Human Papillomavirus Infection in Oral Cavity and Oropharyngeal Cancers: Are They the Same Story?

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** Background:** Nowadays, human papillomavirus (HPV) infection becomes the main risk factor for head and neck cancer development. In Thailand, the related role of this viral infection to head and neck cancer is still unknown and not well established.

**Objective:** To identify different characteristics of oral cavity and oropharyngeal cancer, and to determine the HPV-associated prevalence of these two tumor types in Thailand, which is unlike the Western countries.

**Material and Method:** Between 2010 and 2012, a cross-sectional study was performed in 23 oral cavity and 23 oropharyngeal cancer patients. HPV genome was studied in all of them from pathological confirmed fresh specimens. Risks of HPV infection were collected using self-reported questionnaire.

**Results:** The prevalence of HPV-related oropharyngeal cancer was significantly noted in 26.09% (p = 0.009), while no demonstrable HPV-associated prevalence in oral cavity cancer. In addition, the routes of HPV infection were not identifiable.

**Conclusion:** Oral cavity and oropharyngeal cancers are not only anatomically distinct, but also greatly differed in their characteristics and pathophysiology. The percentage of HPV-related tumors in Thailand is considerably low when compared to the Western countries. However, the impact on treatment modification cannot yet be universally applied.

**Keywords:** Human papillomavirus, Oral cavity, Oropharynx, Head and neck cancer, Thailand, Asia, HPV infection, Oral cancer, Oropharyngeal cancer


Full text, e-Journal: http://www.jmatonline.com

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Head and neck cancer is biologically a pathophysiological group of heterogeneous neoplasm localized in the same region. Epstein-Barr infection is a major carcinogenic factor in the nasopharynx, but not in other subsites of head and neck cancer. While there is a relatively high prevalence of oral cavity and pharyngeal cancers in Asian countries, human papillomavirus (HPV) infection has had a major impact on treatment for both types of cancers in several Western countries[1].

In 2010, a hallmark paper by Ang et al[2] revealed different spectrum of diseases between HPV- and tobacco-associated oropharyngeal cancer, with treatment results subcategorized into low, intermediate, and high risk groups. Despite a favorable prognosis of HPV-associated oropharyngeal cancer, HPV status in overall oropharyngeal cancer needs to be identified, especially in regions with high prevalence of this oncovirus. In Southeast Asia where HPV is not prevalent[3], the impact of HPV infection is still inconclusive.

Currently, the prevalence of HPV detection in each subsite of head and neck squamous cell carcinoma (HNSCCA) and its route of infection are dissimilarly reported[4], possibly due to differences in life style and carcinogen exposure. Anti-smoking policies may successfully decrease the HNSCCA incidence in several Western countries; whereas, betel nuts are still important carcinogens in some Asian countries. Meanwhile, the mandatory identification of HPV effecting to patients at risk can likely improve the quality and outcome of HNSCCA treatment in many other countries, but not practically applied in Thailand. Moreover, many ongoing trials on de-escalation of treatment in HPV positive cancer may not be applicable in developing countries. In the present study, the role of HPV infection in HNSCCA carcinogenesis and its association with oral cavity and oropharyngeal cancers would be thus clearly identified and determined specifically in Thai population.
Material and Method

The present cross-sectional study was approved by the Institutional Review Board (IRB). Patients, aged >20 years, diagnosed as oral cavity and oropharyngeal cancers without any prior treatment between January 2010 and December 2012 were included. As defined, oral cavity extends from the lips to the junction of hard and soft palates, consisting of lips, oral tongue, floor of mouth, hard palate, buccal mucosa, gum, and retro-molar area. While, oropharynx covers the area of posterior to circumvallate papillae with four subsites including soft palate, tonsils, base of tongue, and pharyngeal wall. Primary cancer of unclearly identified epicenter was excluded.

A medical record form of easy Thai-language questionnaire was divided into two parts, 1) demographic data, clinical information, tobacco use, alcohol, and betel nut consumption, 2) the concealed self-administered data about the risks of HPV infection. All questionnaire questions were adapted from the sexually transmitted disease evaluation questionnaires(6,7), including number of lifetime sexual partners, age of the first sexual activity, history of oral sex, and previous sexually transmitted diseases. The risks of HPV infection could be identified from the responded data in the second part of the questionnaire.

A sample size of 23 patients was calculated from the prevalence of 39% HPV positive HNSCCA reported by Termine et al(5). Following the sample size equation, the margin of error for estimated prevalence was 0.2 and the type I error was 0.05. Statistical analysis was performed using SPSS program version 19. A Chi-square test was used to compare demographic data between two anatomical regions. Risks of HPV infection were analyzed using Fisher’s exact test. A p-value <0.05 was considered statistically significant.

DNA extraction was done from histologically confirmed fresh specimens, with subsequent PCR of linear array hybridization for specific HPV testing (Roche USA) in all 46 specimens. Biotinylated DNA was used as a primer for the genotypic test to detect 37 sub-types of HPV including type 6, 11, 16, 18, 26, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6108. The sensitivity and specificity of testing were 96% and 99% respectively(8). Oncogenic types of HPV included type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 73(9,10).

Results

There were 23 patients in each group, and 93.5% (43/46) did the second part of the questionnaire (risk of HPV infection). Remarkably, the prevalence of oral cavity cancer in the present study was predominant in women despite the more commonly found head and neck malignancy in men. Whilst, more advanced stages of oropharyngeal cancer were present due to early regional lymph node metastases. Patients’ characteristics were shown in Table 1.

The present study revealed 26.09% (6/23) HPV positive in the oropharyngeal cancer group. Of these six patients, there were three HPV type 11, two HPV type 16, and one HPV type 26. The found HPV16 positive cancer was tonsillar in origin. Nonetheless, no HPV DNA was detected in the oral cavity group, even in the non-smokers-non-drinkers.

Regarding the risks of HPV infection, the six HPV positive patients denied having any orogenital

<table>
<thead>
<tr>
<th>Variable</th>
<th>Oropharyngeal CA (n = 23)</th>
<th>Oral cavity CA (n = 23)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean ± SD</td>
<td>57.7±10.99</td>
<td>61.5±18.41</td>
<td>0.02</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>Male</td>
<td>20 (87.0)</td>
<td>9 (39.1)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3 (13.0)</td>
<td>14 (60.9)</td>
<td></td>
</tr>
<tr>
<td>Risk, n (%)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol</td>
<td>23 (100)</td>
<td>7 (30.4)</td>
<td></td>
</tr>
<tr>
<td>Tobacco</td>
<td>23 (100)</td>
<td>13 (56.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Betel nut</td>
<td>2 (8.7)</td>
<td>3 (13.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>HPV, n (%)</td>
<td>6 (26.1)</td>
<td>0</td>
<td>0.009</td>
</tr>
<tr>
<td>High risk HPV</td>
<td>2 (8.7)</td>
<td>0</td>
<td>0.489</td>
</tr>
</tbody>
</table>

CA = cancer; HPV = human papillomavirus

<table>
<thead>
<tr>
<th>Risk of HPV infection</th>
<th>HPV positive (n = 6)</th>
<th>HPV negative (n = 17)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of first sexual activity (17-30)</td>
<td></td>
<td></td>
<td>0.643</td>
</tr>
<tr>
<td>≤20 years</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>&gt;20 years</td>
<td>3</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Sexual partner</td>
<td></td>
<td></td>
<td>0.318</td>
</tr>
<tr>
<td>Multiple partner</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Single partner</td>
<td>3</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Oral sex</td>
<td></td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>History of sexually transmitted diseases</td>
<td></td>
<td></td>
<td>0.539</td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Characteristics of oral cavity and oropharyngeal cancer

Table 2. Risk of HPV infection
contact or previous sexually transmitted disease infection. While four of them were at risk for HPV infection, they were not associated with the presence of HPV DNA in oropharyngeal specimens. Specifically, no evidence of viral DNA was shown in the oral cavity tissues.

**Discussion**

Due to the successful anti-smoking policies, there is a downtrend in the overall incidence of head and neck cancer in developed countries. In the United States, tobacco smoking population decreased from more than half of the adult men to less than 25% during the past fifty years (11). Nevertheless, tobacco and alcohol consumption are still the major risks of head and neck cancer in developing countries, with smoking-related oral cavity and oropharyngeal cancer having been reported in 65% of the cases (12). The present study showed the same percentage of tobacco use among the oral cavity cancer patients. However, the smoking rate was much higher in the oropharynx group. In particular, approximately 1/3 of the oral cavity cancer patients were noted without identifiable risk of cancer development.

According to the WHO report 2013, the percentage of Thai male smokers was twice as high when compared with the US. Despite the successful anti-smoking campaigns, such as anti-tobacco on mass media, health warning pictures on tobacco packages, bans on tobacco advertising, and increasing tax on commercial tobacco products, their effects have not yet been identifiable. Moreover, in the population with low level of education, there is a higher prevalence of hand-rolled cigarette and areca nut consumption. Probably, the primary prevention of tobacco associated cancer in Thailand may not yet have a notable in these recent years.

During the past two decades, the prevalence of HPV related oropharyngeal carcinoma has increased more than three folds despite the lower incidence of overall head and neck cancer (13,14). The characteristics of the continuous mucosal lining two sites are greatly different. While palatine and lingual tonsils of tongue base are parts of Waldeyer’s ring, which mostly composed of lymphoepithelial tissues, oral cavity are composed of ciliated columnar epithelium. Hence, it is likely that HPV related oropharyngeal cancer originates from tonsils and base of tongue, with rather small primary sites. Therefore, the epicenter of the tumors could be hard to define. In the present study, the primarily originated oropharyngeal cancer patients tended to present in advanced T stages that involved other nearby subsites, such as nasopharynx, oral tongue, and hypopharynx with more extensive tumors and different carcinogenesis. In a bid to have representative tissues for further study, the inclusion criteria for primary sites of oropharyngeal cancer should be redefined to balance between contaminations from other sites and selective bias.

Following many reports about HPV as a causation of oral cavity cancer (15,16), our study showed a distinction between oral cavity cancer and oropharyngeal cancer, with no detection of HPV DNA in oral cavity cancer patients, similar to the results reported by others (17-20). One rationale for the discrepancy was on how the specimens to be included in each study. As mentioned earlier, the ambiguous term “oral cancer” could be either oral cavity cancer and/or oropharynx cancer. If the tumor involves both oral tongue and base of tongue, it is a question of which primaries should be classified. Practically, those tumors should be biopsied transorally and classified as oral cavity cancer. This is the pitfall in several studies without strict inclusion criteria. There is a probability in retrospective studies that cross contamination commonly occurs between these two regions. Thus, the reported studies on HPV in oral cavity cancer specifically from countries with different cancer registration systems (21,22) might yield equivocal results because the oral tongue and base of tongue are sometimes defined as the same entity (5,21,23).

A detected viral DNA may be involved in carcinogenesis or be only a bystander infection (22). Because only 50% of HPV positive oral cavity cancers express viral mRNA (25,26), molecular markers indicating an oncogenic property of HPV such as expression of viral E6/E7 oncogene is needed (24). Particularly, over expression of p16 protein, as a surrogate marker of transcriptionally active HPV in oropharynx, is not a reliable marker for oral cavity cancer. As suggested in several studies, the overexpression of p16 in oral cavity cancer is caused by non-viral mechanism (27-29). Our findings thus revealed no effects of HPV on oral cavity cancer even in patients without other identifiable risk factors.

Conversely, we detected 26% of HPV DNA for oropharyngeal cancer in subjects with both low and high-risk of HPV infection. Subsequent genotypic study showed only two (8.67%) cases of type-16 HPV positive tumor. HPV-related oropharyngeal cancer was relatively low when compared with data in developed countries. Nevertheless, it is comparable to the mostly
less than 10% from other studies in developing countries\(^{(30-33)}\). As a result of a better prognosis, many experts have suggested to include HPV status for a new staging system. Nonetheless, TNM classification without HPV status may not represent a true prognostic indicator. Meanwhile, de-escalation protocols for treatment of HPV-related oropharyngeal cancer have been proposed to decrease treatment complications. Up until now, it should only be applied in clinical trials with careful monitoring, especially in regions with low HPV prevalence.

As a sexually transmitted disease, presence of HPV infection in oral cancer could relate to high-risk sexual behaviors, including early age of sexual debut, multiple lifetime sexual partners, oral sexual practice, and history of other sexually transmitted diseases\(^{(34,35)}\). Among men denying having oral sexual contact, open mouth kiss could also substantially increase the risk of oral HPV infection\(^{(34,36)}\). In Thailand, there has been no association between these high-risk behaviors and HPV in oral cancer, probably due to the low prevalence of HPV. Our result is concurred by other studies with different demographic profiles. Significantly, lower proportion of HPV is detected in a non-white, old aged, and low socioeconomic patients\(^{(37,38)}\).

With regard to the study questionnaire, it is possible that some data following the past information is inaccurate. For instance, the age at first sexual activity and the number of lifetime partner were only estimated. Hence, it is recommended that investigators need to restrict their analysis only for the variables in those aged less than 60 years\(^{(37)}\). In several studies, statistical significance are instead considered by defining different definitions of “early age” of first sexual activity and “multiple” sexual partners\(^{(34,35,37,39)}\).

### Conclusion

In the present study, HPV infection could be identified in 26.09% of the patients with oropharyngeal cancer, but none in those with oral cavity cancer. Of these six HPV positive patients, there were only two (8.7%) with high-risk HPV infection. Besides, all patients with positive HPV identification did not have their risk of HVP from sexual contact. Risk factors for HPV was identifiable in the oropharyngeal cancer group, while some in the oral cavity cancer group were not at risk for cancer development.

Moreover, the carcinogenesis of SCCA requires multiple abnormal molecular modifications. The present study supports the oncogenic property of HPV in oropharynx, but with limited role. Following a favorable prognosis, de-escalation protocol might improve treatment outcome in subsets of head and neck cancer. For future and universal application, a de-intensification regimen is thus recommended, and should be restricted particularly in developed countries with HPV-positive oropharyngeal cancer. Whilst, in developing countries where oral cancer is the same extensive disease, surgical excision is still a treatment of choice for oral cavity cancer as well as residual/recurrent diseases.

### What is already known on this topic?

Cigarette is still a major risk factor in oral cavity and oropharyngeal cancer in developing countries.

### What this study adds?

1. The first report of prevalence and HPV in oral cavity and oropharyngeal cancer in Thailand.
2. Different pathogenesis of head and neck cancer in both developed countries and developing countries.

### Acknowledgement

The authors would like to extend special thanks to Dr. Chulaluk Komoltri for statistical advices and Ms. Jeerapa Kerdnoppakhun for manuscript assistance.

### Financial support

The present study was funded by the Faculty of Medicine Siriraj Hospital, Mahidol University.

### Potential conflicts of interest

None.

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การตรวจพบดีเอ็นเอของไวรัส HPV ในโรคมะเร็งของคอหอยและช่องปาก: มีความสัมพันธ์กันอย่างไร

วรุณ ทองพิทักษ์, พิชญู โชติผลประสาน, จุฑาทิพย์ เลิศนรงค์, เสนนา หุ่นอาทร, ศิริบัณฑิต, จักริกา คงสวัสดิ<br>

ภูมิหลัง: ปัจจุบันการติดเชื้อ human papillomavirus (HPV) เป็นปัจจัยสำคัญหนึ่งที่ทำให้เกิดมะเร็งศีรษะและลำคอ แต่สำหรับประเทศไทยข้อมูลเกี่ยวกับการติดเชื้อ HPV ในมะเร็งศีรษะและลำคอยังไม่เคยมีการรายงานถึงความสัมพันธ์กัน

วัตถุประสงค์: เพื่อแสดงให้เห็นถึงความแตกต่างระหว่างมะเร็งของปากและมะเร็งคอของส่วนปาก รวมถึงความชุกของการติดเชื้อ HPV ในส่วนต่างๆนี้ ซึ่งมีค่าต่างจากข้อมูลที่เคยมีการรายงานจากต่างประเทศ

วัสดุและวิธีการ: การศึกษานี้รวบรวมผู้ป่วยมะเร็งช่องปากและผู้ป่วยมะเร็งคอของส่วนปากกลุ่มละ 23 ราย ในช่วง พ.ศ. 2553 ถึง พ.ศ. 2555 โดยขึ้นเบื้องต้นจากผู้เข้าร่วมการศึกษาทั้งสองกลุ่มจะได้รับการสกัดสารพันธุกรรมของเชื้อ HPV เพื่อนำมาศึกษาเบื้องต้นก่อนชิ้นเนื้อที่ได้รับจากการศึกษาจะได้รับการสกัดสารพันธุกรรมของเชื้อ HPV

ผลการศึกษา: พบว่าไม่มีการติดเชื้อ HPV ในกลุ่มมะเร็งช่องปาก ส่วนกลุ่มมะเร็งคอของส่วนปากพบว่ามีการติดเชื้อ HPV 26.09% โดยผู้ป่วยกลุ่มนี้ไม่มีปัจจัยเสี่ยงต่อการติดเชื้อสูงที่จะเข้าเลือก

สรุป: มะเร็งช่องปากและมะเร็งคอของส่วนปากมีความแตกต่างกันในบริเวณใกล้เคียงกัน แต่ผู้ป่วยเป็นคนละกลุ่มกัน มีความแตกต่างกันทางพยาธิวิทยา คนไทยมีอัตราการติดเชื้อ HPV ของมะเร็งศีรษะและลำคอค่อนข้างต่ำ ดังนั้นการเปลี่ยนแปลงวิถีการรักษามะเร็ง contrasted ที่พันธุ์จากกลุ่มประชากรที่มีการติดเชื้อ HPV เป็นส่วนใหญ่จึงไม่สามารถนำมาประยุกต์ใช้กับคนไทยได้