Detection of Human Papillomavirus 16 and 18 in the Saliva of Indonesian Dental Residents in Jakarta

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Abstract
Occupational hazard risk related to viral infection are considerably high in dental professionals due to direct contact with saliva during treatment. Recent studies have found that High-Risk (HR) Human Papillomavirus (HPV) types 16 and 18 was detected in the saliva of healthy individuals. The aim of this study was to test for HPV 16 and 18 in the saliva of dentists.

This was a cross sectional study involving the female dental residents in the Faculty of Dentistry, Universitas Indonesia that met the inclusion criteria (n = 73). The subjects were instructed to follow certain protocols before collecting saliva; deoxyribonucleic acid (DNA) was extracted using QiaAmp DNA mini blood kit 250 and the presence of HPV 16 or 18 was detected by polymerase chain reaction. HPV 16 and 18 were not detected. This was inconsistent with the findings of a few previous studies; however, the results were consistent with those of a previous study with similar subjects in Jakarta.

This study did not detect HPV 16 and 18 in the female resident population. Several variables might contribute to this finding, such as good infection control and safe sexual practices. Our findings were inconsistent with the previous study. However, the risk of HPV infection in the dentist or patient due to direct contact cannot be ignored and needs further investigation.

Keywords: HPV 16, HPV 18, saliva.

Introduction
Human papillomavirus (HPV) is a double-stranded deoxyribonucleic acid (DNA) virus. HPV is commonly divided into two groups based on ability in increasing human cell changes into cancer, i.e., Low Risk (LR) HPV and High-Risk (HR) HPV. The HR-HPV group can cause cancers.¹ HR-HPVs have been reported to cause anogenital cancers (anal, vulvar, cervical, vaginal, and penile) as well as head and neck cancer.²,³ However, alcohol and smoking are still considered the main causes of head and neck cancers.⁴ One study has reported an increase in oropharyngeal cancer incidence even after smoking and alcohol consumption decreased. In this case, the suspected cause of cancer apart from alcohol and smoking was HPV.⁵ The most dominant HR-HPV strains suspected of causing oropharyngeal cancer are HPV 16 and 18.⁵,⁶,⁷ In addition to being a cause, these types of HPV can also increase the severity of oropharyngeal cancer triggered by smoking and alcohol.⁵ Other studies have also reported the occurrence of oral cavity cancer in individuals without a smoking or alcohol risk.⁵,⁸

Two different studies conducted with female dentistry students showed the presence of HPV in those subjects.⁵,⁹ Dental professionals are often exposed to infectious agents such as viruses and bacteria from patients’ saliva and blood. With this elevated possibility of non-sexual HPV transmission, the practice of infection control should attract more attention, particularly for dentists in order to prevent HPV transmission from patients to dentists or vice versa.¹⁰ HPV transmission occurs through direct contact in minor trauma, particularly through sexual...
However, HPV transmission through non-sexual or indirect contact remains controversial. This possibility was considered because HPV has properties similar to that of a common virus, which can survive at room temperature and is resistant to certain disinfectants, such as alcohol.

One study detected the presence of HPV 16 and 18 in the saliva of patients with head and neck cancer. Because of these findings, another study was conducted to determine the possibility of early detection of head and neck cancer through the detection of HPV 16 and 18 in the saliva of healthy individuals. The aim of this study was to confirm the possibility of HPV transmission through saliva; i.e. detect the presence of HPV in the saliva of female dentists that have a lot of contact with the patient’s saliva.

Materials and methods

Subjects
Subjects (n = 73) were female dental residents of the Dental Faculty of Universitas Indonesia who work at the Dental Hospital of Universitas Indonesia; informed consent was obtained from all the subjects. Inclusion criteria were no history of anti HPV vaccination and no previous diagnosis of anogenital or oropharyngeal cancers.

Saliva Collection
Subjects were asked to collect stimulated saliva in 15 ml tubes while chewing 1×1-inch paraffin wax for 3 minutes. Subjects were not allowed to eat, drink, brush teeth, smoke, or mouth wash one hour before the collection time. After the collection of saliva, tubes were kept in a cooler box and stored at −20°C at the Oral Biology laboratory.

DNA Extraction
The DNA from the saliva were extracted using QiaAmp DNA mini blood kit 250 (Qiagen/ Cat no./ID.51306) as per the manufacturer’s instructions (available online at http://emerald.tufts.edu/~mcourt01/Documents/QIAGEN_protocol.pdf). The DNA was resuspended in water and its purity was determined using a spectrophotometer (Metertech UV/VIS SP-8001) by measuring absorbance at 260 and 280 nm (the average A260/280 ratio was 2.8).

Polymerase Chain Reaction (PCR)
PCR was performed using primers for HPV16, HPV18, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) whose sequences are shown in Table 1. PCR was performed using Dream Taq Green PCR Master Mix (Cat #K1081, Thermo Scientific) and T100™ Thermal Cycler (Biorad). PCR conditions for HPV 16 were as follows: 120 s at 95°C; 34 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 54.2°C, extension for 60 s at 72°C, and final extension for 5 min at 72°C. PCR conditions for HPV 18 were as follows: 60 s at 95°C; 32 cycles of denaturation for 60 s at 95°C, annealing for 60 s at 58°C, extension for 60 s at 72°C, and final extension for 5 min at 72°C. PCR conditions for GAPDH were as follows: 120 s at 95°C; 40 cycles of denaturation for 60 s at 95°C, annealing for 60 s at 58°C, extension for 60 s at 72°C, and final extension for 5 min at 72°C.

The 1.5% Agarose gels electrophoresis (Thermo scientific TopVision Agarose) were used to separate the PCR reaction products. UV illumination (Gel Doc Bio-rad) and 1D Image Analysis Software (Eastman Kodak: Rochester, NY) were used to observe the bands of PCR products of HPV16, HPV 18 and GAPDH.

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Primers</th>
<th>Base pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV 16&lt;sup&gt;14&lt;/sup&gt;</td>
<td>TCAAAGGCACCTGTGTTCTCTG</td>
<td>120</td>
</tr>
<tr>
<td>HPV 18&lt;sup&gt;15&lt;/sup&gt;</td>
<td>ATGGCCGCCCTTTTGGAGATCC</td>
<td>188</td>
</tr>
<tr>
<td>GAPDH&lt;sup&gt;5&lt;/sup&gt;</td>
<td>ATCTTCCAGGAGCGAGATCC</td>
<td>503</td>
</tr>
</tbody>
</table>

Table 1. PCR primers<sup>9</sup>

Controls
The housekeeping gene GAPDH was used as a control for DNA integrity, whereas CaSki cell line DNA, containing HPV16 and HeLa cell line DNA, containing HPV18, were
used as positive controls for PCR analysis.

**Statistical analysis**

Descriptive analysis was done to report the percentage of HPV 16 & 18 status in Indonesians female dental residents of Dental Hospital Universitas Indonesia.

**Results**

From 73 total subjects in this study, only 66 subjects completed the social data form (Table 2). Sexual practice before marriage was not found in these subjects.

<table>
<thead>
<tr>
<th>Age</th>
<th>30(25–44)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marital Status</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>40</td>
</tr>
<tr>
<td>Single</td>
<td>26</td>
</tr>
<tr>
<td>Years in Dental Practice</td>
<td>5 (2–19)*</td>
</tr>
</tbody>
</table>

* Median (min-max)

Table 2: Social data of the subjects (n = 66).

A total 73 saliva samples were collected and analyzed for HPV 16 & 18 PCR. All the saliva samples were positive for GAPDH, but negative for HPV 16 & 18.

From the upper panel of the gel electrophoresis of PCR products (Figure 1), GAPDH was positive in positive control (lane 1), positive with ladder (lane 2), negative in negative control (lane 3), positive in sample (lane 4). From the middle panel, HPV 16 was positive in positive control (lane 1), positive with ladder (lane 2), negative in negative control (lane 3), negative in sample (lane 4). In the lower panel, HPV 18 was positive in positive control (lane 1), positive with ladder (lane 2), negative in negative control (lane 3), negative in sample (lane 4).

**Discussion**

The prevalence of HPV in patients with HPV-associated head and neck cancer is higher than that in healthy individuals. However, Zhao et al. found that HPV is not all detected in the saliva of HPV-associated head and neck cancer patients.\(^{15}\) Frequently, HPV detection in the saliva of healthy individuals was lower than in patients with head and neck cancer.\(^{13,15}\) The subject used in this study were healthy, which may have resulted in a lower or even no percentage of HPV in their saliva.

Previous studies of HPV detection in the saliva of healthy dental students’ also found a low HPV prevalence.\(^5,9\) A study by Wimardhani et al. revealed no HPV in the saliva samples of Indonesian healthy dental students\(^9\) which is consistent with the results of this study. Another study detected the presence of HPV in healthy individuals which was believed to be caused by sexual relations with multiple partners.\(^16\) Data regarding sexual behavior of subjects was difficult to obtain in our study because of their eastern culture background.

Additionally, the laboratory technique used in this study may have contributed to the negative PCR result. The HPV 16 primers in this study were detecting the E6 oncogene of HPV 16. According to Peixoto et al., the correct primer for detecting HPV 16 is one for the viral L1 gene.\(^{17,18}\) It is possible that the L1 primer is more sensitive in detecting HPV 16 than the one used in this study. Besides that, the PCR technique used in detecting HPV 18 in these subjects, were less sensitive than the test that was done by Raji et al. A fast PCR-ELISA assay designated as DIAPOPS (Detection of Immobilized Amplified Products in a One Phase System) for detection of HPV 18 is more sensitive, specific, and rapid in detecting HPV 18.\(^{19}\)

Another factor that might have produced false-negative HPV results in our subjects’ saliva could also have been the effective infection control practices at Dental Hospital of Universitas Indonesia that could have prevented cross infection between patients and dentists.\(^{20}\)

**Conclusions**

This study did not detect HPV 16 & 18 in the saliva of dentists. Several variables might have contribute in this negative result, such as good infection control, safe sexual practice, and less sensitive laboratory technique. Futher study using highly sensitive HPV 16 & 18 primers were suggested.
Declaration of Interest

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References