RESEARCH ARTICLE

Saliva-Based Screening of High-Risk Human Papillomavirus Strains: Detection in Female Indonesian and Thai Dental Students

Yuniardini Septorini Wimardhani[1]*, Harum Sasanti[1], Indriasti Indah Wardhany[1], Afi Savitri Sarsito[1], Siti Aliyah Pradono[1], Gus Permana Subita[1], Anandina Irmagita Soegyanto[1], Febrina Rahmayanti[1], Nutchapon Chamusri[2], Anak Iamaroon[2]

Abstract

Background: Currently it is believed that human papillomaviruses (HPV) are associated with the development of some oral/oropharyngeal cancers. It has been suggested that these viruses influence carcinogenesis in both smokers and non-smokers. Data on the prevalence of HPV in healthy adults are thus needed to estimate the risk of oral/oropharyngeal cancer. The aim of this study was to assess the prevalence of oral HPV in healthy female adults in Indonesia and Thailand. Materials and Methods: Healthy female students from the Faculties of Dentistry of Universitas Indonesia and Chiang Mai University were asked to participate in this pilot study. DNA was extracted from saliva specimens and screened for HPV16 and HPV18 using PCR. Results: The age, marital status and sexual experience of the subjects between the two countries were not significantly different. Eight (4%) and 4 (2%) samples were positive for HPV16 and HPV18, respectively. Fisher's Exact test found a significant difference between HPV16 positivity in subjects who were married and had sexual intercourse but not for HPV18. Conclusions: This study successfully detected presence of HPV16 and HPV18 DNA in a number of saliva samples from female dental school students. Marital status, experience of sexual intercourse and safe sexual practice are related to the possibility of finding HPV DNA finding in saliva. Dentists, physicians and other health care professionals may gain significant value from the findings of this study, which provide an understanding of the nature of HPV infection and its risk to patient health and disease.

Keywords: Oral cancer - human papilloma virus - saliva - PCR - detection

Asian Pac J Cancer Prev, **16 (13)**, 5525-5529

Introduction

Oral/oropharyngeal cancer represents one of the most common types of head and neck cancer. Worldwide estimated incidence of oral/oropharyngeal cancer is more than 250,000 new cases per year. The estimated incidence of oral/oropharyngeal cancers in Southeast Asian countries in 2008 was 15,300 new cases. Indonesia ranked as the country having the sixth highest incidence of oral/oropharyngeal cancer, with an estimated incidence of approximately 5,000 new cases, followed by China, Taiwan and Thailand with more than 4,000 new cases. (Bray et al., 2013) According to the Thailand Cancer Registry, the Northeast region of Thailand has the highest incidence of oral/oropharyngeal cancer, with increased incidence rates in females. (Vatanasapt et al., 2011)

It is generally accepted that the most common risk factors for oral/oropharyngeal cancers are tobacco and heavy alcohol usage. Evidence shows that HPV may influence carcinogenesis in some tobacco- and/or alcohol-induced oral/oropharyngeal cancers, or may be the main risk factor for non-smoker- and non-drinker-related oral/oropharyngeal cancers. (Rampias et al., 2014) Consistently, previous epidemiological studies have revealed increasing rates of oral/oropharyngeal cancers between 1997 and 2005, despite decreasing usage of tobacco and alcohol. (Bunnell et al., 2010)

The implication of as the causative organism of almost all cervical cancers worldwide has been documented (Suh et al., 2015). HPV is a non-enveloped DNA virus and has many family members. Once HPV invades the targeted host tissue, it can integrate into the host genome or be arranged in an episomal form or in a combination form. (Suh et al., 2015) Epithelial cells are the most commonly affected cells and the infection to the cells accounts for HPV-related cancers. (de Vuyst et al., 2013) Infection of

*[1]Department of Oral Medicine, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia, [2]Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand *For correspondence: yuniardini@ui.ac.id*

HPV to host epithelial cells have been reported to cause cell transformation. (Diao et al., 2015) Some studies found that compared to normal mucosa, the presence of HPV was three times and five times more frequently found in oral pre-cancer and oro-pharyngeal cancer lesions, respectively. (Rampias et al., 2014) The most commonly found HPV strains in oral biopsies, in order of occurrence are HPV16 and HPV18. (Gan et al., 2014) The presence of these high-risk HPV strains in the oropharyngeal epithelial cells may influence the carcinogenesis and cancer progression in already-developed oropharyngeal cancer. (Bunnell et al., 2010)

Previous studies using biopsy samples from healthy individuals revealed that the prevalence of HPV ranged from 0 to 15%. (Mao et al., 1995; do Sacramento et al., 2006; Lang et al., 2013) The invasiveness of the biopsy procedure limited the number of samples for analysis in these studies, whereas other studies using saliva samples showed higher prevalence rates between 2.6 to 25%. (Turner et al., 2011) According to previous studies, the presence of high-risk HPV in normal subjects was quite varied, and has not been shown any studies on South East Asian normal subjects. (Osazuwa-Peters et al., 2015) Another finding of increasing incidence of oral/oropharyngeal cancer in females, despite a low incidence of tobacco smoking and alcohol consumption, is also interesting. (Auluck et al., 2014) Therefore, estimating the prevalence of oral HPV among female healthy adults in this region is needed to evaluate oral risk and to know the conditions compared to other regions worldwide. Possible different patterns of findings between South East Asian countries and other countries worldwide, or between Thailand and Indonesia might also be expected.

Advance of performing the research collaboration is publishing results of the prevalence of high-risk HPV strains in subsets of healthy female population of two most largest SEA countries which has not been available in the literature. The results of this study should be comparable to those of previous studies performed in countries in different parts of the world, providing possible findings of geographic differences.

Materials and Methods

Subjects

Female subjects inform consented and agreed to participate voluntarily in this study were be included. Sample size for this study is determined as 200, based on calculation of previous study in Nevada. 100 female dental students from each dental school (Faculty of Dentistry Universitas Indonesia (UI) and Faculty of Dentistry of Chiang Mai University (CMU)) were be recruited. Inclusion criteria: subject is 18 years of age or older with no previous diagnosis of oral/oropharyngeal cancer.

Saliva collection protocol

In brief, subjects who agreed to participate were be ask to collect saliva into a 50mL tube while chewing paraffin wax for 5 minutes. Eating or drinking are not allowed 30 minutes prior collection. Samples were stored on ice until transport to a biomedical laboratory for analysis. Each saliva sample from the subject was numbered randomly preventing possible research bias. Socio-demographic data of the subjects was collected. This included age, gender, marital status, sexually activity, and ethnicity.

DNA isolation

All saliva samples we centrifuged for 10 minutes at 2,000 g and the pellet washed with 1X phosphate-buffered saline (PBS) and resuspended in 5 mL of 1X PBS. DNA was isolated from the saliva sample using the GenomicPrep DNA isolation kit (Amersham Biosciences: Buckinghamshire, UK), using the procedure recommended by the manufacturer. DNA of Thai dental students was be pelleted and brought to Oral Biology Laboratory for further analysis. The DNA was resuspended in water and the DNA purity was calculated using ratio measurements of absorbance at 260 and 280 nm using spectrophotometer (A260/A280 ratio between 1.7 and 2.0).

Polymerase chain reaction (PCR)

DNA from each sample was then used to perform PCR using the following primers for HPV16 (O'Leary et al., 1994), HPV18, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Turner et al., 2011). One microgram of DNA was used for each reaction. PCR reaction was be optimized for this study, however previous condition used by other study would also be referred. (Initial denaturation: 3 mins at 94°C; 30 cycles: denaturation 30 secs at 94°C, annealing 60 secs at 58°C, and extention 30 secs at 72°C; Final extension : 5 mins at 72°C). The PCR reaction products were separated by gel electrophoresis using Reliant 4% NuSisseve® 3:1 Plus Agarose gels (Lonza: Rockland, ME). Bands were visualized by UV illumination of ethidium-bromide-stained gels and captured using a Kodak Gel Logic 100 Imaging System and 1D Image Analysis Software (Eastman Kodak: Rochester, NY). PCR product was purified and sequenced for confirmation of HPV16, HPV18 dan GAPDH gene.

Analytic sensitivity

The CaSki cell line DNA (containing of HPV16) and HeLa cell line DNA (containing HPV18) were used as positive control for PCR analysis. For control of DNA integrity, housekeeping gene of GAPDH was used.

Statistical analysis

Chi-Square and Fisher exact were used for data analysis. $P<0.05$ was considered to be statistically significant.

Results

A total of 200 saliva samples were collected from 100 female dental students of Universitas Indonesia and Chiang Mai University between November 2013 and January 2014. The age, marital status and sexual experience of the subjects whom samples were collected and screened. These data were not different statistically between the two universities (Table 2). Only 1% (n=2/200) of subjects were married and 2.5% (n=5/200) had already

Table 1. Primer Sequences of HPV16, HPV18 and GADPH Used for PCR

Target Gene	Primers		Products (bp)
	Forward	Reverse	
HPV16	TCAAAAGCCACTGTGTCCTG	CGTGTTCTTGATGATCTGCAA	120
HPV18	ATGGCGCGCTTTGAGGATCC	GCATGCGGTATACTGTCTCT	188
GADPH	ATCTTCCAGGAGCGAGATCC	ACCACTGACACGTTGGCAGT	503

Table 2. Demographic Characteristic

Variable	Answer	UI	CMU	Both	Statistical analysis
Age (year)		21.71±3.53	21.23±2.82	21.5±1.41	p=0.2894
Marital Status (%)	Y	2	0	1	
	N	98	100	9	p=0.49
Have ever had sexual intercourse (%)	Y	3	2	2.5	
	N	97	98	97.5	p=1
Having multiple sexual partners (%)	Y	0	0	0	
	N	97	98	100	

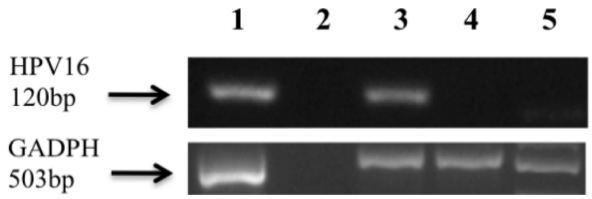

Figure 1. Upper panel: Gel electrophoresis of PCR product of HPV16 (120bp): Lane 1 positive control for CasKi cell; Lane 2 negative control; Lane 3 positive sample; Lane 4 negative sample; Lane 5 molecular weight marker, **Lower panel:** Gel electrophoresis of PCR product of GADPH (503bp): Lane 1 positive control for CasKi cell; Lane 2 negative control; Lane 3 positive sample; Lane 4 negative sample; Lane 5 molecular weight marker

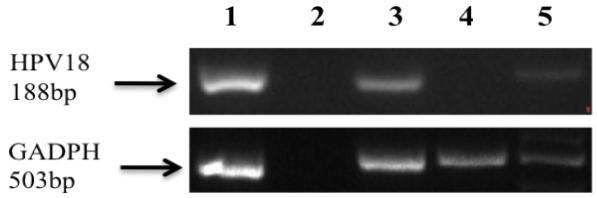

Figure 2. Upper panel: Gel electrophoresis of PCR product of HPV18 (188bp): Lane 1 positive control for HeLa cell; Lane 2 negative control; Lane 3 positive sample; Lane 4 negative sample; Lane 5 molecular weight marker, **Lower panel:** Gel electrophoresis of PCR product of GADPH (503bp): Lane 1 positive control for HeLa cell; Lane 2 negative control; Lane 3 positive sample; Lane 4 negative sample; Lane 5 molecular weight marker

Table 3. Analysis of HPV Screening

Variable		n	HPV16	Statistical analysis	HPV18	Statistical analysis
Marital status (%)	Married	2	2	p=0.0075	0	p>0.05
	Not	98	6	RR=0.51, OR=0.03	4	
Having ever had sexual intercourse (%)	Had	5	6	p=0.024	0	p>0.05
	Never	195	2	RR=0.71, OR=0.07	4	
Having multiple sexual partners (%)	Yes	0	2	p=0.001	0	NA
	No	200	6	RR=0, OR=0.006	4	

had sexual intercourse, however all subjects did not have multiple sexual partners.

The extracted DNA was screened for the presence of HPV16 and HPV18 using PCR (Figure 1). The screening showed that 12 saliva samples were HPV-positive, represented 6% of the total samples (n=12/200). Eight out of 12 (4%) samples were HPV16 positive and 4 (2%) samples were HPV18 positive. Seven out of 8 HPV16 positive samples and all samples with HPV18 were saliva samples from Indonesian students. None saliva samples from Thai students were positive for HPV18. None saliva samples that were positive for HPV16 were also positive for HPV18 and vice versa. The PCR products had been sequenced and confirmed for HPV16 and HPV18 gene.

This screening determined 12 samples (6%) out of 200 samples harboring HPV16 or HPV18 DNA, a simple statistical analysis was performed using Fisher's Exact test that also analyze the relative risk an odds ratio. The analysis revealed that HPV16 were found more in subjects who had married and had sexual intercourse and the difference was statistically significant (Table 3). In contrast, no difference was found between finding of HPV18 with the same parameters. Risk of detection of HPV16 was also increased with the above mentioned parameters (Table 3).

Discussion

The aim of this study was to estimate the presence of high-risk HPV in normal female subjects in two South East Asian countries to start a basic information about HPV prevalence among healthy subjects in this world region. Female subjects were chosen as the population of this study since the incidence of oral/oropharyngeal cancer is

increasing in female despite the tobacco and alcohol usage.

This study successfully collected and screened a total of 200 saliva samples from healthy female dental students of Universitas Indonesia and Chiang Mai University. The students were all healthy and the age was older than18 year old. The participation to this study was voluntary and the usage of saliva as the biological specimens resulted in good participation rate to the study. This study revealed 6% prevalence of high risk HPV in the saliva of healthy females adults. The result was approximately similar with previous studies on healthy adults, which ranged from 1.3-7%. (Chow et al., 2007; Flake et al., 2012) This study only included small number of female subjects in a dental setting situation, further investigation in larger groups of female to determine more information on HPV prevalence in the oral cavity is required.

Data on marital status, experience of sexual intercourse and safe sexual practice were collected and related to the HPV screening results. Viral exposure and infection are most commonly found in sexually active individuals. (Lima et al., 2013) The prevalence of this viral infection is declining with increasing age, as there are reduction in sexual activity and immune response development that cleared the infection. (Sellors et al., 2003) The characteristic of sexual transmission of HPV infection and its high prevalence in young adults makes the rationale of inclusion of female in their active sexual age in this study. Only 1% of subjects were married and 2.5% subjects of this study have had sexual intercourse. This study revealed that HPV16 were found more in subjects who were married, had sexual intercourse and had multiple sex partner than those who were not (Table 3). However, the results of only 6% subjects with HPV could not be used for drawing any boarder conclusion. The result that showed that HPV16 was more prevalently detected from the samples was similar to other studies (Flake et al., 2012; Gan et al., 2014)

Of all 12 samples that were positive for HPV, 10 (83%) samples were collected from subjects who were not married and had never had sexual intercourse. There is increasing evidence about nonsexual transmission of HPV in the literature. (Ryndock et al., 2014) A study reported possible nonsexual transmission of HPV by finding of positive HPV in genital samples of female virgins. It showed that half of the subjects who had no sexual intercourse were positive for HPV, while 70% of subjects who were sexually active. Status of female virginity is considered a very delicate issue to be uncovered, especially in eastern culture. (Galazios et al., 2008) Therefore, possible inaccuracy of virginity data may be present and this might have happened in this study. Nonsexual transmission of HPV also includes vertical transmission and direct or indirect contact with objects or surfaces that contaminated with the virus. (Hager et al., 2009; Ryndock et al., 2014) Previous study showed that HPV16 can survive after treatment of several clinical disinfectants. (Meyers et al., 2014) This study used saliva as biological specimen for detecting HPV. Self inoculation of the virus might be responsible for the positive finding in students with no history of sexual intercourse. Previous studies in a hospital and a university revealed positive HPV DNA detection in the fingers of infected individuals, supporting the possible transmission to other individuals via surface contact. (Sonnex et al., 1999; Winer et al., 2010)

The presence of oncogenic HPV in the saliva of the subjects in this study by means of PCR for E6 region of the virus is not necessarily infection by the virus. The positivity of this test is still a subject of discussion, since there is still unclear whether the subjects with positive result would develop oral/oropharyngeal cancer, although risk of developing HPV-related cancers and move for referral and monitoring are suggested. In the United States, it was estimated that there were 6 millions new infections occurred every year, while only 0.5% would develop HPV-related malignancy. (Braakhuis et al., 2004) The most important factor in HPV infection is the presence of epithelium breach that allow the acquired HPV to transmit to basal layer of the tissue. (Conway et al., 2009) It was postulated that for HPV to be oncogenic and be able to induced cancer, is related to its condition in the host genome. Integrated form instead of episomal form of HPV DNA in the host genome would facilitate the virus to be oncogenic. The success rate of integration of the HPV DNA into the host genome is quite low, therefore the presence of salivary HPV DNA does not always mean that a patient is in higher risk of developing cancer. There is also theory of HPV clearance by the host immune systems that could prevent the virus to be integrated to the host genome.

Several limitations were present in this study. The number of individuals screened for high risk HPV in this study was relatively smaller than previous studies (Chen et al., 2012; Chen et al., 2013) and chosen using convenient sampling method. However, the number of healthy individuals used in this study was still acceptable compares to previous studies ranging from 16-151 subjects (Zhao et al., 2005; Sayyah-Melli et al., 2011; Turner et al., 2011; Ahn et al., 2014). Demographic data of the subjects of this study was limited and did not include information regarding social status and behavioral data such as smoking and or alcohol use. Inclusion of such data in future study may provide additional profound understanding of the pattern of HPV infection. (Tachezy et al., 2009) Finally, further investigation on the subjects who had HPV in their saliva would be interesting to gain insights on HPV pattern of infection and if possible detecting or preventing presence of disease progression. (Chen et al., 2013)

In conclusion, this study recruited 200 healthy female adults for saliva collection and screening for high-risk HPV DNA. Successfully detected a number of saliva with the presence of HPV16 and HPV18 DNA in these dental school students. Several variables consisted marital status, experience of sexual intercourse and safe sexual practice are related to the possibility of HPV DNA finding in the saliva. As the practicing professionals, dentists and physicians and other health care professionals may gain significant value from the finding of this study, which is understanding the nature of HPV infection and its risk to patients' health and disease.

Acknowledgements

This study was supported by Global Research Initiative Funding 2013 (No.5625/H2.R12/HKP.05.00/2013) from the Universitas Indonesia. The authors sincerely thankful for Ms. Dessy Ashari, Ms. Maysaroh, Dr. Pareena, and Dr. Ahmad Ronal assistance in the study.

References

Ahn SM, Chan JY, Zhang Z, et al (2014). Saliva and plasma quantitative polymerase chain reaction-based detection and surveillance of human papillomavirus-related head and neck cancer. *JAMA Otolaryngol Head Neck Surg*, **140**, 846-54.

Auluck A, Walker BB, Hislop G, et al (2014). Population-based incidence trends of oropharyngeal and oral cavity cancers by sex among the poorest and underprivileged populations. *BMC Cancer*, **14**, 316.

Braakhuis BJ, Snijders PJ, Keune WJ, et al (2004). Genetic patterns in head and neck cancers that contain or lack transcriptionally active human papillomavirus. *J Natl Cancer Inst*, **96**, 998-1006.

Bray F, Ren JS, Masuyer E, et al (2013). Global estimates of cancer prevalence for 27 sites in the adult population in 2008. *Int J Cancer*, **132**, 1133-45.

Bunnell A, Pettit N, Reddout N, et al (2010). Analysis of primary risk factors for oropharyngeal cancer from select US states with increasing rates. *Tob Induc Dis*, **8**, 5.

Chen KM, Stephen JK, Ghanem T, et al (2013). Human papilloma virus prevalence in a multiethnic screening population. *Otolaryngol Head Neck Surg*, **148**, 436-42.

Chen KM, Worsham MJ, Stephen JK, et al (2012). HPV Prevalence in a Multi-ethnic Screening Population. *Otolaryngol Head Neck Surg*, **147**, 59-60.

Conway MJ, Meyers C (2009). Replication and assembly of human papillomaviruses. *J Dent Res*, **88**, 307-17.

de Vuyst H, Alemany L, Lacey C, et al (2013). The burden of human papillomavirus infections and related diseases in sub-saharan Africa. *Vaccine*, **31**, 32-46.

Diao MK, Liu CY, Liu HW, et al (2015). Integrated HPV genomes tend to integrate in gene desert areas in the CaSki, HeLa, and SiHa cervical cancer cell lines. *Life Sci*, **127**, 46-52.

do Sacramento PR, Babeto E, Colombo J, et al (2006). The prevalence of human papillomavirus in the oropharynx in healthy individuals in a Brazilian population. *J Med Virol*, **78**, 614-8.

Flake C, Arafa J, Hall A, et al (2012). Screening and detection of human papillomavirus (HPV) high-risk strains HPV16 and HPV18 in saliva samples from subjects under 18 years old in Nevada: a pilot study. *BMC Oral Health*, **12**, 43.

Gan LL, Zhang H, Guo JH, et al (2014). Prevalence of human papillomavirus infection in oral squamous cell carcinoma: a case-control study in Wuhan, China. *Asian Pac J Cancer Prev*, **15**, 5861-5.

Galazios G, Tsikouras P, Liberis V, et al (2008). Attitudes towards contraception in three different populations. *Clin Exp Obstet Gynecol*, **35**, 22-6.

Hager WD (2009). Human papilloma virus infection and prevention in the adolescent population. *J Pediatr Adolesc Gynecol*, **22**, 197.

Lang Kuhs KA, Gonzalez P, Struijk L, et al (2013). Costa Rica Vaccine Trial Group. Prevalence of and risk factors for oral humanpapillomavirus among young women in Costa Rica. *J Infect Dis*, **208**, 1643-52.

Lima EG, de Lima DB, Miranda CA, et al (2013). Knowledge about HPV and screening of cervical cancer among women from the Metropolitan Region of Natal, Brazil. *ISRN Obstet Gynecol*, **2013**, 930479.

Mao EJ (1995). Prevalence of human papillomavirus 16 and nucleolar organizer region counts in oral exfoliated cells from normal and malignant epithelia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, **80**, 320-9.

Meyers J, Ryndock E, Conway MJ, et al (2014). Susceptibility of high-risk human papillomavirus type 16 to clinical disinfectants. *J Antimicrob Chemother*, **69**, 1546-50

O'Leary JJ, Browne G, Johnson MI, et al (1994). PCR in situ hybridisation detection of HPV16 in fixed CaSki and fixed SiHa cell lines. *J Clin Pathol*, **47**, 933-8.

Osazuwa-Peters N, Wang DD, Namin A, et al (2015). Sexual behavior, HPV knowledge, and association with head and neck cancer among a high-risk group. *Oral Oncol*, **51**, 452-6.

Rampias T, Sasaki C, Psyrri A (2014). Molecular mechanisms of HPV induced carcinogenesis in head and neck. *Oral Oncol*, **50**, 356-63.

Rushatamukayanunt P, Morita K, Matsukawa S, et al (2014). Lack of association between high-risk human papillomaviruses and oral squamous cell carcinoma in young Japanese patients. *Asian Pac J Cancer Prev*, **15**, 4135-41.

Saheb Jamee M, Boorghani M, Ghaffari SR, et al (2009). Human papillomavirus in saliva of patients with oral squamous cell carcinoma. *Med Oral Patol Oral Cir Bucal*, **14**, 525-8.

Sayyah-Melli M, Kazemi-Sheshvan M, Bonyadi M, et al (2011). Detection of human papillomavirus in the saliva of women with concurrent human papillomavirus related genital lesions. *Saudi Med J*, **32**, 141-6.

Sellors JW, Karwalajtys TL, Kaczorowski J, et al (2003). Survey of HPV in Ontario Women Group. Incidence, clearance and predictors of human papillomavirus infection in women. *CMAJ*, **168**, 421-5.

Sonnex C, Strauss S, Gray JJ (1999). Detection of human papillomavirus DNA on the fingers of patients with genital warts. *Sex Transm Infect*, **75**, 317-19.

Suh DH, Lee KH, Kim K, Kang S, Kim JW (2015). Major clinical research advances in gynecologic cancer in 2014. J Gynecol Oncol, **26**, 156-67.

Syrjänen S, Puranen M (2000). Human papillomavirus infections in children: the potential role of maternal transmission. *Crit Rev Oral Biol Med*, **11**, 259-74.

Tachezy R, Klozar J, Rubenstein L, et al (2009). Demographic and risk factors in patients with head and neck tumors. *J Med Virol*, **81**, 878-87.

Turner DO, Williams-Cocks SJ, Bullen R, et al (2011). High-risk human papillomavirus (HPV) screening and detection in healthy patient saliva samples: a pilot study. *BMC Oral Health*, **11**, 28.

Vatanasapt P, Suwanrungruang K, Kamsa-Ard S (2011). Epidemiology of oral and pharyngeal cancers in Khon Kaen, Thailand: a high incidence in females. *Asian Pac J Cancer Prev*, **12**, 2505-8.

Winer RL, Hughes JP, Feng Q, et al (2010). Detection of genital HPV types in fingertip samples from newly sexually active female university students. *Cancer Epidemiol Biomarkers Prev*, **19**, 1682-5.

Zhao M, Rosenbaum E, Carvalho AL, et al (2005). Feasibility of quantitative PCR-based saliva rinse screening of HPV for head and neck cancer. *Int J Cancer*, **117**, 605-10.