SURVEY OF HUMAN PAPILLOMAVIRUS PREVALENCE IN THE ORAL CAVITY OF THAI DENTAL PATIENTS

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Abstract. Human papillomavirus (HPV) is associated with oral cancer but it is unclear whether the normal oral cavity is a reservoir for this virus. We aimed to determine the prevalence of HPV in the oral cavity of Thai dental patients in order to better understand the risk for oral cancer in this patient population. We randomly recruited dental patients from those attending the Faculty of Dentistry Dental Clinic, Mahidol University. Inclusion criteria for study subjects were dental patients aged 15-79 years with no oral pathologic lesions who agreed to participate in this study. Exclusion criteria were those who had serious systemic diseases, oral soft tissue pathological lesions, used antibiotics or antiseptic mouth rinse during the 7-day period before sample collection or had problems with communication. A minimum sample size was calculated and determined to be 180 subjects. Oral mucosal cell samples were collected using a soft bristle toothbrush and examined for the presence of HPV using polymerase chain reaction (PCR) analysis for the presence of HPV DNA. HPV isolates were then typed using DNA sequence analysis. Selected subject characteristics were recorded and associations between these characteristics and the presence of HPV was determined. A total of 210 subjects were included in the study; 74% females. The mean (±standard deviation) age of study subjects was 31.7 (±14.1) (range 17-72) years. Of the 210 subjects, 2 (1%) had a positive PCR test for HPV. The typing of both samples was HPV 18. On analysis, only male gender (both positive subjects were male) was significantly ($p = 0.017$) associated with HPV infection. The prevalence of HPV in our study subjects was low. Further studies involving larger numbers of subjects from a variety of populations are needed to determine whether the oral cavity can act as a reservoir for HPV.

Keywords: Human papillomavirus, oral cavity, Nested PCR, Thai dental patients

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INTRODUCTION

The World Health Organization International Agency for Research on Cancer (IARC) reported the incidence of reported cancers in Thailand during 2018 was 170,495 cases; lip and oral

cancers were the tenth most prevalent that year (Ferlay *et al*, 2019). Oral cancer is associated with tobacco and alcohol use (Lambert *et al*, 2011) but some studies have reported that human papillomavirus (HPV) infection may also be associated with oral cancers (Chang *et al*, 1991; Shaikh *et al*, 2015).

HPV is a double-stranded DNA virus causing infections only in the stratified squamous epithelium (zur Hausen, 2000). There are many strains of HPV; these may be divided into those with a low- or high-risk for oncogenesis (Syrjanen, 2005). While both groups of HPV can cause abnormal cellular proliferation, cancerous lesions have only been associated with high-risk strains (Syrjanen, 2005). The E6 and E7 genomic regions of these high-risk virus encode oncoproteins that target tumor suppressor proteins, p53 and pRb respectively, which subsequently lead to carcinogenesis (Munger and Howley, 2002). High-risk HPV strains 16 and 18 are responsible for about 70% of all cervical cancer cases world-wide with the prevalence of HPV 16 being 50% and of HPV 18 being 14% (Bosch *et al*, 1995).

Sexual intercourse is the primary mode of transmission of HPV (zur Hausen, 2009). It has been suggested that oral HPV infection occurs through oral sex (Emmett *et al*, 2018). Having an HPV-related tumor of the oral cavity or oropharynx is associated with: being married or having been married previously ($p = 0.046$), multiple numbers of kissing partners ($p = 0.046$), having given oral sex ($p = 0.0007$) and a large number of oral sex partners ($p = 0.0015$) (Emmett *et al*, 2018).

Our previous study conducted in Thailand found the prevalence of HPV infection in oral squamous cell carcinomas to be 3%, which is low compared to some other studies (Khovidhunkit *et al*, 2008). Since the presence of HPV in the oral cavity may be a risk factor for oral cancers and the oral cavity may be a reservoir for HPV, it is important to determine the prevalence of HPV among people with apparently normal oral cavity. The prevalence of detecting HPV in apparently normal healthy subjects varies considerably by study (Esquenazi *et al*, 2010). In a review of the literature, one study reported the prevalence to vary from 0 to 100% (Esquenazi *et al*, 2010). A study from Japan using polymerase chain reaction (PCR) analysis to detect HPV in an apparently normal oral cavity among children reported a prevalence of 48.1% (Kojima *et al*, 2003). Another study from Pakistan among dental patients with an apparently normal oral cavity using PCR analysis to detect HPV reported a prevalence of 24.5% (Gichki *et al*, 2012). These data suggest the prevalence of HPV infection in apparently normal oral mucosa varies by country and study. Therefore, we aimed to determine the prevalence of HPV in Thai dental patients with an apparently normal oral cavity and to examine any demographic factors associated with that infection in order to determine the patient risk for oral cancer.

MATERIALS AND METHODS

Study population

The minimum number of study subjects was calculated to give adequate power to the study based on the prevalence of HPV infection in the oral mucosa of 13.5% reported in a meta-analysis of 1026 subjects (Miller and White, 1996). This minimum number was determined to be 180. Study subjects were randomly chosen from patients attending Dental Clinic at the Faculty of Dentistry, Mahidol University, Bangkok, Thailand. Inclusion

criteria for study subjects were dental patients aged 15-79 years who had no pathological oral cavity lesions who were willing to participate in this study. Exclusion criteria for study subjects were dental patients who had serious systemic diseases, such as leukemia, any type of malignancy, renal failure, and serious cardiac problems, who had visible soft tissue pathological lesions in the oral cavity, had used antibiotics within the previous 7 days or who had problems with communication.

Each subject was requested to complete a questionnaire asking about socio-demographics and other selected factors including a history of smoking, drinking alcohol or chewing betel nut. We then examined the oral cavity of each subject and obtained a sample of their oral mucosal cells as described below.

Collection of oral samples

Oral mucosal cells were collected using a soft-bristled toothbrush; brushing 3 strokes at each studied location. The studied areas were the hard palate, buccal mucosa and the dorsal tongue. Samples were collected bilaterally. The toothbrush was then rinsed in a tube containing phosphate buffered saline (PBS) solution and the collected samples were then frozen at -20 °C until DNA extraction.

DNA extraction

DNA was extracted from the collected samples using the QIAamp DNA Mini Kit (QIAgen®, Hilden, Germany) following the manufacturer's instructions. The collected samples were centrifuged at 3000 rpm for 5 minutes; the supernatant was discarded and PBS was added to the remaining sample. Proteinase K solution (QIAgen®, Hilden, Germany) and lysis buffer AL were then added to the sample. The suspension was then incubated for 10 minutes at 56°C and then absolute ethanol was added to the suspension. The obtained mixture was then placed in the QIAamp spin column and then the column was washed with wash buffer AW1 and centrifuged at 8,000 rpm for 1 minute. Wash buffer AW2 was then added and the suspension was then centrifuged again at 14,000 rpm for 3 minutes. The remaining substrate consisting of the DNA from each column was then incubated with elution buffer AE at 65°C for 5 minutes and again centrifuged at 8000 rpm for 1 minute. The processed DNA was kept at -20 °C until further use.

Nested PCR to determine the presence of HPV

Amplification was performed in duplicate using three sets of primers: KM29/KM138 primers to detect the DNA integrity of the house-keeping *β-globin* gene; MY09/11, to detect the L1 region of HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 58 and 68; HPV1003/1004 to detect the L1 region of HPV types 1, 6, 8, 11, 13, 16, 18, 30, 31, 32 and 33 (Table 1).

The PCR mixture to detect the *β-globin* gene contained 1 µl of 2.5 µM primer, 2 µl of 5X Green GoTaq™ reaction buffer (Promega®, WI), 1 µl of 25 mM $MgCl_2$, 1 µl of the investigated DNA, 0.2 µl of 10 mM dNTPs, 1 unit of *i*-Taq® DNA polymerase (Intron Biotechnology, Gyeonggi-do, Korea) and sterile distilled water to give a total volume of 10 µl. DNA extracted from Hela cells was used as a positive control and de-ionized sterile water was used as a negative control. All *β-globin*-positive samples were evaluated using nested PCR to determine the presence of HPV. The PCR mixture for the MY09/11 outer primers contained the same reagents as the PCR mixture used to detect the *β-globin* gene except 1.5 µl of 25 mM $MgCl_2$ was used instead of 1 µl. The PCR product from

Table 1
Primer sequences and thermocycling regimens used for study subjects.

Primer	Amplicon size (base pairs)	Target region	Sequences	Thermocycling regimens
KM29	262	*β-globin*	GGTTGGCCAATCTACTCCCAGG	95°C for 15 minutes / 35 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds / 72°C for 5 minutes
KM138			TGGTCTCCTTAAACCTGTCTTG	
MY09*	450	L1 HPV	CGTCCMARRGGAWACTGATC	95°C for 15 minutes / 35 cycles of 95°C for 30 seconds, 44°C for 30 seconds, 72°C for 30 seconds / 72°C for 5 minutes
MY11*			GCMCAGGGWCATAAYAATGG	
HPV1003	150	L1 HPV	TTTGTTACTGTGGTAGATA	95°C for 15 minutes / 35 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds / 72°C for 5 minutes
HPV1004			GAAAAATAAACTGTAAATC	

* Degenerate code indicated M = A+C, R = A+G, W = A+T and Y = C+T.

the first analysis were then diluted to a concentration of 1:30 and used as a template for the second analysis. The second PCR analysis used for the HPV1003/1004 inner primers was the same mixture used to detect the *β-globin* gene. The PCR product was placed on 1.5% agarose gel and stained using ethidium bromide and analyzed under UV transillumination (Geldoc, Bio-Rad®, Hercules, CA). The expected size of the PCR product of the *β-globin* gene was 262 base pairs. The sizes of the PCR products amplified using the MY09/MY11 and HPV1003/HPV1004 primers were expected to be 450 and 150 base pairs, respectively (Table 1).

HPV typing

The PCR product was sent to Macrogen Company, Seoul, Korea to obtain the DNA sequence analysis. The generated sequence was analyzed using Basic Local Alignment Search Tool (BLAST) to identify the type of HPV DNA.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Inc, Chicago, IL). Descriptive statistics were used to classify socio-demographic factors. Associations between independent variables and having HPV infection were determined using the Pearson chi-square. Statistical significance was set at $p < 0.05$.

Ethical considerations

The study was approved by the Ethics Committee of the Faculty of Dentistry/Faculty of Pharmacy (MU-DT/PY-IRB 2013/010.1902). Each subject gave written informed consent prior to participation in the study.

RESULTS

Participant characteristics

A total of 210 subjects were included in the study, 74.0% female. The mean (±standard deviation) age of study subjects was 31.7 (±14.1) (range: 17-72) years. Ninety-one percent of subjects were non-smokers, 6.0% were current smokers and 3.0% were former smokers. Seventy-six percent of subjects were non-drinkers, 20.0% were current drinkers and 4.0% were former drinkers (Table 2).

DNA integrity

Among the 210 subjects, all samples were positive for the ***β-globin*** gene using KM29/KM138 primers (Fig 1). Hence, all samples were of good quality and used for the HPV analysis.

Nested PCR analysis to detect the presence of HPV

Two samples (1%) were positive for HPV (Fig 2). On the first step of PCR analysis, no detectable bands were seen in the positive samples but on the second step, bands were seen.

HPV typing

Both PCR positive samples were HPV type 18.

Characteristics of subjects with HPV infection.

The characteristics of subjects with HPV infection were: male gender (both were male), non-smokers (both were non-smokers) and non-drinkers (both were non-drinkers). One HPV positive subject was married and one was divorced. Neither subjects answered the question of whether they had ever had oral sex in the past (Table 2).

Association between HPV infection and studied factors

Only male gender was significantly associated with HPV infection ($p = 0.017$) (Table 2).

DISCUSSION

In this study, we investigated the

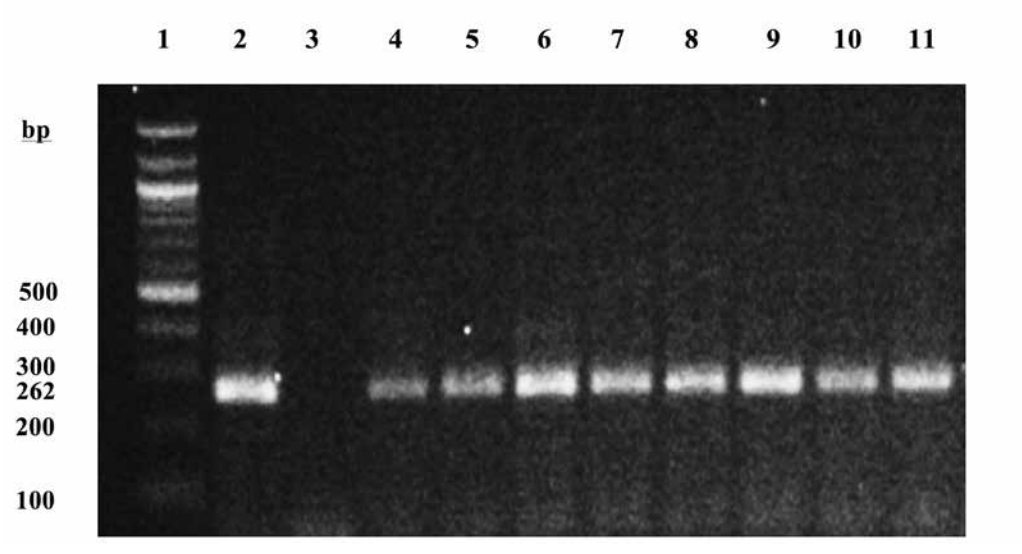

Fig 1-Agarose gel electrophoresis of PCR products using KM29/KM138 primers.

Lane 1: 100-bp molecular marker; Lane 2: HeLa DNA positive control; Lane 3: Negative control; Lanes 4-11 : ***β-globin*** positive samples.

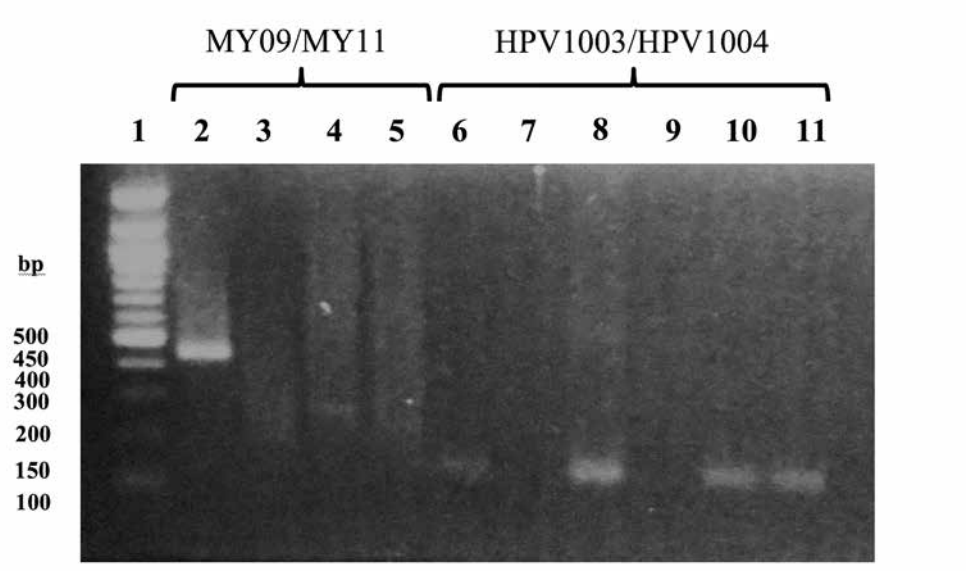

Fig 2-Agarose gel electrophoresis of the PCR products using MY09/MY11 primers followed by HPV1003/HPV1004 primers.

Lane 1: 100-bp molecular marker; Lane 2: HeLa DNA positive control of MY09/MY11 primers; Lane 3: Negative control of MY09/MY11 primers; Lanes 4-5: MY 09/11 positive samples; Lane 6: HeLa DNA positive control of HPV1003/HPV1004 primers (non-nested); Lane 7: Negative control of HPV1003/HPV1004 primers (non-nested); Lane 8: HeLa DNA positive control of nested PCR; Lane 9: Negative control of nested PCR; Lanes 10-11: Nested PCR positive samples.

prevalence of HPV among study subjects using nested PCR and found 1.0% was positive. A low prevalence of HPV examined using PCR of DNA from oral cells retrieved from apparently normal oral cavity was also reported in a study from Iowa, USA: 8.7% among children aged <7 years, 5.2% of adolescents aged 13 to 20 years and 0% among children aged 7-12 years (Summersgill *et al*, 2001).

However, some studies have reported higher prevalence of HPV infection in apparently normal oral cavity among subjects. A study from Japan (Terai *et al*, 1999) using PCR and DNA sequencing for type specific HPV in scraping samples of buccal mucosa reported a prevalence of 80% among adults aged 22-48 years. Another study from Japan (Kojima *et al*, 2003) using PCR and DNA sequencing reported a prevalence of 48.1% among children aged 3-5 years when swabs of oral epithelial cells were examined. A study from Mongolia (Sosorbaram *et al*, 2006) reported a prevalence of HPV infection to be 25.0% among subjects aged 1-20 years in swabs of oral epithelial cells using PCR analysis. A study from Pakistan (Gichki *et al*, 2012) using real-time PCR analysis to detect HPV DNA from oral mucosal cells from dental subjects reported a prevalence of 24.5%.

In the present study, the only strain of HPV isolated was HPV 18. However, Sugiyama *et al* (2003) found HPV 16 in 36.0% of study subjects and did not find any HPV 18. A study from Pakistan (Gichki *et al*, 2012) reported finding HPV 18 in 5.7%

Table 2
Evaluation of selected factors potentially associated with HPV status.

Variables	Classifications	HPV+ *n* (%)	HPV- *n* (%)	Total	%	*p*-value
Age in years	16-19	0 (0.0)	64 (100.0)	64	30.0	0.098
	20-29	0 (0.0)	48 (100.0)	48	23.0	
	30-39	1 (2.0)	42 (98.0)	43	20.0	
	40-49	0 (0.0)	25 (100.0)	25	12.0	
	50-59	0 (0.0)	18 (100.0)	18	9.0	
	60-79	1 (8.3)	11 (91.7)	12	6.0	
Sex	Male	2 (4.0)	53 (96.0)	55	26.0	0.017
	Female	0 (0.0)	155 (100.0)	155	74.0	
Marital status	Single	0 (0.0)	152 (100.0)	152	72.0	0.168
	Married	1 (2.0)	46 (98.0)	47	22.0	
	Divorce/Widow	1 (9.1)	10 (90.9)	11	6.0	
Smoking status	Non-smoker	2 (1.0)	190 (99.0)	192	91.0	0.910
	Former smoker	0 (0.0)	6 (100.0)	6	3.0	
	Current smoker	0 (0.0)	12 (100.0)	12	6.0	
Alcohol drinking status	Non drinker	2 (1.0)	157 (99.0)	159	76.0	0.557
	Former drinker	0 (0.0)	9 (100.0)	9	4.0	
	Current drinker	0 (0.0)	42 (100.0)	42	20.0	
Betel nut chewing status	Non chewer	2 (1.0)	208 (99.0)	210	100.0	N/A
	Former chewer	0 (0.0)	0 (100.0)	0	0.0	
	Current chewer	0 (0.0)	0 (100.0)	0	0.0	
History of HPV vaccination	No	2 (1.0)	192 (99.0)	194	92.0	0.683
	Yes	0 (0.0)	14 (100.0)	14	8.0	
History of having oral sex	No	0 (0.0)	164 (100.0)	164	78.0	0.770
	Yes	0 (0.0)	14 (100.0)	14	7.0	
	No answer	2 (6.0)	30 (94.0)	32	15.0	

HPV+: positive for human papillomavirus; HPV-: negative for human papillomavirus.

and HPV 16 in 2.1% of study subjects. A study from Japan (Terai *et al*, 1999) reported finding HPV 18 in 86.7% of study subjects. Our results show high risk HPV strains can be found in the oral cavity.

In this study, 2 subjects with HPV infection were in the age groups of 30-39 and 60-79 years old. A study from Pakistan (Gichki *et al*, 2012) reported the prevalence of HPV infection among subjects aged 30-39, 40-49 and 50-59 years were 26.3%, 29.2% and 35.7%, respectively. A meta-analysis from 94 studies (Miller and Johnstone, 2001) reported the prevalence of HPV infection among subjects aged 40-49 and 50-59 years were 29.2% and 35.77%, respectively, suggesting the prevalence of HPV infection increases with increasing age.

In this study, one of the subjects was married and the other was divorced. It has been postulated that HPV positivity increases in prevalence by cumulative

lifetime exposures and episodes of sexual activity (Summersgill *et al*, 2001). HPV infection is transmitted by sexual contact or through autoinoculation from one body area to another in the same individual (Kellokoski *et al*, 1992). It has also been reported infections increase with sexual activity (Schwartz *et al*, 1998; D'Souza *et al*, 2007). In this study, both subjects had sexual exposure risk due to previously being married.

In this study, HPV infection was significantly associated with male gender, similar to previous studies (Gichki *et al*, 2012; Sanders *et al*, 2012). Male subjects are more likely to have risk of HPV transmission (Reiter *et al*, 2010).

The technique used to detect HPV infection may influence the HPV infection detection rate (Gravitt *et al*, 2008). Husnjak *et al* in 2000 reported nested PCR (GP5/GP6) amplification, compared to single PCR amplification, increases the detection rate by 38.8%. When primer sets MY09/MY11 and GP5/GP6 were used to conduct a nested PCR assay, the sensitivity of HPV detection increased compared to the conventional PCR assay (Chaiwongkot *et al*, 2007; Winder *et al*, 2009). This is the reason why we used nested PCR analysis in our study. In this study, no PCR products were visible in the first step of nested PCR analysis. This may be due to the low number of HPV copies. However, with the second round of PCR using HPV1003/HPV1004, there were 2 positive samples with bands with high intensity. This result suggests single PCR analysis may not be sufficient to detect HPV infection in an apparently normal oral cavity and nested PCR analysis may be more suitable for detecting oral HPV infection in an apparently normal oral cavity (Chaiwongkot *et al*, 2007; Winder *et al*, 2009).

The sample collection method used in our study may also have influenced the prevalence of HPV in our study. One study reported the sensitivities of detecting HPV infection in apparently normal oral cavities (Lawton *et al*, 1992) and reported the following: 3% sucrose mouthwash detected HPV infection in 51.1%, mucosal scraping in 45% and buccal mucosal biopsies in 12%. Future studies should use the mouthwash collection technique.

A weakness of our study was that about half our study subjects were aged <30 years; most were single and most were female. Future studies should include older subjects, more male subjects, more married subjects and more subjects with a history of having oral sex. Future studies should also be conducted in other parts of Thailand and in other institutions.

In summary, we found a low prevalence of oral HPV infection (1%) among dental patients with apparently normal oral cavities. The only factor associated with HPV infection was male gender. Future studies in broader subject groups and more locations are needed to confirm these results.

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REFERENCES

Bosch FX, Manos MM, Munoz N, *et al*. Prevalence of human papillomavirus in

cervical cancer: a worldwide perspective. International Biological Study on Cervical Cancer (IBSCC) Study Group. *J Natl Cancer Inst* 1995; 87: 796-802.

Chaiwongkot A, Pientong C, Ekalaksananan T, *et al*. Evaluation of primers and PCR performance on HPV DNA screening in normal and low grade abnormal cervical cells. *Asian Pac J Cancer Prev* 2007; 8: 279-82.

Chang F, Syrjanen S, Kellokoski J, Syrjanen K. Human papillomavirus (HPV) infections and their associations with oral disease. *J Oral Pathol Med* 1991; 20: 305-17.

D'Souza G, Kreimer AR, Viscidi R, *et al*. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med* 2007; 356: 1944-56.

Emmett S, Boros S, Whiteman DC, Porceddu SV, Panizza BJ, Antonsson A. Sexual behaviour, HPV status and p16(INK4a) expression in oropharyngeal and oral cavity squamous cell carcinomas: a case-case comparison study. *J Gen Virol* 2018; 99: 783-9.

Esquenazi D, Bussoloti Filho I, Carvalho Mda G, Barros FS. The frequency of human papillomavirus findings in normal oral mucosa of healthy people by PCR. *Braz J Otorhinolaryngol* 2010; 76: 78-84.

Ferlay J, Colombet M, Soerjomataram I, *et al*. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer* 2019; 144: 1941-53.

Gichki AS, Buajeeb W, Doungudomdacha S, Khovidhunkit SO. Detection of human papillomavirus in normal oral cavity in a group of Pakistani subjects using real-time PCR. *Asian Pac J Cancer Prev* 2012; 13: 2299-304.

Gravitt PE, Coutlee F, Iftner T, Sellors JW, Quint WG, Wheeler CM. New technologies in cervical cancer screening. *Vaccine* 2008; 26 (Suppl 10): K42-52.

Husnjak K, Grce M, Magdic L, Pavelic K. Comparison of five different polymerase chain reaction methods for detection of human papillomavirus in cervical cell specimens. *J Virol Methods* 2000; 88: 125-34.

Kellokoski JK, Syrjanen SM, Chang F, Yliskoski M, Syrjanen KJ. Southern blot hybridization and PCR in detection of oral human papillomavirus (HPV) infections in women with genital HPV infections. *J Oral Pathol Med* 1992; 21: 459-64.

Khovidhunkit SO, Buajeeb W, Sanguansin S, Poomsawat S, Weerapradist W. Detection of human papillomavirus in oral squamous cell carcinoma, leukoplakia and lichen planus in Thai patients. *Asian Pac J Cancer Prev* 2008; 9: 771-5.

Kojima A, Maeda H, Kurahashi N, *et al*. Human papillomaviruses in the normal oral cavity of children in Japan. *Oral Oncol* 2003; 39: 821-8.

Lambert R, Sauvaget C, de Camargo Cancela M, Sankaranarayanan R. Epidemiology of cancer from the oral cavity and oropharynx. *Eur J Gastroenterol Hepatol* 2011; 23: 633-41.

Lawton G, Thomas S, Schonrock J, Monsour F, Frazer I. Human papillomaviruses in normal oral mucosa: a comparison of methods for sample collection. *J Oral Pathol Med* 1992; 21: 265-9.

Miller CS, Johnstone BM. Human papillomavirus as a risk factor for oral squamous cell carcinoma: a meta-analysis, 1982-1997. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001; 91: 622-35.

Miller CS, White DK. Human papillomavirus expression in oral mucosa, premalignant conditions, and squamous cell carcinoma: a retrospective review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1996; 82: 57-68.

Munger K, Howley PM. Human papillomavirus immortalization and transformation functions. *Virus Res* 2002; 89: 213-28.

Reiter PL, Brewer NT, Smith JS. Human papillomavirus knowledge and vaccine acceptability among a national sample of heterosexual men. *Sex Transm Infect* 2010; 86: 241-6.

Sanders AE, Slade GD, Patton LL. National

prevalence of oral HPV infection and related risk factors in the U.S. adult population. *Oral Dis* 2012; 18: 430-41.

Schwartz SM, Daling JR, Doody DR, *et al*. Oral cancer risk in relation to sexual history and evidence of human papillomavirus infection. *J Natl Cancer Inst* 1998; 90: 1626-36.

Shaikh MH, McMillan NA, Johnson NW. HPV-associated head and neck cancers in the Asia Pacific: A critical literature review & meta-analysis. *Cancer Epidemiol* 2015; 39: 923-38.

Sosorbaram K, Sugita Y, Suzumura Y, Sato E, Maeda H, Kameyama Y. Human papillomavirus infection in the normal oral cavity of young people in Mongolia. *Oral Med Pathol* 2006; 11: 105-12.

Sugiyama M, Bhawal UK, Dohmen T, Ono S, Miyauchi M, Ishikawa T. Detection of human papillomavirus-16 and HPV-18 DNA in normal, dysplastic, and malignant oral epithelium. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003; 95: 594-600.

Summersgill KF, Smith EM, Levy BT, Allen JM, Haugen TH, Turek LP. Human papillomavirus in the oral cavities of children and adolescents. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001; 91: 62-9.

Syrjanen S. Human papillomavirus (HPV) in head and neck cancer. *J Clin Virol* 2005; 32 (Suppl 1): S59-66.

Terai M, Hashimoto K, Yoda K, Sata T. High prevalence of human papillomaviruses in the normal oral cavity of adults. *Oral Microbiol Immunol* 1999; 14: 201-5.

Winder DM, Ball SL, Vaughan K, et al. Sensitive HPV detection in oropharyngeal cancers. *BMC Cancer* 2009; 9: 440.

zur Hausen H. Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis. *J Natl Cancer Inst* 2000; 92: 690-8.

zur Hausen H. Papillomaviruses in the causation of human cancers - a brief historical account. *Virology* 2009; 384: 260-5.