Prevalence of Oncogenic Human Papillomavirus 16 and 18 in the Palatine Tonsils of the General Adult Population

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Objective: To determine whether there has been a demonstrable increase in the prevalence of human papillomavirus (HPV)-infected palatine tonsils corresponding to the increase in incidence of HPV-positive oropharyngeal squamous cell carcinoma (SCC) over time.

Design: Review of archived, paraffin-embedded, noncancerous palatine tonsils.

Setting: A single institution in El Paso County, Colorado.

Patients: Age- and sex-matched patients 21 years and older from 2 different periods: January 1, 1979, to December 31, 1982, (group A) and January 1, 1997, to December 31, 2001 (group B).

Main Outcome Measures: Prevalence of oncogenic HPV-16 and HPV-18 in noncancerous palatine tonsils in relation to the incidence of HPV-positive oropharyngeal SCC.

Results: All specimens in both groups were negative for HPV-16 and HPV-18. Thus, the prevalence of HPV infection in the palatine tonsils of the general adult population was zero in both group A and group B.

Conclusions: This analysis shows a low prevalence of HPV infection in the palatine tonsils of the general adult population in a single county in Colorado known to have an increasing rate of HPV-positive oropharyngeal SCC. Analysis of oropharyngeal tissues from individuals at highest risk of developing HPV-positive oropharyngeal SCC (middle-aged men) is likely to provide a higher prevalence rate.

associated with the clinical finding of chronic tonsillitis, obstructive adenotonsillar hypertrophy, or obstructive sleep apnea. This tissue had been removed surgically between the periods January 1, 1979, to December 31, 1982, and January 1, 1997, to December 31, 2001. Before we engaged in this review, we obtained permission from the institutional review board of the above-mentioned hospital system. We consulted the privacy officer of the same institution to ensure compliance with Health Insurance Portability and Accountability Act regulations. All specimens were deidentified. We found pathology specimens by pathology number and then recorded patient age, patient sex, and histologic findings from the pathology reports. We chose specimens based on an age of 21 years or older, both sexes, and benign histologic findings. Complete clinical history was unavailable. We divided the specimens by age into 2 subgroups: 21 through 35 years of age and 36 year or older. Because there was a predominance of tonsils in women in the younger group, and in an effort to keep specimens from each sex relatively equal, we excluded half of the specimens from women in the 21-through 35-years age group. Because the specimens were sequentially listed in the pathology records, this result was accomplished by the elimination of every other specimen from women. The paraffin blocks were obtained and the tissue examined for quality as criterion for inclusion. The DNA was extracted, and DNA-PCR analysis for HPV-16 and HPV-18 was undertaken using type-specific primers.

DNA EXTRACTION

Specimen scrolls were taken from the cross-sections of the tonsils, which on gross inspection included both crypt and surface epithelia and the underlying lymphoid tissue. Paraffin-embedded tissue DNA was isolated using the Puregene DNA Purification Kit (Qiagen, Germantown, Maryland) extraction method for 5- to 10-mg paraffin-embedded tissue with a few minor adjustments. Concentration of the DNA was measured using spectrophotometry (Beckman DU 640 Spectrophotometer; Beckman Coulter, Fullerton, California) and diluted to approximately 50 ng/μl for the PCR reactions. The technique is more fully described in a previous work.3

ISOLATION AND AMPLIFICATION OF THE HPV GENOME

Qualitative type-specific PCR was used to test for HPV-16 and HPV-18 in all samples with a technique previously described.3 All primers used in the PCR were purchased from Integrated DNA Technologies, Inc (Coralville, Iowa). The human prothrombin gene and the β-globin gene were used as internal controls. Only samples with internal control positivity for both genes were used in the study. Although some of the samples were more than 25 years old, this practice allows us to use only reliable DNA material.

STATISTICAL ANALYSIS

The specimens were chosen by date of surgical procedure from 2 periods: January 1, 1979, to December 31, 1982 (group A), and January 1, 1997, to December 31, 2001 (group B). The hypothesis was that specimens that contained HPV positive material (designated HPV positive) would be proportionally greater in group B than in group A. This hypothesis may be represented as (1) an HPV-positive proportion in group A greater than or equal to the HPV-positive proportion in group B and (2) an HPV-positive proportion in group A less than the HPV-positive proportion in group B. In this manner of presentation, we intend to refute the first theory and show the second theory is plausible. Group A and group B were then subdivided by age and sex. The subcategories by age were previously defined herein. The groups were then matched by age and sex as well as the study material would allow.

RESULTS

We identified 119 specimens in group A and 107 specimens in group B. Table 1 demonstrates the age and sex distribution in each period. We were unable to disprove the first theory in this analysis because no specimen demonstrated the HPV genome. The prevalence in both study groups was zero. From this sample analysis, the prevalence of oncogenic HPV-16 and HPV-18 oropharyngeal infection is presumed to be less than 1 in 119 (0.84%) in the 1979 to 1982 period and less than 1 in 107 (0.95%) in the 1997 to 2001 period in El Paso County, Colorado.

This study was conducted with the same testing methods as our previously reported analysis on the presence of the HPV-16 and HPV-18 in a group of 72 patients with oropharyngeal SCC.1 In that analysis, 50 specimens (69%) tested positive for the presence of HPV-16. For that reason and on the basis of the knowledge that our controls showed quality DNA, we believe these results are valid.

COMMENT

The incidence of oropharyngeal SCC is increasing in the United States.3 One potential way to explain this increase would be to demonstrate an increasing prevalence of oncogenic HPV in palatine or lingual tonsil tissue over time. In this study, we were not able to demonstrate an increase in prevalence. In fact, we found none of the specimens contained the HPV-16 or HPV-18 genome in either period.

This finding differs from those of several other studies in which oncogenic HPV was identified in paraffin-embedded palatine tonsil tissue by PCR, in situ hybridization, or Southern blot testing. Syrjanen7 summarized several studies and determined a 6.0% (12 of 200) HPV positivity rate in archived paraffin-embedded palatine tonsil tissue. The geographic sites reported in this analysis were the United States, Japan, and Western Europe. A
Table 2. Summary of Studies to Determine Human Papillomavirus Oropharyngeal Prevalence by the Use of Archived Pathology Tissue and Genetic Analysis (Polymerase Chain Reaction or In Situ Hybridization)

<table>
<thead>
<tr>
<th>Source</th>
<th>Age, y</th>
<th>No. (%) of Patients Testing Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syrjänen et al.</td>
<td>≥21</td>
<td>12/200 (6.0)</td>
</tr>
<tr>
<td>Marmas et al.</td>
<td>&lt;21</td>
<td>6/106 (5.7)</td>
</tr>
<tr>
<td>Sisk et al.</td>
<td>&lt;21</td>
<td>0/50 (0)</td>
</tr>
<tr>
<td>Chen et al.</td>
<td>&gt;4</td>
<td>13/206 (6.3)</td>
</tr>
<tr>
<td>Current study</td>
<td>≥21</td>
<td>0/226 (0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>31/788 (3.9)</td>
</tr>
</tbody>
</table>

Table 3. Summary of Studies to Determine Human Papillomavirus Oropharyngeal Prevalence by the Use of Exfoliated Cells From a Brushing or Rinsing Technique and Genetic Analysis (Polymerase Chain Reaction or Hybrid Capture Technique)

<table>
<thead>
<tr>
<th>Source</th>
<th>Age, y</th>
<th>No. (%) of Patients Testing Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hansson et al.</td>
<td>&gt;21</td>
<td>3/320 (0.9)</td>
</tr>
<tr>
<td>D’Souza et al.</td>
<td>&gt;21</td>
<td>8/200 (4.0)</td>
</tr>
<tr>
<td>Smith et al.</td>
<td>&lt;21</td>
<td>10/1235 (0.8)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>21/1755 (1.2)</td>
</tr>
</tbody>
</table>

Greek study found 6 of 106 tonsils (5.7%) from children to be HPV-16 positive. A recent study from the United States revealed a 0% HPV-16 positivity rate in 50 children. A Scandinavian report found 13 of 206 tonsils (6.3%) to be HPV-16 positive, but 1 of the 13 specimens were from children or young adults younger than 25 years. The significance of the identification of oncogenic HPV in the tonsils of children is not known but raises the possibility that orogenital sexual contact may not be the only way oncogenic HPV is acquired in the oropharynx. Table 2 lists the available studies on prevalence determined by this method. Overall, these studies suggest that there is a 3.9% prevalence of oncogenic HPV in normal tonsils of the general population.

Another way to determine the prevalence of oncogenic HPV in noncancerous oral and oropharyngeal tissue is to use PCR analysis or a hybrid capture technique of genetic material acquired by screening methods, such as oral rinses or brushing. A recent Swedish study investigated 320 control patients by collecting oral rinse samples and using PCR to determine the presence of oncogenic HPV genomic material. They found 3 patients who tested positive for HPV, for a prevalence of 0.94%. D’Souza et al. found a 4.0% (8 of 200) prevalence of HPV-16 in a control group of 200 patients as determined by quantitative real-time PCR in which the samples were obtained by a brushing technique. Smith et al. studied a large group of children younger than 21 years and found HPV material in 10 of 1235 individuals. These studies are summarized in Table 3.

Another potential method of prevalence determination is that of serologic identification of antibodies to HPV oncoproteins (E6, E7, or L1) or to HPV-like particles. Human papillomavirus oncoproteins are believed to be present only when oncogenesis has occurred, and thus they would not be suitable as infection markers. HPV-like particles are present after HPV infection, but the antibody response is dependent on viral copy number and persistence. Therefore, neither serologic approach is reliable as a marker for HPV infection as an isolated testing method.

Lastly, Hobbs et al. recently reported a technique in which tonsil T-cell immunity was ascertained by a T-cell proliferation assay technique. In their analysis, noncancerous tonsil specimens demonstrated a heightened proliferation response to HPV-16 antigen, which suggests prior exposure to the virus. This is a labor-intensive test that is useful in the determination of prior HPV exposure but is not applicable to large population studies.

The factors that may influence the determination of HPV prevalence in a population are the accuracy of the testing methods and the regional or demographic differences in the populations studied. Although PCR analysis is a sensitive test, it is dependent on the relative amounts of tissue analyzed when 2 different types of cells are present. The HPV genome is found in the epithelial, not the lymphoid, portion of the study specimen. We grossly assessed the paraffin-embedded tissue and obtained complete cross-sections of the tonsils, including the epithelium and the underlying lymphoid tissue. We did not microdissect the specimen, and, therefore, both epithelial and lymphoid tissues were analyzed. This practice may have produced a disproportionately high lymphoid tissue to epithelium ratio, which could dilute the viral genome.

The use of fresh tonsillectomy specimens would provide the most reliable genetic material for both PCR and in situ hybridization. However, fresh specimen analysis has the inherent drawback of not easily allowing historical tissue analysis.

Although the population we studied, from El Paso County, Colorado, has experienced an increasing incidence of HPV-positive oropharyngeal SCC, the incidence rate is still low (average annual incidence of 3.47 per 100,000 population in Colorado from January 1, 1991, to December 31, 2001) compared with the United States as a whole (average annual incidence of 13.93 per 100,000 population in US men older than 50 years from January 1, 1991, to December 31, 2001). In addition, we studied all adults older than 21 years and both sexes. Studying populations in regions of the United States and specific demographic groups with the highest rates of HPV-positive oropharyngeal SCC will likely yield a higher prevalence rate of oncogenic HPV infection in the oropharynx.

Future investigations into the prevalence of oropharyngeal HPV infection should consider 2 observations from our study. The first is methodologic. When PCR is used, investigators should obtain epithelial tissue from the study specimen for genetic analysis. Microdissection of the specimen to achieve this objective is advised. The second recommendation is epidemiologic in nature. We recommend focusing on the high-risk groups in which the prevalence of oncogenic HPV infection is...
likely to be the highest. On the basis of recent studies regarding the changing incidence of HPV-positive oropharyngeal SCC, men with a mean age of 53 years are the most likely to manifest this disease process. It is likely that this demographic group, and particularly those with a remote history of frequent orogenital sexual contact, will manifest the highest prevalence of oncogenic HPV infection of the oropharynx.

With a clear understanding of the prevalence of oncogenic HPV in a specific population, an estimate of the progression rate from HPV infection to HPV-positive carcinoma may be derived. This effort could help guide screening and prevention strategies in the future.

In conclusion, this analysis provides insight into the prevalence of oncogenic HPV infection of the oropharynx in the general adult population. We found no specimens that tested positive for HPV-16 or HPV-18 in 119 specimens from January 1, 1979, to December 31, 1983, and 107 specimens from January 1, 1999, to December 31, 2001. This study was conducted in a community where the rate of HPV-positive oropharyngeal SCC has been shown to be increasing since 1995.3 Future investigators of HPV prevalence may consider the microdissection of oropharyngeal specimens to preferentially obtain epithelial tissue and the study of demographic groups that are at highest risk of harboring oncogenic HPV in the oropharynx.

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REFERENCES