Oral Squamous Cell Carcinoma Positive for HPV Genotypes 16 and 18: A Retrospective Clinicopathologic Study in Yogyakarta, Indonesia

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Abstract

Human papillomavirus (HPV) is closely related with the incidence of OSCC. Few studies conducted in Indonesia have focused on the association of HPV genotypes with OSCC. In this study, the prevalence of HPV infection and correlate it with the clinicopathologic features of OSCC patients was investigated.

This retrospective study involved 64 FFPE blocks which diagnosed as OSCC in the year of 2011–2015. DNA extraction was conducted with a commercial kit. HPV-positive samples were genotyped using a commercial kit. Bivariate analysis was conducted to correlate clinicopathologic features with HPV genotypes.

Thirty-five out of 64 (54.7%) OSCC samples were positive for HPV. Of these cases, eight (22.86%) and 3 (8.57%) cases were positive for HPV genotypes 18 and 16, respectively. HPV infection was found in 23 out of 38 (60.50%) male patients and 22 out of 36 (61.10%) female patients aged ≥55 years old. HPV DNA was detected in 22 out of 37 (59.5%) tongue samples and in 28 out of 49 (57.1%) samples with well-differentiated histopathologic features.

Results suggested that 54.7% of OSCC patients were HPV infected. The tongue was the most common anatomical site for HPV-positive OSCC. The prevalence of well-differentiated HPV-positive OSCC was 57.1%.

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Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most commonly reported malignancy in the world and is particularly prevalent in Southeast Asia.[1,2] *Human papillomavirus* (HPV) has an important role in the carcinogenesis of several malignancies, including OSCC.[3,4] HPV is member of the *Papillomaviridae* family. It is a non-enveloped, small, circular double-stranded DNA virus approximately 8000 base pairs in length. It infects the epithelial tissues of the epidermis and mucosa. High risk (HR) genotypes are 2.8 times more commonly detected in OSCCs than low risk (LR) genotypes.[5,6] A recent study reported that HR genotypes, especially HPV genotype 16, is an etiological factor of OSCC.[1]

Epidemiological studies have revealed the high prevalence of HPV-induced OSCC in Asian countries. Asian countries have the highest HPV prevalence (0-100%) followed by Europe (0-95%), the United States of America (0-70%), and Africa (0-11.9%).[7] Ndiaye et al. (2014) reviewed 72 papers published during the period of 2000-2012 and found that HPV-positive OSCC has a prevalence of 24.2%.[8] Zhu et al. (2012) conducted a meta-analysis of 18 case control studies published during 1994-2011 and found

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that the prevalence of HPV-positive OSCC among the Chinese population is 58%.[9] A recent meta-analysis revealed that the prevalence of HPV-positive OSCC among Asia-Pacific countries is 37.55%.[10]

The high prevalence of HPV-positive OSCC in Asia indicates that the presence of this virus induces mutations during carcinogenesis. However, the relation between HR-HPV and OSCC in Indonesian patients, particularly patients in Yogyakarta, remains unknown. This study investigated the prevalence of HPV infection in Yogyakarta, Indonesia, as well as the relation of HPV with the clinicopathologic profiles of patients with OSCC.

Materials and methods

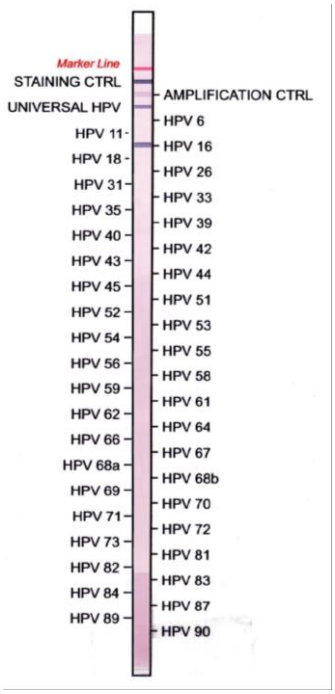
Material

Sixty-four FFPE blocks were obtained from three anatomical pathology laboratories in Yogyakarta, Indonesia. All samples were collected from patients who were diagnosed with OSCC during the period of January 2011 to December 2015. The clinical data (sex, age, anatomical site) of the patients were collected. The histopathologic features of OSCC were classified in accordance with WHO classification. This study received ethical approval from the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Gadjah Mada University (KE/FK/294/EC/2016).

Method

DNA extraction and HPV detection

A microtome was used to cut FFPE blocks into five slices. Each slice had a thickness of 5 μm. The slices were placed in a 1.5 mL microcentrifuge tube. DNA extraction was performed in accordance with the Gene All Exgene Clinic SV mini protocol (catalog No. 108_101). Samples were screened for HPV DNA using a universal primer pair synthesized from L1 ORF (F:5'-GAATATGATTTACAGTTATTTT TCA-3',R:5'-GAAAACTTTTCCTTTAAAT-3').[11] HPV DNA was amplified using the following thermocycler program: 93 °C for 5 min, 35 cycles at 93 °C for 1 min, 46.8 °C for 30 seconds, 72 °C for 1 minute, and 1 cycle at 72 °C for 1 minute. Electrophoresis was performed using 1.5% agarose gel at 100 V for 30 min. The sample was considered positive if a 250-bp band was present. All samples with positive HPV DNA bands were genotyped with Ampliquality HPV-type Express, Single-step PCR & Reverse Line Blot (AbAnalitika, catalog No. 2292-150825). Figure 1 shows the genotyping result for HPV16 positive, Figure 2 result for HPV18 positive and Figure 3 result for HPV positive, undetermined genotype.



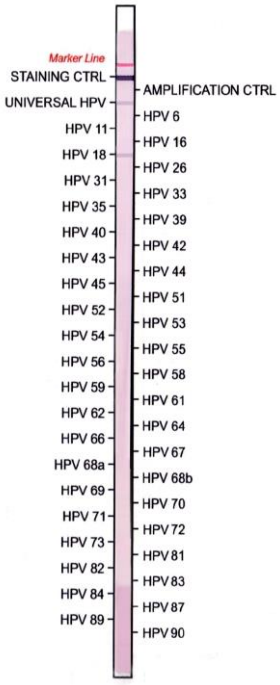


Figure 1. HPV16 Positive. **Figure 2.** HPV18 Positive.

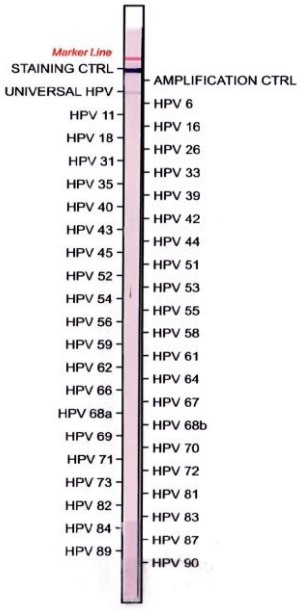


Figure 3. Genotyping of HPV Positive, Undetermined Genotype.

Data analysis

All clinicopathological feature was compared with the presence of HPV and HPV genotype. Chi-square test was carried out to evaluate the association between variables. To evaluate the strength of associations between presence of HPV and the clinicopathologic features, odds ratios (ORs) and 95% confidence interval (CIs) were calculated using binary logistic regression analysis. The statistical significance was considered at *P* value < 0.05. Statistical analysis was conducted with the IBM SPSS version 22.0 program.

Results

Characteristics of OSCC samples

Table 1 shows the distribution of clinicopathologic profiles by gender. The female-to-male ratio of recruited patients was 1:1.46. The age, anatomic location, and histopathologic features of male and female patients were not significantly different.

Prevalence and association of HPV with the clinicopathologic features of patients with OSCC

HPV DNA was detected in 35 out of 64 (54.7%) OSCC samples. Table 2 shows the relation between the clinicopathologic profiles (sex, age, anatomical site, and histopathologic features) of patients and HPV infection status. Binary logistic regression analysis showed no statistically significant relation between HPV infection status with gender, age, anatomical site, or histopathologic features (*P*>0.05)

The distribution of HPV genotypes is summarized in Table 3. HPV genotypes 16 and 18 were detected in 3 (8.6%) and 8 (22.8%) HPV-positive OSCC samples, respectively. The clinicopathologic profiles associated with these two HPV genotypes were not significantly different (*P*>0.05). Table 4 shows the clinicopathologic profile of 11 OSCC samples positive for HPV genotypes 16 and 18. The mean age of 8 patients with HPV genotype 18 was lower than that of the 3 patients with HPV genotype 16.

Discussion

This retrospective study, which was conducted in Yogyakarta, showed that OSCC is more prevalent in males than in females (female-to-male ratio = 1:1.46). This finding is similar to that reported by Rao et al. (2013), who suggested that males are more likely to have OSCC.[12] Patients recruited in the present study had a mean age of 54.58 years. The tongue is the most commonly affected cancer site in the recruited patients. The present results for age and anatomical site are similar to those of previous studies conducted in other Asian countries; these studies reported that patients with OSCC fell in the age range of 51-55 years and that the tongue is the most common anatomical site for oral cancer.[12] A report from a cancer hospital in Jakarta, Indonesia (2014) showed that tongue cancer is more prevalent in males than in females; tongue cancer is also more prevalent in the age group of 30-40 years than in other age groups.[13]

The prevalence of HPV-positive OSCC varies and is influenced by several factors, including geographic region, sample types, and HPV detection methods. Shaikh et al. (2015) suggested that among Asia-Pacific countries, Southeast Asia has the highest prevalence of HPV-positive OSCC.[10] Gan et al. (2014) and Chen et al. (2016) respectively reported that the prevalence of HPV in China is 14.04% and 27.50%.[14,15] Studies from India have shown that 7.0-9.2% of OSCC samples are positive for HPV.[16,17] One recent publication from Malaysia reported high HPV positivity OSCC compared with oral potentially malignant disorders (34.78%, 3.12%).[18] The prevalence of HPV-positive OSCC in Yogyakarta is higher than that in other regions. However, a similar study by Phusingha et al. (2016) reported that the prevalence of HPV in Thailand is 56.2%.[19]

Patients with HPV-positive or HPV-negative OSCC exhibited different profiles for gender, age, and anatomical site distribution. Males are more likely to have HPV-positive OSCC than females, although several studies have also shown that females are more likely to have HPV-positive OSCC than males.[10,19,20] Saini et al. (Malaysia, 2011) and Phusingha et al. (Thailand, 2016) reported that the mean age of patients with HPV-positive OSCC is >50 years.[19,20] Other reports have shown that HPV-

positive OSCC is highly prevalent among patients <50 years of age.[16,17,21] The present study found that the majority of patients with HPV-positive OSCC were male and ≥55 years old. Several lifestyle components are related to the high prevalence of oral HPV infection among males. Global data have previously shown that the high prevalence of HPV-positive head and neck cancer among male patients is related with the limited or absent tobacco and/or alcohol consumption. However, recently published data from Asian countries have shown that the high incidence of oral HPV infection among males is associated with tobacco and/or alcohol consumption.[10] The tobacco and alcohol consumption changes the cellular immune response to bacterial and viral infection. Tobacco contains carcinogens that modify immune cell response; thus, individuals who consume tobacco become highly susceptible to viral infection, including HR-HPV.[22] Indonesia has the second-highest highest smoking rates among males in the world.[2]

HPV genotype 16 is the most commonly detected HPV genotype in patients with OSCC. Shaikh et al. (2015) reported that in the Asia-Pacific region, the prevalence of HR-HPV genotype 16 in patients with OSCC is 50-68%, followed by that of HPV genotype 18.[10] Oral infection with HPV genotype 16 is strongly related with oropharyngeal cancer, head and neck cancer, and oral cancer.[12,23] The ORs of OSCC and HPV genotype 16 reported by these studies are higher than those reported by Zhu et al.[9] HPV genotypes 16 and 18 have been detected in the saliva samples of 10 (41.7%) and 13 patients (54.2%), respectively.[24] Out of 178 OSCC cases, Chen et al. (2016) detected HPV genotypes 18 and 16 in 19 (10.67%) and six cases (3.37%), respectively.[15] Interestingly, the results of the present study showed that HPV genotype 18 is more prevalent than HPV genotype 16. HPV genotype 18 was detected in eight cases, whereas HPV genotype 16 was detected only in three cases. This study showed that the mean age of patients positive for HPV genotype 18 was lower than that of patients positive for HPV genotype 16. Twenty-four cases were undetermined HPV genotypes. Therefore, future investigations are required to determine HPV genotypes using sequencing methods.

Conclusions

HPV DNA was detected in 35 out of 64 OSCC cases (54.7%). The tongue was the most affected site of HPV-positive OSCC. Histopathologic feature of well-differentiated was prominent in HPV-positive OSCC. A large-scale cohort study should be conducted to confirm the results of the present study.

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Declaration of Interest

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	Total (n = 64)		Female (n = 26)		Male (n = 38)		*p*-value
	n	%	n	%	n	%	
Age							
< 55 years	28	43.7	10	35.7	18	64.3	0.48[a]
≥ 55 years	36	56.3	16	44.4	20	55.6	
Mean ± SE	54.58±1.60		56.31±2.93		53.39±1.82		0.07
Anatomic site							
Lips	7	10.9	5	71.4%	2	28.6%	0.06[b]
Tongue	37	57.8	16	43.2%	21	56.8%	
Gingival and palate	11	17.2	0	0.0%	11	100%	
Buccal mucosa	9	14.1	5	55.6%	4	44.4%	
Histopathologic feature							
Well-differentiated	49	76.6	22	44.9%	27	55.1%	0.21[a]
Moderately-poorly differentiated	15	23.4	4	26.7%	11	73.3%	

a: x-square test; b: Mann-Whitney test

Table 1. Distribution of Clinicopathologic Features in Male and Female Patients.

	HPV negative (n = 29)		HPV positive (n = 35)		*P*-value*	OR (95% CI)*
	n	%	n	%		
Gender						
Female (reff)	14	53.8	12	46.2	0.26	1.79 (0.65-4.91)
Male	15	39.5	23	60.5		
Age						
< 55 years (reff)	15	53.6	13	46.4	0.24	1.81 (0.67-4.93)
≥ 55 years	14	38.9	22	61.1		
Mean±SE	53.38 ± 2.45		55.57± 2.15			
Anatomical site						
Lips, gingival, palate, buccal mucosa (reff)	14	51.9	13	48.1	0.37	1.58 (0.58-4.92)
Tongue	15	40.5	22	59.5		
Histopathologic features						
Well-differentiated	21	42.9	28	57.1	0.48	1.52 (0.48-4.87)
Moderately-poorly differentiated (reff)	8	53.3	7	46.7		

- logistic regression

Table 2. Clinicopathologic Features Based on HPV Infection Status.

	Genotype 16 (n = 3)		Genotype 18 (n = 8)		Undetermined genotype (n = 24)		Total (n = 35)	*P* value*
	n	%	n	%	n	%	n	
Gender								
Male	1	4.3	4	17.4	18	78.3	23	0.08
Female	2	16.7	4	33.3	6	50.0	12	
Age								
<55 years old	0	0.0	5	55.6	4	44.4	9	0.16
≥55 years old	3	11.5	3	11.5	20	76.9	26	
Anatomic site								
Tongue	1	4.5	5	22.7	16	72.7	22	0.41
Other site in the oral cavity (gingival, buccal mucosa)	2	15.4	3	23.1	8	61.5	13	
Histopathologic features								
Well differentiated	2	7.1	5	17.9	21	75.0	28	0.12
Moderately-poorly differentiated	1	14.3	3	42.9	3	42.9	7	

- Mann Whitney test

Table 3. Association Between Clinicopathologic Features and HPV Genotype.

HPV	Age	Anatomic site	Gender	Histopathologic feature	Mean age±SE
HPV genotype 16	63	Palate	Male	Poorly differentiated	67.67±6.77
	59	Tongue	Female	Well differentiated	
	81	Lips	Female	Well differentiated	
HPV genotype 18	30	Buccal mucosa	Female	Well differentiated	48.13±6.04
	34	Tongue	Female	Well differentiated	
	34	Tongue	Female	Well differentiated	
	40	Tongue	Male	Moderately differentiated	
	49	Tongue	Male	Moderately differentiated	
	65	Palate	Male	Well differentiated	
	67	Tongue	Female	Well differentiated	
	70	Buccal mucosa	Female	Moderately differentiated	

Table 4. Profile of Patients with OSCC Positive for HPV Genotypes 16 or 18.

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