Detection of Human Papilloma Virus in the Tonsils of Children Undergoing Tonsillectomy

Kátia Moreira Xavier Ribeiro¹, Janaina Maria Alvez², Shirley S.N.Pignatari¹ and Luc Louis Maurice Weckx¹

¹Division of Pediatric Otolaryngology, ²Division of Infectious Diseases; Paulista School of Medicine, Federal University of São Paulo, SP, Brazil

Human papilloma virus (HPV) is related to respiratory mucosal diseases, such as recurrent respiratory papillomatosis, as well as to upper-respiratory-tract malignancies. There are few reports concerning the prevalence of HPV in the upper respiratory tract of non-affected individuals. We examined the prevalence of HPV in the tonsils of children of the general population scheduled for tonsillectomy. Samples were taken from the tonsils of 100 children undergoing tonsillectomy and were then tested for HPV with the polymerase chain reaction (PCR) technique, utilizing the generic primers MY09 and MY 11. The study excluded children known to have HPV and HIV-related diseases. Parents and legal guardians completed a standardized socio-demographic questionnaire. The questionnaire revealed that 84% of the mothers had at least one risk factor for genital HPV. None of the tonsil samples were positive for HPV. Apparently HPV does not commonly colonize the tonsils of children undergoing routine tonsillectomy.

Key Words: HPV, tonsils, children.

Human papilloma virus is currently classified as a DNA virus with more than 90 distinct types. These types are classified according to their relationship with benign and malignant lesions into low and high-risk HPV respectively [1]. HPV is also related to certain skin lesions and other lesions of the respiratory, genitourinary and gastrointestinal tracts. The skin lesions include common warts (verruca vulgaris), plantar warts (verruca plana) and verruciform epidermal dysplasia (epidemodyplasia verruciformis). In the respiratory tract, HPV is related to papillomatous lesions that occur from the nasal to the bronchial mucosa. In the larynx, this virus is responsible for recurrent respiratory papillomatosis (RRP) in adults and children. HPV also has been linked to several head and neck carcinomas [2-10]. Gastrointestinal tract manifestations include papillomas, focal epithelial hyperplasia and leucoplakia. In the genitourinary tract, HPV has been detected in cervical, vaginal and penile mucosa and in perianal skin. It is linked to the development of condyloma acuminatum, flat warts (verruca plana) and verruciform epidermal dysplasia (epidemodyplasia verruciformis). In the respiratory tract, HPV is related to papillomatous lesions that occur from the nasal to the bronchial mucosa. In the larynx, this virus is responsible for recurrent respiratory papillomatosis (RRP) in adults and children. HPV also has been linked to several head and neck carcinomas.

Material and Methods

This study was carried out at the University Hospital of the Federal University of São Paulo. Surgeons performed routine tonsillectomies on 100 children between ages 2 and 13 years and removed a small portion of the tonsils with a surgical scalpel. The physician then the specimen in a cryo-tube containing 5 mL of a media transport solution (tris-HCl 10mM, pH 7.5, EDTA 5 mM, Na Cl 150 mM and sodium azide 0.1%), and arranged for the specimen to be taken immediately to the Molecular Biology Laboratory for storage at minus 70° C.

Before participation in the study, the legal guardians of each child read, discussed and signed an informed consent. They also reviewed specific information about risks for the transmission of HPV and completed a standardized socio-demographic questionnaire designed to evaluate the presence or absence of risk factors. The study excluded any child known to be HIV positive, or having any HPV-related disease and any child born to an HIV carrier.

Laboratory procedures. DNA extraction was accomplished by using a commercial kit for extraction of nucleic acid from tissue “NucleoSpin Tissue Kits”, CLONTECH, by following the manufacturer’s procedures.

Polymerase Chain Reaction. 5mL of the extracted DNA was added to 45mL of buffer solution [1X], MgCl2 [1.25mM], dNTP (dATP, dCTP, dGTP, dTTP) [200mM/base], 4 pmol/ mL of each
Detection of HPV in Children

BJID 2006; 10 (June)

The presence of HPV in the oral cavity and upper respiratory tract mucosa is of great importance, since several studies have demonstrated an association of HPV with a great variety of benign and malignant lesions. Some of the most frequent benign lesions of the oral mucosa related to HPV are oral papilloma, focal epithelial hyperplasia and leukoplakias [13-15]. Frequency of HPV in head and neck cancer patients varies between 8 and 50%; it is believed that its detection may be dependent on several factors including the molecular technique utilized (Southern blot hybridization, PCR, in situ hybridization), the treatment of the sample material (fresh, frozen, paraffin embedded), ethnic and geographical differences, and the anatomical site of the lesion [2-10,13,18].

Because 60% to 64% of individuals with carcinoma of the tonsil have detectable HPV, some researchers have investigated the prevalence of HPV in the oral cavity mucosa [16,17]. An overview of the medical literature results of HPV detection in the oropharynx is shown in Table 1 [1,18-34].

One hundred children previously scheduled for tonsillectomy participated in this research. The size of the cohort was based on the number calculated to achieve statistical significance. Since the aim of the study was to determine the frequency of HPV in healthy children, we excluded all patients with HPV or HIV-related diseases, because HPV prevalence is known to be higher in these groups than in the general population [21,35]. The socio-demographic questionnaire included elements related to the most relevant risk factors for maternal genital HPV infection, including smoking and sexual history [35].

Most of the mothers had stopped their education before completing elementary school, which may mean a lack of knowledge about sexual diseases and preventive methods. The low median family income (approximately 300 US dollars a month) suggests the possibility of poor hygiene, nutrition and limited access to health care.

According to the literature [35], smoking is also a risk factor for HPV infection in women. Nearly 40% of the women were smokers. It is known that starting sexual activity and giving birth at an early age, having numerous sexual partners, and using oral contraceptives correlate with a higher risk for HPV infection [35]. In our study, maternal sexual activity started between 11 and 30 years of age (median 18 years); 9% delivered their first child before age 18; and the number of sexual partners ranged between 1 and 11. Thirty-eight of the mothers were regularly taking contraceptive pills.

Although sexually transmitted diseases were reported by only 5.4% of the mothers, this figure may underestimate the true prevalence because of inadequate medical information and access. It is not clear if delivery by caesarian section acts as a protective factor against HPV transmission. In our study, about 40% of the children were born via caesarian section. The final analysis of the risk factors showed that 84% of the mothers presented at least one risk factor for HPV infection.

The existing reports on the detection of HPV in swabs of oral mucosa and in the nasopharynx of children and newborns using PCR and DNA hybridization techniques show highly variable results, ranging from 0 to 73% [20,21,25-28,30,3-39]. In adults, the results are also variable, ranging from 5.2% to 81% [1,23,29,31-33,34,40]. This suggests that the oral mucosal swab may not yield a reliable sample for detecting HPV, regardless of the technique employed.

On the other hand, biopsies of normal oral mucosa in adults have revealed less variability, with PCR techniques detecting HPV in 3.8% [31] to 23.1% [32], and hybridization techniques...
detecting the virus in 15.6% [32], 41.6% [33] and 41% [34]. Based on these results, we elected to use tissue biopsies for our study.

The choice of PCR in-house generic primers MY09 and MY11 was based on the high sensitivity of this method, which allows amplification of small quantities of viral DNA, and also on its specificity compared to other techniques [41-43].

The negative results in all our samples may reflect the fact that when HPV is present in oral mucosa, it might not be due only to colonization, but could be mostly related to disease problems. Possibly, the tonsils are not a frequent site of colonization; concomitant oral swabs might have been useful. Further studies of other areas of the respiratory tract in healthy children and a long-term follow-up are warranted to clarify whether or not the presence of HPV in tissue is a risk factor for the development of HPV-related diseases. We also need to know whether hypertrophic or chronically infected tonsils are refractive to HPV infection.

References


Table 1. Literature findings of human papillomavirus detection in oral mucosa

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age</th>
<th>Site</th>
<th>Samples</th>
<th>Technique</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>[18]</td>
<td>Adults</td>
<td>Mouth</td>
<td>Biopsy</td>
<td>Hybridization</td>
<td>41.6%</td>
</tr>
<tr>
<td>[19]</td>
<td>Adults</td>
<td>Mouth</td>
<td>Biopsy</td>
<td>Hybridization</td>
<td>41%</td>
</tr>
<tr>
<td>[20]</td>
<td>Children</td>
<td>Mouth</td>
<td>Swab</td>
<td>PCR</td>
<td>24% &amp; 19%*</td>
</tr>
<tr>
<td>[21]</td>
<td>Newborns</td>
<td>Naso-oropharynx</td>
<td>Suction</td>
<td>Hybridization</td>
<td>2.8%</td>
</tr>
<tr>
<td>[22]</td>
<td>Adults</td>
<td>Mouth</td>
<td>Biopsy</td>
<td>PCR</td>
<td>3.1%</td>
</tr>
<tr>
<td>[23]</td>
<td>Adults</td>
<td>Mouth</td>
<td>Swab</td>
<td>PCR</td>
<td>43%</td>
</tr>
<tr>
<td>[24]</td>
<td>Adults</td>
<td>Mouth</td>
<td>Biopsy</td>
<td>PCR</td>
<td>23.1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hybridization</td>
<td>15.6%</td>
<td></td>
</tr>
<tr>
<td>[25]</td>
<td>Children</td>
<td>Mouth</td>
<td>Swab</td>
<td>PCR</td>
<td>31.6%</td>
</tr>
<tr>
<td>[26]</td>
<td>Children</td>
<td>Mouth</td>
<td>Swab</td>
<td>PCR</td>
<td>35.4%</td>
</tr>
<tr>
<td>[27]</td>
<td>Children</td>
<td>Mouth</td>
<td>Swab</td>
<td>Hybridization</td>
<td>41.6%</td>
</tr>
<tr>
<td>[28]</td>
<td>Children</td>
<td>Mouth</td>
<td>Swab</td>
<td>PCR</td>
<td>0%</td>
</tr>
<tr>
<td>[29]</td>
<td>Adults</td>
<td>Mouth</td>
<td>Swab</td>
<td>PCR</td>
<td>81.1%</td>
</tr>
<tr>
<td>[30]</td>
<td>Children</td>
<td>Mouth</td>
<td>Swab</td>
<td>PCR</td>
<td>51.7%</td>
</tr>
<tr>
<td>[31]</td>
<td>Adults</td>
<td>Mouth</td>
<td>Swab</td>
<td>PCR</td>
<td>0.6%</td>
</tr>
<tr>
<td>[32]</td>
<td>Adults</td>
<td>Mouth</td>
<td>Swab</td>
<td>PCR</td>
<td>2.4%</td>
</tr>
<tr>
<td>[33]</td>
<td>Adults</td>
<td>Mouth</td>
<td>Swabs</td>
<td>PCR</td>
<td>51.1%</td>
</tr>
<tr>
<td>[34]</td>
<td>Adults</td>
<td>Mouth</td>
<td>Biopsy</td>
<td>PCR</td>
<td>26%</td>
</tr>
</tbody>
</table>


