Prevalence of Human Papillomavirus 16&18 in Oral Cavity and Oropharyngeal Squamous Cell Carcinoma in Rajavithi Hospital

Manus Potaporn MD*, Porn-Ake Apipan MD*, Suchada Suphanpayak MSc**

** Otolaryngologist at ENT Department, Rajavithi Hospital, Bangkok, Thailand*
*** Department of Pathology, Rajavithi Hospital, Bangkok, Thailand*

Background: *Recently, the incidences of oral cavity and oropharyngeal squamous cell carcinomas have increased and they are common in several regions of the world. Many risk factors are attributed to oral cavity and oropharyngeal cancers, including betel nut chewing, tobacco exposure, oral hygiene, alcohol consumption and infection by the human papilloma virus 16 and 18.*
Objective: *To determine the prevalence of HPV16&18 in the oral cavity and oropharyngeal squamous cell carcinoma in Rajavithi Hospital.*
Material and Method: *The examination samples were collected from Formalin-fixed, paraffin-embedded (FFPE) tissue blocks from 78 Patients at Rajavithi Hospital, between 1st September 2011 and 30th September 2013. The detection of HPV16 and HPV18 was carried out using real time PCR technique.*
Results: *HPV DNA was detected in 78 samples (in 7 oropharyngeal specimens and 71 in the oral cavity). Three cases were positive for HPV16 and only 1 case was positive for HPV18. HPV16&18 were detected in 3.85% and 1.28% of all samples respectively. The only factor associated with HPV 16&18 infection was age.*
Conclusion: *The prevalences of human papilloma virus (HPV16 and 18) infection in the oral cavity and oropharyngeal squamous cell carcinoma in this study were found to be low (5.13%). Human papilloma virus 16 and 18 infection may not play an important role in this group of Thai patients.*

Keywords: *Human papillomavirus 16 and 18, Oral cavity, Oropharyngeal, Squamous cell carcinoma*

J Med Assoc Thai 2018; 101 (Suppl. 2): S1-S8
Full text. e-Journal: *http://www.jmatonline.com*

The majority of head and neck cancers are associated with high tobacco and alcohol consumption. However, increasing trends in the incidence at specific sites suggest that other etiological factors are involved. Infection by certain high-risk types of human papillomavirus (i.e. HPV 16) has been reported to be associated with head and neck cancers, in particular with oropharyngeal cancer. Current evidence suggests that HPV 16 is associated with tonsil, base of tongue and other oropharyngeal cancers. Associations with other head and neck cancer sites such as oral cancer are neither strong nor consistent when compared to molecular-epidemiological data on HPV with oropharyngeal cancer while the situation with regard to laryngeal cancer is still unclear (IARC Monograph Vol 100B)[1].

Correspondence to:
Potaporn M, Otolaryngologist at ENT Department, Rajavithi Hospital, Bangkok 10400, Thailand.
Phone: +66-2-3548108, Fax: +66-2-3548146
E-mail: manus60@yahoo.com

Head and neck cancers are serious public health problems in many parts of the world. Mortality from cancer was found 0.9% in Africa, 1.5% in America, 2.4% in Asia, 2.7% in Europe and 1.3% in Oceania. Moreover, the incidence of cancer in South-East Asia constitutes 2.1% of cancers among men and 0.5% cancers among women[1].

The etiology of oral cavity and oropharyngeal squamous cell carcinoma (OSCC) is considered to be multifactorial and the potential factors include environmental factors such as life style, infectious agents and genetics. The main factors are tobacco and alcohol abuse[2]. A study of the carcinogenicity of HPV in humans was conducted by the International Agency for Research on Cancer (IARC) in 2007 and 2012[3,4]. The IARC Monograph concluded that in the oral cavity, there was sufficient evidence for the carcinogenicity of HPV16, and limited evidence for that of HPV 18.

The human papilloma virus (HPV) is one of the most common viruses in the world, affecting the skin and mucosa of the body. Different types of HPV

are known to infect different parts of the body. Genital warts technically known as condylomata acuminatum are generally associated with two HPV types, 6 and 11, both of which can be sexually transmitted. Two other types of genital tract HPV, HPV16 and HPV18, are known to cause up to 95% of cervical cancers, and new studies have shown that they may be linked to oral cancer as well. Both of these genital viruses are spread through sexual contact.

In 1983, Syrjanen et al implicated HPV as a risk factor in the development of oral cancer[5]. Since then, several studies have focused on HPV detection in oral cancer[6]. A more recent study by Syrjanen K and Syrjanen S showed a strong association between the presence of HPV DNA, especially HPV16, and OSCC[7]. This meta-analysis showed that HPV significantly increased the risk of OSCC compared with controls. The prevalence of HPV-positive oropharyngeal cancer varied according to the geographical region, i.e. in North America 56%, in Japan 52%, in Australia 45%, and in Northern and West Europe 38%[8].

In Thailand, the prevalence of HPV infection in each subsite of head and neck squamous cell and its route of infection are still unclear, possibly due to differences in life style and carcinogen exposure including different detection techniques.

Different techniques have been used to detect HPV, including in situ hybridization, Southern blot hybridization, dot blot hybridization, Hybrid Capture 2 (HC_2), conventional polymerase chain reaction (PCR), and real-time PCR[9]. In situ hybridization, Southern blot and dot blot hybridization are time-consuming procedures that require relatively large amounts of purified DNA[9]. Hc2 assay cannot genotype single HPV subtypes[9]. Of these methods, studies using PCR techniques have reported a higher sensitivity for HPV detection[10]. However, conventional PCR assay was found to have a lower sensitivity of 92% and a specificity of 97% in detecting HPV, and it is able to genotype and quantify HPV viral load[11].

The aim of this study was to determine the prevalence of HPV16&18 in the oral cavity and oropharyngeal squamous cell carcinoma in Rajavithi Hospital using a real time PCR technique, and to specify its correlation with demographic data, possible risk factors and histological variables.

Material and Method

This cross-sectional study was performed in Rajavithi Hospital. The study protocol was reviewed and approved by the institute's Ethics Committee. A total of 115 participants were enrolled from the department of ENT at Rajavithi Hospital between September 1st 2011 and September 30th 2013. The inclusion criteria were as follows: patients who were new cases of oral cavity and oropharyngeal squamous cell carcinoma, and who signed the consent form. The exclusion criteria were as follows: history of chemotherapy, radiation therapy, recurrence of tumor, or other types of cancer. Tissue samples were sent to the Department of Pathology, and histological diagnoses were made by a pathologist.

DNA extraction from Formalin-fixed, paraffin-embedded (FFPE) tissue

Sections were cut from FFPE tissue, and DNA was extracted using a QIAamp DNA FFPE kit for DNA extraction.

The extraction protocol was as follows:

1) Using a scalpel, trim excess paraffin off the sample block.

2) Cut up to 8 sections 5 to 10 µm thick.

3) Immediately place the sections in a 1.5 or 2 ml microcentrifuge tube and add 1 ml xylene to the sample. Close the lid and vortex vigorously for 10 seconds.

4) Centrifuge at full speed for 2 minutes at room temperature (15 to 25°C).

5) Remove the supernatant by pipetting. Do not remove any of the pellets.

6) Add 1 ml ethanol (96 to 100%) to the pellet, and mix by vortexing. The ethanol extracts residual xylene from the sample.

7) Centrifuge at full speed for 2 minutes at room temperature.

8) Remove the supernatant by pipetting. Do not remove any of the pellets.

9) Open the tube and incubate at room temperature or up to 37°C. Incubate for 10 minutes or until all residual ethanol has evaporated.

10) Resuspend the pellet in 180 µl buffer ATL. Add 20 µl proteinase K, and mix by vortexing.

11) Incubate at 56°C for 1 h (or until the sample has been completely lysed).

12) Incubate at 90°C for 1 hour.

13) Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.

14) Add 200 µl buffer AL to the sample, and mix thoroughly by vortexing. Then add 200 µl ethanol (96 to 100%), and mix again thoroughly by vortexing.

15) Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.

16) Carefully transfer the entire lysate to the QiAamp MinElute column (in a 2 ml collection tube) without wetting the rim, close the lid, and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the flow-through in the collection tube.

17) Carefully open the QIAamp MinElute column and add 500 µl buffer AW1 without wetting the rim. Close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

18) Carefully open the QIAamp MinElute column and add 500 µl Buffer AW2 without wetting the rim. Close the lid and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

19) Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 minutes to dry the membrane completely.

20) Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute column and apply 20 to 100 µl buffer ATE to the center of the membrane.

21) Close the lid and incubate at room temperature for 1 min. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 min.

We tested the quality of DNA using a spectrophotometer (ratio 260/280 nm).

Real time PCR

We tested real time PCR using CFX 96, Biorad. For this step, we used HPV 16 and 18 real time PCR kit, Shanghai ZJ Biotech Co. Ltd. (Cat. No. TD-0030-02). Real time PCR was performed according to the kit protocol: 35 µl reaction mix +0.4 µl enzyme mix +1 µl internal control +4 µl DNA template, programmed for real time as follows: 37°C for 2 minutes 1 cycle, 94°C for 2 minutes, 1 cycle, 93°C for 15 seconds, 40 cycles and 60°C for 1 minute, 40 cycles. VIC channels were selected for target nucleic acid, FAM channel of fluorescence, and for internal control. The cycle threshold (CT) value for the test was <35 and for the internal control was 25-33.

Statistical analysis

Data were analyzed using SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA). Baseline characteristics were analyzed using descriptive statistics such as number, percentage, mean and standard deviation, minimum and maximum. Chi-square test was used to compare the categorical variables and frequency difference. Independent t-test was used to compare continuous variables. A *p*-value less than 0.05 was considered as statistically significant.

Results

One hundred and fifteen patients participated in this study, but only 74% (86/115) completed the questionnaires while 6.9% (8/115) had no tumor lesion in FFPE tissue; thus, only 78 patients were enrolled in this study. All patients' characteristics, risk factors, sites of tumor and sexual history are shown in Table 1 and 2.

All patients in this study were Thai, and 46 patients (58.96%) were male. The mean age of the studied patients was 55.57±13.58 years in the HPV negative group and 72.00±11.34 years in the HPV positive group. Patients in the HPV positive group were significantly older (*p*-value = 0.027). The majority of the patients were farmers (agriculturists) and other people with lower education (primary school) levels. Interestingly, the majority of the patients in this group neither had repeated trauma (75 patients) nor genital warts (77 patients). There were 58 patients who had no cancer history in their family members, and another 58 had poor oral hygiene.

From a total of 78 patients, 38 were former smokers and 35 were drinkers. Sixty-two and 71 patients had no history of betal nut chewing and tobacco chewing respectively.

A total of 78 patients with oral cavity cancer (71) and oropharyngeal cancer (7) were included in this study. The most frequent site of oral cavity cancer was the tongue (31 out of 71, 43.66%).

The risk factors for HPV infection were evaluated by questionnaire, the mean age of 1st sexual intercourse was 18 to 25 years, 62 patients had multiple sexual partners and 68 patients had no history of oral sex.

Real time PCR results showed that of all the patients in this group, 3.85% (3/78) had positive results for HPV16 and 1.28% (1/78) was positive for HPV18. Amplication curves for HPV 16 and 18 are shown in Fig. 1 A, B and 2.

In this study, HPV 16&18 infection were identified in 5.13% of the patients with oral cavity squamous cell carcinoma (4 out of 71), but none in those with oropharyngeal squamous cell carcinoma (0

Table 1. Characteristics (n = 78)

Variables	HPV 16&18		*p*-value
	Negative (n = 74)	Positive (n = 4)	
Age (years)	55.57±13.58	72.00±11.34	0.027*
Sex			0.710
Male	44 (59.5)	2 (50.0)	
Female	30 (40.5)	2 (50.0)	
Education			0.840
Primary or lower	52 (70.3)	3 (75.0)	
Secondary or upper	22 (29.7)	1 (25.0)	
Occupation			0.582
None	12 (16.2)	0 (0.0)	
Agriculture	31 (41.9)	3 (75.0)	
Labor	15 (20.3)	1 (25.0)	
Merchant	10 (13.5)	0 (0.0)	
Officer	5 (6.8)	0 (0.0)	
Others	1 (1.4)	0 (0.0)	
Cancer in Member family			0.976
No	55 (74.3)	3 (75.0)	
Yes	19 (25.7)	1 (25.0)	
Oral hygiene			0.976
Good	19 (25.7)	1 (25.0)	
Poor	55 (74.3)	3 (75.0)	
Repeated Trauma			1.000
No	71 (95.9)	4 (100)	
Yes	3 (4.1)	0 (0.0)	
Genital warts			0.815
No	73 (98.6)	4 (100.0)	
Yes	1 (1.4)	0 (0.0)	

Values are represented as n (%), mean ± SD, * = Significant at $p<0.05$.

out of 7). In all of the HPV positive patients with oral cavity squamous cell carcinoma, three cases were positive for HPV16 and only one case was positive for HPV18.

Discussion

In the past few decades, there has been speculation worldwide about the role of HPV in the pathogenesis of head and neck squamous cell carcinoma (HNSCC). The most commonly detected HPV, HPV16, accounts for 90% of the HPV DNA-positive cases in HNSCC, followed by HPV18 and other high-risk subtypes[12]. However, the detection rate of HPV in oral squamous cell carcinoma (OSCC) and oral potentially malignant disorders (OPMD) varies widely and remains controversial[13,14]. This variation may be due to differences in the types of sample, detection methods and geographic locations[13,15]. Therefore, confirming the HPV infection rate in OSCC and OPMD cases may contribute to the study of carcinogenesis in the oral cavity[16,17]. In this study, we used real time PCR to detect HPV16 and HPV18 in FFPE samples of Thai oral cavity and oropharyngeal squamous cell carcinoma patients.

We found that four of the patients with oral cavity squamous cell carcinoma had HPV16 & HPV18. The low prevalence in our sample implies that HPV 16&18 infection may not be common in Thai patients suffering from oral cavity squamous cell carcinoma.

Yadav et al showed that the HPV DNA detection limit for conventional PCR was 200 copies, whereas for real time PCR, which has a higher sensitivity, detecting HPV DNA required only 1 copy[18]. Lingen et al detected high-risk HPV DNA in 9.8% of OSCC cases using consensus primer PCR, but the positive rate was 6.6% using real time PCR[19]. Scapoli et al found the detection rate of HPV16 to be 2% in OSCC with real time PCR[20]. Real time PCR shows a

Table. 2 Association between risk factors and HPV16 &18 infection (n = 78)

Risk factors	HPV 16&18		*p*-value
	Negative (n = 74)	Positive (n = 4)	
Betal nut chewing			0.470
No	59 (79.7)	3 (75.0)	
Yes	10 (13.5)	0 (0.0)	
Ex-chewer	5 (6.8)	1 (25)	
Tobacco chewing			0.379
Yes	7 (9.5)	0 (0.0)	
No	67 (90.5)	4 (100)	
Tobacco exposure			0.591
Non-smoker	29 (39.2)	2 (50.0)	
Former smoker	36 (48.6)	2 (50.0)	
Current smoker	9 (12.2)	0 (0.0)	
Alcohol drinking status			0.668
Never drank	30 (40.5)	2 (50.0)	
Ex-drinker	34 (45.9)	1 (25.0)	
Current drinker	10 (13.6)	1 (25.0)	
Site of tumor			
Oral cavity			1.000
Yes	70 (94.6)	4 (100.0)	
No	4 (54.4)	0 (0.0)	
Oropharyngeal			1.000
Yes	7 (9.5)	0 (0.0)	
No	67 (90.5)	4 (100.0)	
Sexual history			
Age at 1st sex			0.862
Never	6 (8.1)	0 (0.0)	
Under 18 years	15 (20.3)	1 (25.0)	
18 to 25 year	41 (55.4)	2 (50.0)	
Over 25 years	12 (16.2)	1 (25.0)	
Number of partners			1.000
Never	8 (10.8)	0 (0.0)	
1 to 4	58 (78.4)	4 (100)	
5 to 10	5 (6.8)	0 (0.0)	
>10	3 (4.1)	0 (0.0)	
Oral sex			0.429
Never	65 (87.8)	3 (75.0)	
Yes	9 (12.2)	1 (25.0)	
Number of oral sex partner			0.525
Never	62 (83.8)	3 (75.0)	
1	5 (6.8)	0 (0.0)	
2 to 4	6 (8.1)	1 (25.0)	
≥5	1 (1.4)	0 (0.0)	

Values are represented as n (%), * = Significant at $p<0.05$

higher sensitivity and specificity than conventional PCR assay[18,19,21]. In the current study, we utilized real time PCR and found a 5.13% detection rate of HPV 16&18 by real time PCR, which was a reliable method and provided further understanding of HPV infection in Thai patients. However, there have been no correlations between HPV 16&18 infections and demographic data, risk factors, or histological grading.

The population has also been considered to be another factor affecting rate diversification. Several

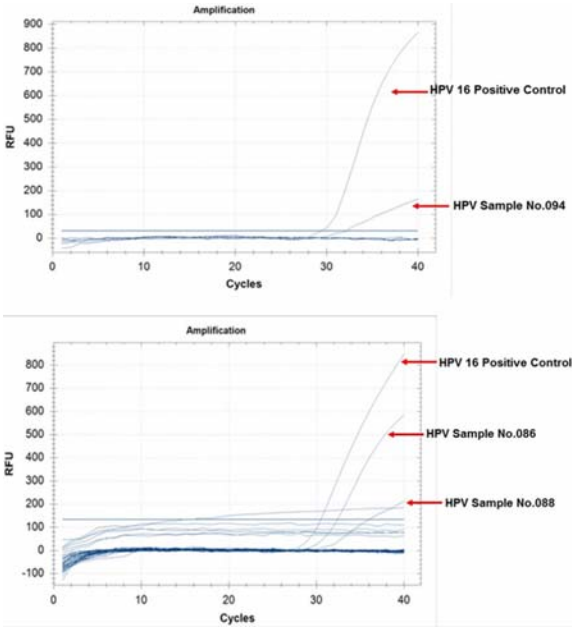

Fig. 1 Amplification curves for HPV 16 with real time PCR, showing positive samples (HPV Sample No. 094, 088 and 086) are positive for HPV 16.

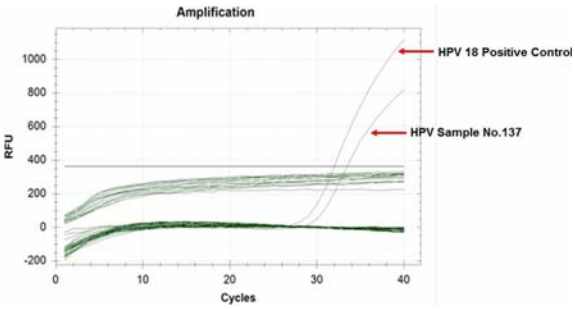

Fig. 2 Amplification curves for HPV 18 with real time PCR showing a clear positive sample (HPV Sample No. 137) together with the positive control.

countries including India[22-24], Brazil[25], Japan[26], Mozambique[27] and China[28] have revealed a zero detection rate of OSCC. Other reported detection rates were 6.6% in America[19], 5% in Mexico[29], 39.4% in Spain[30] and 66.7% in Sudan[31].

In Thailand, Khovidhunkit SO et al investigated the presence of HPV in 65 patients with oral squamous cell carcinoma, leukoplakia and lichen planus using PCR. They found only one sample of HPV positive (1/65)[31].

Results of studies performed in Thailand have varied because of the different types of samples and different methods used for detection of HPV infection. Limitations of this study were its small sample size and the small proportion of completed questionnaires.

Conclusion

The prevalence of human papilloma virus (HPV16 and 18) infection in oral cavity and oropharyngeal squamous cell carcinoma in this study was found to be low (5.13%). Human papilloma virus 16 and 18 infection, therefore, may not play an important role in this group.

What is already known on this topic?

HPV 16&18 infection may not be common in Rajavithi patients with oral cavity squamous cell carcinoma. Real-time PCR for HPV 16&18 detection is an effective method (high sensitivity and specificity).

What this study adds?

This is the first report of the prevalence of HPV16&18 in oral cavity squamous cell carcinoma using Real time PCR technique.

Acknowledgements

The authors would like to extend special thanks to Dr. Charuwan Manmee for statistical advice and approving the final manuscript, and Ms. Siriporn Monyarit for data management.

Potential conflict of interest

None.

References

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v 1.0, Cancer incidence and mortality worldwide: IARC Cancer Base No.11. Lyon, France: International Agency for Research on Cancer; 2013.
2. Garbuglia AR. Human papillomavirus in head and neck cancer. Cancers 2014; 6: 1705-26.
3. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 90. Human papillomaviruses. Lyon, France: International Agency for Research on Cancer; 2007.
4. IARC Monographs on the evaluation of carcinogenic risks to humans. A review of human carcinogens, Biological agents. Lyon: World Health Organization 2012; 255.
5. Syrjanen K, Syrjanen S, Lamberg MA, Pyrhonen S. Human papillomavirus (HPV) involvement in squmous cell lesions of the oral cavity. Proc Finn Dent Soc 1983; 79: 1-8.

6. Jalouli J, Ibrahim SO, Mehrotra R, Jalouli MM, Sapkota D, Larson PA, et al. Prevalence of viral (HPV, EBV, HSV) infections in oral submucous fibrosis and oral cancer from India. Acta Otolaryngol 2010; 130: 1306-11.
7. Syrjanen K, Syrjanen S. Detection of human papillomavirus in sinonasal carcinoma: systematic review and meta-analysis. Hum Pathol 2013; 44: 983-91.
8. Gillison M, Castellsague X, Chaturvedi A, Goodman M, Smijders P, Tommasino MA, et al. Comparative epidemiology of HPV infection and associated cancers of the head and neck and cervix. Int. J Cancer 2014; 134: 497-507.
9. Abreu AL, Souza RP, Gimenes F, Consolaro ME. A review of methods for detect human papillomavirus infection. Virol J 2012; 9: 262.
10. Ha PK, Pai SI, Westra WH, Gillison ML, Tong BC, Sidransky D, et al. Real time quantitative PCR demonstrates low prevalence of human papillomavirus type 16 in premalignant and malignant lesions of the oral cavity. Clin Cancer Res 2002; 8: 1203-9.
11. Venuti A, Paolini F. HPV detection methods in head and neck cancer. Head Neck Pathol 2012; 6: 63-74.
12. Gillson ML, Alemany L, Snijders PJ, Chaturvedi A, Steinberg BM, Schwartz S, et al. Human papillomavirus and diseases of the upper airway: head and neck cancer and respiratory papillomatosis. Vaccine 2012; 30: 34-54.
13. Gupta S, Gupta S. Role of human papillomavirus in oral squamous cell carcinoma and oral potentially malignant disorders: A review of the literature. Indian J Dent 2015; 6: 91-8.
14. Ha PK, Califano JA. The role of human papilllomavirus in oral carcinogenesis. Crit Rev Oral Biol Med 2004; 15: 188-96.
15. Campisi G, Panzaarella V, Giuliani M, Lajolo C, Di Fede O, Falaschini S, et al. Human papillomavirus: its identity and controversial role in oral oncogenesis, premalignant and malignant lesions (review). Int J Oncol 2007; 30: 813-23.
16. Chai RC, Lambie D, Verma M, Punyadeera C. Current trends in the etiology and diagnosis of HPV-related head and neck cancers. Cancer Med 2015; 4: 596-607.
17. Sathish N, Wang X, Yuan Y. Human papillomavirus (HPV)-associated oral cancers and treatment strategies. J Dent Res1 2014; 93: 29-36.
18. Yadav R, Paria A, Mankame S, Makesh M, Chaudhari A, Rajendran KV. Development of SYBR green and Tagman quantitative real time PCR assays for hepatopancreatic parvovirus (HPV) infecting Penaeus monodon in India. Mol Cell Probes 2015; 29: 442-8.
19. Lingen MW, Xiao W, Schmitt A, Jiang B, Pickard R, Kreinbrink P, et al. Low etiologic fraction for high risk human papillomavirus in oral cavity squamous cell carcinomas. Oral Oncol 2013; 49: 1-8.
20. Scapoli L, Palmieri A, Rubini C, Martinelli M, Spinelli G, Ionna F, et al. Low prevalence of human papillomavirus in squamous-cell carcinoma limited to oral cavity proper. Mod Pathol 2009; 22: 366-72.
21. Laprise C, Madathil SA, Allison P, Abraham P, Raghavendran A, Shahul HP, et al. No role for human papillomavirus infection in oral cancers in a region in southern India. Int J Cancer 2015; 138: 912-7.
22. Patharre SM, Gerstung M, Beerenwinkel N, Schaffer AA, Kannan S, Pai P, et al. Clinicopathological and prognostic implications of genetic alterations in oral cancers. Oncol Lett 2011; 2: 445-51.
23. Patel KR, Vajaria BN, Begum R, Desai A, Patel JB, Shad FD, et al. Prevalence of high-risk human papillomavirus type 16 and 18 in oral and cervical cancers in population from Gujarat, West India. J Oral Pathol Med 2014; 43: 293-7.
24. Rivero ER, Nunes FD. HPV in oral squamous cell carcinoma of a Brazillian population: amplification by PCR. Braz Oral Res 2006; 20: 21-4.
25. Maruyama H, Yasui T, ishikawa-Fujiwara T, Morii E, Yamanmoto Y, Yoshii T, et al. Human papillomavirus and p53 mutations in head and neck squamous cell carcinoma among Japanese population. Cancer Sci 2014; 105: 409-17.
26. Blumberg J, monjane L, Prasad M, Carrlho C, Judson BL. Investigation of the presence of HPV related oropharyngeal and oral tongue squamous cell carcinoma in Mozambique. Cancer Epidemiol 2015; 39: 1000-5.
27. Chen Xiao-Jie, Kai Sun, Wei-Wen Jiang. Absence of high-risk HPV 16 and 18 in Chinese patients with oral squamous cell carcinoma and oral potentially malignant disorders. Virology Journal 2016; 13: 81.
28. Gonzalez-Ramirez I, Irigoyen-Camacho ME, Ramirez-Amador V, Lizano-Soberon M, Carrillo-Garcia A, Garcia-Carranca A, et al. Association between age and high risk human papilloma virus in Mexican oral cancer patients. Oral Dis 2013; 19: 796-804.

29. Llamas-Martinez S, Esparza-Gomez G, Campo-Trapero J, Cancela-Rodriguez P, Bascones-Martinez A, Moreno-Lopez LA, et al. Genotypic determination by PCR-RFLP of human papillomavirus in normal oral mucosa, oral leukoplakia and oral squamous cell carcinoma samples in Madrid (Spain). Anticancer Res 2008; 28: 3733-41.
30. Babiker Ay, Eltom FM, Abdalaziz MS, Rahmani A, Abusail S, Ahmed HG. Screening for high risk human papilloma virus (HR-HPV) subtypes, among Sudanese patients with oral lesions. Int J Clin Exp Med 2013; 6: 275-81.
31. 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. Asia Pac J Cancer Prev 2008; 9: 771-5.