1. Introduction

Growing evidence suggests that infection with high-risk types of human papillomavirus (hrHPV), particularly HPV16, is associated with oropharyngeal squamous cell carcinoma (OPSCC) (D'Souza et al., 2007). The most recent data from North America indicate that up to 70% of these cancers are caused by HPV Mehanna et al. (2013). hrHPV is typically regarded by the public as a sexually transmitted infection associated with cervical cancer. However, based on current trends, it is estimated that, by the year 2020, the incidence of oropharyngeal cancer will surpass that of cervical cancer. In fact, over the past 15 years, the incidence of hrHPV-positive OPSCC has increased by 225% (Chaturvedi et al., 2011).

The oropharynx is composed of the tonsils, base of tongue, posterior pharyngeal wall, and soft palate. The tonsils are composed of full-thickness crypts that are lined by a reticulated squamous epithelium with an incomplete basement membrane, which allows the passage of lymphocytes and antigen-presenting cells. It is postulated that this porous basement membrane permits hrHPV to infect the tonsillar basal cells (Pai and Westra, 2009).

Despite a growing body of literature about hrHPV-related OPSCC, little is known about the natural course of oropharyngeal hrHPV infection. A large US-based study showed an overall oral HPV prevalence of 6.9% with 3.7% harboring a high-risk strain (Gillison et al., 2012). In this study, oral hrHPV infection was independently associated with age (30–34 and 60–64 years), male sex, increasing number of lifetime sexual partners, and current number of cigarettes smoked per day in multivariate models (Gillison et al., 2012). It is also unclear whether infection may occur with open-mouth kissing (D'Souza et al., 2009) or vertical transmission (Koskimaa et al., 2012). Fortunately, the majority of hrHPV infections will clear within 12 months (Kreimer et al., 2013), and only about 11,000 of the over 300 million Americans will develop HPV-related OPSCC each year (CDC, 2014a).

The majority of oropharyngeal cancers arise from tonsillar tissue. However, most oral hrHPV prevalence studies have consisted of an oral rinse and gargle, which sample the entire oral cavity and oropharynx. Few studies address the prevalence of hrHPV infection in the tonsillar tissue itself (Chen et al., 2005; Duray et al., 2011; Ernster

☆ All work was performed at the above address.

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et al., 2009; Mammas et al., 2006; Palmer et al., 2014; Ribeiro et al., 2006; Sisk et al., 2006; Syrjanen, 2004). Furthermore, existing tonsillar studies have used formalin-fixed, paraffin-embedded (FFPE) (Duray et al., 2011; Ernster et al., 2009; Mammas et al., 2006; Palmer et al., 2014; Syrjanen, 2004) or frozen tonsillectomy specimens (Chen et al., 2005; Palmer et al., 2014; Ribeiro et al., 2006; Sisk et al., 2006), and some studies used only tissue from a very small portion of the tonsil (Chen et al., 2005; Ribeiro et al., 2006) or mucosa surrounding the tonsil (Sisk et al., 2006) which may not have included the tonsillar crypt cells known to harbor hrHPV infection. We hypothesized that testing a large portion of fresh tonsillar tissue may provide improved detection of hrHPV infection.

Most existing tonsillar studies have also relied on in-house laboratory-developed polymerase chain reaction (PCR) methods for hrHPV detection. Laboratory-developed methods may vary significantly in accuracy, sensitivity, and ease of use and make it difficult to compare data between studies. Recently, nucleic acid amplification methods such as the Roche cobas® (Indianapolis, IN, USA) and Aptima® (Hologic, Bedford, MA, USA) hrHPV tests have become commercially available for detection of hrHPV genotypes and have been widely adopted by diagnostic microbiology laboratories in the United States, Canada, and Europe. Although these tests are Food and Drug Administration (FDA)–approved for use with endocervical specimens in PreservCyt® media, they may provide an option for hrHPV studies of the head and neck given that there is no universally accepted testing platform for oropharyngeal specimens.

Given the existing gaps in the literature, the aim of this study was to determine the prevalence of hrHPV infection in tonsillar swabs and fresh, nonfrozen benign tonsillectomy specimens in pediatric and adult patients using the Roche cobas® HPV test for detection of HPV DNA and the Hologic Aptima® HPV test for detection of HPV E6/E7 messenger RNA (mRNA).

2. Methods

2.1. Subjects

This study was performed at Mayo Clinic in Rochester, Minnesota. Prior to enrollment, the study was approved by the Institutional Review Board at Mayo Clinic. Subjects were recruited in an outpatient otolaryngology clinic setting. Inclusion criteria were patients undergoing routine tonsillectomy for hypertrophy, chronic tonsillitis, or sleep disordered breathing, ages 1–100 years old. The recruitment period was from July 2012 to January 2014. Exclusion criteria were subjects with a history of or clinical concern for oropharyngeal malignancy. Informed consent was obtained either from subjects or, in the case of minors, the subject's parent or guardian. Oral assent was obtained from children ages 7–12 years old. Participants were not informed of their test results. Age, indication for tonsillectomy, and sex were collected for each patient. Patients were stratified into 2 groups, pediatric (ages 1–12 years) and adults (ages 13 years and older) based on the age of potential sexual debut (Aral et al., 2005).

2.2. Specimen collection and processing

After induction of anesthesia and insertion of a mouth gag, a flocked swab (FLOQSwabs, regular, molded bp 100 mm; Copan Diagnostics, Corona, CA, USA) was used to swab both palatine tonsils, with particular attention to the crypt areas, and placed into Copan universal transport media. The tonsils were then removed in the standard fashion using electrocautery. Each tonsil was cut in half, labeled left or right, and sent to the laboratory for processing. The cauterized portion of the tonsil tissue was removed, and the remaining tissue was minced using a sterile scalpel blade, being sure to sample the tonsillar crypts. The specimens were then placed in separate 10-mL conical tubes, and 5 mL of 1× Tris-EDTA buffer, 500 μL of 10% sodium dodecyl sulfate, and 500 μL of proteinase K were added to each tube. Both tubes were placed in a thermomixer (Eppendorf, Hauppauge, NY, USA) at 550 °C and shaken overnight at 700 rpm. Dissolved tissue specimens were then diluted 1:10 in 1× Tris-EDTA buffer to prevent assay run failures that were observed on the cobas assay due to excess internal control target (human β-globin gene).

One milliliter of each patient's processed left and right tonsil tissues, as well as the tonsillar swab, was aliquoted into appropriate tubes for testing, for a total of 3 samples per patient for each assay (6 tests total per patient). Specimens were aliquoted into Aptima specimen transfer kit collection devices for the GenProbe Aptima assay and into sterile 13-mL (Sarstedt Newton, NC, USA) aliquot tubes (round bottom, 101 × 16.5 mm) for the Roche cobas HPV test.

2.3. HPV detection

Testing was performed using the Aptima HPV assay on the Tigris system (Hologic) and by the cobas HPV assay on the cobas 4800 system (Roche) according to the manufacturers' instructions (cobas® 4800 HPV Test US package insert; Aptima HPV Assay product insert, 2014).

The Aptima HPV assay detects hrHPV mRNA types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 and utilizes 3 main principles for hrHPV detection: 1) target capture, 2) target amplification of RNA sequences by transcription-mediated amplification, and 3) detection of the amplification products by the hybridization protection assay. The assay incorporates an internal control to monitor nucleic acid capture, amplification, and detection, as well as operator or instrument error (Aptima HPV Assay product insert, 2014). The manufacturer-determined lower limit of detectable DNA is 49 HPV 16+ SiHa (cervical SCC) cells/mL.

The Roche cobas HPV test detects hrHPV DNA types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 and utilizes 2 major processes for detection: 1) automated specimen preparation to simultaneously extract hrHPV and 2) cellular DNA and PCR amplification of target DNA sequences using target selection, target amplification, automated real-time detection, and selective amplification. The Roche cobas assay also includes an internal control targeting human β-globin (cobas® 4800 HPV Test US package insert). The manufacturer-determined lower limit of detectable DNA is 600 HPV 16+ SiHa cells/mL.

2.4. Protocol validation

Limit-of-detection studies were performed by preparing a 2-fold dilution series of the Acrometrix™ HPV-16 Genotype Control (Thermo Fischer Scientific, Benicia, CA, USA) SiHa cells in negative processed tonsillar specimens in Tris-EDTA (TE) buffer. The dilutions were then tested by the Roche cobas test and the Hologic Aptima HPV test.

To further validate the tonsillar protocol, 5 archived oropharyngeal cancer specimens that were positive for hrHPV by histology and immunohistochemical staining were obtained. These specimens had been freshly frozen at the time of initial collection and stored in a −150 °C freezer for approximately 1–3 years. The specimens were thawed and tested using the tonsillar protocol described above with the Roche cobas assay.

3. Results

3.1. Demographics

Informed consent was obtained for 234 subjects; however, specimens were not collected on 41 subjects, and therefore, 193 subjects were included in the final data analysis. The pediatric group comprised 129 subjects (42% female and 58% male) with an age range of 1–12 years (median age, 5 years). In our pediatric cohort, 6 patients (4.7%) had received the HPV vaccine, with 4 being fully vaccinated (received all 3 vaccinations). The adult group comprised 64 subjects