The Prevalence of High-Risk HPV in Male Patients with a Smoking Habit Who Attend the Dental Hospital of Universitas Indonesia

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Abstract

The high-risk types of the human papillomavirus (HPV) playa role in the carcinogenesis of oral cancer. Some sexual and non-sexual habits are known to increase the prevalence of high-risk HPV in the oral cavity. The role of smoking as one of the risk factors for HPV remains in conclusive. This study aimed to evaluate the prevalence and risk factors associated with HPV 16 and 18 in the oral cavity of male smokers who attend the Dental Hospital of the Faculty of Dentistry, Universitas Indonesia. The smoking habit and other risk factors were recorded for 200 subjects. DNA was extracted from the collected stimulated saliva samples. The DNA was then subjected to the conventional polymerase chain reaction in order to detect the presence of HPV16 and 18.The prevalence of HPV18 DNA was 1.08% in the subjects who smoke, while no HPV DNA was found in the non-smokers. A history of previous oral surgery, early sexual debut, and multiple sexual partners were some of the risk factors revealed and related to the smoking habit. This study determined the low prevalence of high-risk HPV in the oral cavity of male smokers; thus, the role of smoking in increasing the prevalence of high-risk HPV in the oral cavity could not be clarified.

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Introduction

Human papillomavirus (HPV) can potentially contribute to oral carcinogenesis.[1] HPV is a double-stranded, non-enveloped DNA virus that has a lifecycle in the skin or mucosa. A HPV strain can be categorized as either a high-risk or oncogenic strain or a low-risk or non-oncogenic strain.[2] The role of HPV in head and neck carcinogenesis has been established, including the coordinated targeting of several pathways by the HPV oncoproteins E5, E6, and E7.[3]

Some of the risk factors for oral HPV infection are related to sexual behavior and tobacco exposure.[4] Several types of sexual behavior could influence the prevalence of HPV, including the infrequent use of barrier protection during sex, frequent oral-genital and/or oral-anal contact, a high number of lifetime sexual partners, an early sexual debut, and having a sexual partner who was diagnosed with cervical dysplasia.[5]

Importantly, HPV transmission is caused by the direct contact of skin or mucosa with an infected lesion.[6,7] To enter the human body, HPV requires a cut in the epithelial surface that enables the virus to penetrate the basal cell epithelia.[3] Therefore, HPV could also be transmitted via non-sexual routes, for example, autoinoculation[7] and nosocomial transmission.[6]

Smoking can induce immuno suppression, which leads to a greater risk of HPV infection.[8] However, from another perspective, smoking has a preventive effect against HPV infection because it increases mucosal keratinization, thereby creating a more trauma-resilient surface that is less susceptible to HPV infection in the basal cell layer.[9] Hence, the association between smoking and HPV prevalence is not conclusive.

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Based on Global Adult Tobacco Survey, Indonesia is the third-largest cigarette consumer in the world. Further, the number of male smokers in Indonesia is about 30 times higher than the number of female smokers. The prevalence of smoking in Indonesia is 67.0% (57.6 million) among men and 2.7% (2.3 million) among women.[10]

No data have yet described the prevalence of oral HPV among Indonesian male smokers, with data concerning the oncogenic types (HPV 16 and 18) being particularly lacking. It therefore remains unclear whether smokers represent one of the population groups that have a higher risk of being infected with oral oncogenic HPV.

The aim of this study is to evaluate the prevalence and risk factors associated with HPV 16 and 18 in the oral cavity of male smokers who attend the Dental Hospital of the Faculty of Dentistry, Universitas Indonesia.

Material and Methods

This cross-sectional study was approved by the Ethics and Research Committee of the Faculty of Dentistry, Universitas Indonesia (FoDUI). Approximately 200, 18- to 60-year-old male subjects were recruited from the Dental Hospital of FoDUI between August and November 2016. Individuals who engage in high-risk sexual behavior (e.g., homosexual, bisexual), have systemic diseases or oral lesions, and have a history of alcohol and/or drug addiction were excluded from the study. The subjects were equally divided into the smoker and non-smoker groups. Each subject signaled their voluntarily participation by signing the informed consent form. Questionnaires asking about the subjects' demographic data, smoking habit, self-hygiene habits, and sexual behavior were distributed.

Stimulated saliva was taken from the subjects after paraffin wax chewing. The saliva was collected within a sterile 15 ml polypropylene tube container. An intraoral examination was also performed for each subject in order to record caries, plaque, and the calculus index. The collected saliva was then stored at -20^{o}C until the DNA extraction phase. The saliva DNA extraction was performed with a QIAamp DNA Blood Mini Kit (Qiagen, Germany) using the spin protocol (DNA purification from bodily fluid). The extracted DNA was stored in a 200 ml elution buffer within a 1.5 ml micro tube at -20^{o}C until the polymerase chain reaction (PCR) analysis.

DNA from each sample was amplified with conventional PCR (Bio-Rad, Hemel Hempstead, UK) using primers for HPV16,[11] HPV18, and glyceraldehydes–3-phosphate dehydrogenase (GAPDH).[12]

Prior to the detection of the high-risk HPV DNA, the DNA integrity was controlled using GAPDH as the housekeeping gene. For each PCR reaction, there was a procedural control. For HPV 16, the positive control was CaSki cell line DNA, while for HPV 18, the positive control was HeLa cell line DNA. The negative control was obtained from a PCR mixture that did not contain a DNA template. The PCR conditions are presented in Table 1.

The electrophoresis of the PCR products was performed in 1.5% agarose gel (Top Vision Agarose, Thermo Scientific, Waltham, MA) that had been stained with Peq Green RNA and DNA dye (PeqLab, Radnor, PA). The gel analysis was performed using UV visualization in Gel Doc 2000 (Bio-Rad Quantity One). All statistical analyses were completed using SPSS version 23.0 software. A p-value of less than 0.05 was considered to be statistically significant.

Table 1. PCR condition for each primer used in the study.

PCR condition	GAPDH		HPV16		HPV18	
Initial Denaturation	1x 95°C	2 min	1x 95°C	2 mins	1x 95°C	1 min
Denaturation	40 x 95°C	1 min	34 x 95°C	30 secs	32 x 95°C	1 min
Annealing	58°C	1 min	54.2°C	30 secs	58°C	1 min
Extension	72°C	1 min	72°C	1 min	72°C	1 min
Final extension	1 x 72°C	5 mins	1 x 72°C	5 mins	1 x 72°C	5 mins
Infinite hold	4°C	∞	4°C	∞	4°C	∞

Results

The mean age of the subjects was 39.80 ±10.45 years, with the mean age of the smokers being 39.26±11.32 years and the mean age of the non-smokers being 40.34±9.55 years. The smoking habit had a strong association with the level of education (p=0.001), with the smokers having a lower level of education than the non-smokers. All the subjects were sexually active. The smokers had the higher rate of sex before marriage as well as the highest divorce rate (p=0.027). The incidence of the smoking habit is presented in Table 2.

Table2. The pattern of smoking habit of the subjects

	Smoker	
	N	%
Duration of smoking		
0–2 years	5	5.1
2–5 years	5	5.1
5–10 years	23	23.5
> 10 years	65	66.3
Not answering	2	
Cigarette types		
Kretek	77	78.6
Non-Kretek	11	11.2
Electric	0	0
Kretek and non-Kretek	10	10.2
Not answering	2	
Number of cigarettes/day		
1–5	23	23.5
5–10	29	29.6
10–20	37	37.8
>20	9	9.2
Not answering	2	
Filtered cigarettes		
Yes	77	78.6
No	18	18.4
Not answering	2	
Sharing the same cigarettes		
Yes	26	26.5
No	72	73.5
Not answering	2	

Former smokers accounted for 32% of the non-smoker group, and they had mostly (28.1%) stopped smoking for 6–10 years. The majority of the smokers (66.3%) had been smoking for more than 10 years. About 78.6% of the smokers used “Kretek” cigarettes, a local cigarette that contains cloves in addition to tobacco.

The majority of the smokers smoked 11–20 cigarettes per day. Filtered cigarettes were used by 78.6% of smoker, and the majority of smokers (72%) never shared the same cigarettes with other smokers. This study also investigated various sexual and non-sexual risk factors for HPV transmission that were seen in the smoker and non-smoker groups (Table 3).

There was no significant association between the smoking habit and non-sexual transmission factors such as thumb sucking or nail biting and poor personal hygiene, for example, public use of a razor blade, towel, and tooth brush. The non-smoker group tended to have a higher rate of oral and pharyngeal surgery (p=0.002).

The smoking habit also had no significant association with sexual risk factors such as the total number of lifetime partners, having a sexual partner with cervical dysplasia, having a former sexual partner, condom usage, and oral sex. In terms of the oral sex habit, the non-smokers tended to perform it more frequently than the smokers in this study. However, there was a significant association between the smoking habit and marital status (Chi-square, p=0.03) and age of sexual debut (Kolmogorov-Smirnov, p=0.01).

About 29.7% of the subjects engaged in oral sex. Most of the subjects who engaged in sex before marriage tended to perform oral sex. The sex before marriage group mostly made their sexual debut at somewhere between 18 and 24 years old, which represented an earlier start than that seen in the married group. The majority of subjects(78.7%) preferred not to use a condom. Only a few subjects (18.5%) admitted to experiencing problems in the genital area.

GAPDH is a house keeping gene that is often used as a reference for the quantification of gene expression. GAPDH serves as a marker of the integrity of the extracted DNA. In this study, GAPDH (503bp) was found in 92 smokers’ samples and 94 non-smokers’ samples. The detection of HPV 16 and 18 DNA was only performed in the GAPDH-positive samples. The HPV DNA band is located at the 188 base pair.

In this study, there was only one positive HPV 18 DNA sample found in the smoker group (1/92 or 1.08%), while HPV 16 DNA was not found in any of the smokers’ samples. In the non-smoker group, no HPV DNA was found.

Table 3. Non-sexual and sexual risk factors for oral HPV

	Smoker		Non-smoker		Total		*P*
	N	%	N	%	N	%	
Nail biting/thumb sucking							
Yes	12	12	6	6	18	9	0.14*
No	88	88	94	94	182	91	
Sharing same eating utensils							
Yes	56	56	57	57	113	56.5	0.89*
No	44	44	43	43	87	43.5	
Sharing same razor blade							
Yes	38	38	48	48	86	43.3	0.15*
No	62	62	52	52	114	57	
Sharing same toothbrush							
Yes	23	23	25	25	48	24	0.74*
No	77	77	75	75	152	76	
Oral/oropharyngeal surgery							
Yes	13	13	26	26	39	19.5	0.02*
No	87	87	74	74	161	80.5	
Former sexual partner							
Yes	32	32.3	18	18,2	50	25.4	0.02*
No	67	67.7	81	81.8	147	80.5	
Not answering	1		1		2		
Sexual debut							
12–18years old	5	5,2	0	0	5	2,6	0.01‡
18–24 years old	42	43.3	25	25.3	67	3.2	
> 25 years old	50	51.5	74	74.7	124	63.3	
Not answering	3		1				
Homosexual							
Yes	0	0	0	0	0	0	-
No	100	100	99	100	199	100	
Not answering	0	0	1		1		
Total no. of sexual partners							
1	76	77,5	83	85.6	159	82.4	0.09*
2	4	4.1	7	7.2	11	5.7	
≥3	18	18.4	7	7.2	23	11.9	
Not answering	2		3		5		
Partner with cervical cancer							
Yes	1	1	2	2	3	1.5	1.00§
No	99	99	97	98	196	98.5	
Not answering	0	0	1	0	1		
Condom use							
Yes	24	24.5	17	17.2	41	24.8	0.95‡
No	73	74.5	82	82.8	155	78.7	
Yes and No	1	1	0	0	1	0.5	
Not answering	2		1		3		
Oral Sex							
Ever	25	26	33	33.3	58	29.7	0.26*
Never	71	74	66	66.7	137	70.3	
Not answering	4		1				
Genital symptoms							
Yes	19	19.6	17	18.2	36	18.5	0.69[a]
No	78	80.4	81	81.8	159	81.5	
Not answering	3		2				

*= Chi-square test; ‡= Kolmogorov-Smirnov test; §= Fisher test; p<0.05

Discussion

The fact that the majority of smokers in this study smoked Kretek cigarettes (78.6%) confirms the claim that Kretek cigarette dominates the tobacco consumption, accounted for some 88% of the total (machine-made) market volume in 2010.[10] The majority of smokers in this study smoke 11–20 cigarettes per day, which is consistent with the finding of

the Global Adult Tobacco Survey that mention the mean number of cigarettes of the Indonesian men smoked per day is 12.[10]

HPV transmission is caused by the direct contact of the skin or mucosa with an infected lesion.[6,7] HPV therefore requires damage to the epithelial surface so that the virus can enter the basal cell epithelium.[3] Some non-sexual habits that create contact with the mucosa were observed in this study, which were mostly related to poor personal hygiene, for example, nail biting or thumb sucking and sharing the same eating utensils, razor blade, or toothbrush.

This study was conducted in a metropolitan area, but the habit of sharing the same eating utensils and the same toothbrush proved surprisingly high (56.5% and 24%, respectively). This sharing habit also represents an important risk factor for the transmission of other microorganisms.

There was no significant association found between the smoking habit and the non-sexual risk factors in this study, except that the non-smokers were more likely to have a history of surgery in the oral/or opharyngeal area. Such surgery could cause further mucosal injury in the long term, thereby heightening the risk of HPV transmission if other high-risk practices were engaged in during that time. Moreover, the non-smokers tended to engage in oral sex more often than the smokers in this study.

This study revealed that 10% of men from among the total subjects had engaged in sex before marriage, and they had mostly had more than one sexual partner. Among all the subjects, approximately 11.9% had 3 or more sexual partners. Based on the Chi-square analysis, the smoker sex habited a tendency to engage in sex before marriage as well as to get divorced (p=0.03). They also tended to have had a former sexual partner (p=0.02) and an earlier sexual debut (Kolmogorov-Smirnov, p=0.01).

The majority of subjects in this study had their first sexual encounter at the age of 25 years or above. Compared to other Asian countries, this was slightly older than China who has mean age of male sexual debut at 22.5 years.[13] However, Thailand males have much younger sexual debut, in which the mean age of first sex at age group 25-34 is 18.3 years, and become lower with decreasing age. [14]

Of the100 smokers, only 92 samples were GAPDH-positive, while among the 100 non-smokers, 94 samples were GAPDH-positive. GAPDH as a housekeeping gene, shows the variability in the level of expression in different tissues. The concentration of GAPDH varies between individuals, between different stages of the cell cycle, in the presence of certain medications, and during growth.[15]

No HPV 16 DNA was found in this study, while HPV 18 DNA was found in only one smoker (1/92 or 1.08%). In this study, as there was only one positive sample, it was difficult to determine which factor is the most important in increasing the prevalence of high-risk oral HPV in smokers. The correlation between the variables is too complex to be established.

A similar study conducted in 200 Indonesian and Thai female dental students showed a different result. HPV 16 DNA was found in 8 Indonesia and Thai dental students, while HPV 18 DNA was found in 4 Indonesian dental students. It was revealed that HPV 16 was more commonly detected in subjects who had engaged in sexual intercourse, with the difference being statistically significant. The difference was not found in the case of HPV 18 with the same parameters.[16]

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Based on are cent meta-analysis of oral HPV prevalence among healthy subjects, the variability in 20 studies regarding HPV detection by means of PCR was about 0.67–34%.[17]

Inconsistencies were also seen in several studies concerning the association between smoking and high-risk oral HPV prevalence. This variability might be related to the different samples' characteristics. Some large-scale studies that detect quite a high number of high-risk oral HPV cases involved a very large sample size, sometimes involving millions of people.

The subjects were also not restricted based on their high-risk sexual behavior, which meant that LGBTQ individuals could take part in those studies. Oral sex, anal sex, and free sex

were also not restricted in such studies.

Oral HPV is less commonly found than HPV in the anogenital region, even though there are similarities between the oral mucosal and the anogenital epithelium. This might be related to the oral specimen collection method, which could dilute the oral HPV DNA, or due to the low viral load found in the oral specimen.[18] The viral nucleic acid concentration is also variable, depending on the amount of infection and the viral shedding. In this case, it is important to recognize the sensitivity of PCR.[19]

Furthermore, oral immune surveillance could also contribute to the lower prevalence of oral HPV.[18] Oral HPV is less commonly found than HPV in the anogenital region, even though there are similarities between the oral mucosal and the anogenital epithelium. This might be related to the oral specimen collection method, which could dilute the oral HPV DNA, or due to the low viral load found in the oral specimen.[18] The viral nucleic acid concentration is also variable, depending on the amount of infection and the viral shedding. In this case, it is important to recognize the sensitivity of PCR.[19] Furthermore, oral immune surveillance could also contribute to the lower prevalence of oral HPV.[18]

This study had a number of limitations. The cross-sectional study design can influence the interpretation of the results, since HPV could only being measured at one point in time. It is also difficult to identify which cases are actually in recurrence or will enter recurrence, which are self-limiting or will be persistent, and whether the subject was actually in a persistence condition when the study was conducted. Additionally, it is difficult to determine the former oral HPV status of HPV-negative subjects.[2] There was hence a possibility of undetectable latent HPV infection.

The presence of HPV DNA in this study does not indicate that the subject who tested positive was infected with or will certainly develop oral cancer. Further, the presence of HPV DNA does not signify a viral integration, since it could also occur in episomal form.[20]

A persistent HPV infection (latent or chronic) is needed to produce HPV-induced cellular transformation.[3] HPVDNA is detected in 24% of oral cancer cases, although the presence of HPV DNA is not sufficient to establish a causal relation from the molecular perspective. The etiologic fraction of high-risk DNA that induces oral cancer is only 5.9%, which is categorized as a low level.[21] The presence of HPV DNA in this study does not indicate that the subject who tested positive was infected with or will certainly develop oral cancer. Further, the presence of HPV DNA does not signify a viral integration, since it could also occur in episomal form.[20] A persistent HPV infection (latent or chronic) is needed to produce HPV-induced cellular transformation.[3] HPVDNA is detected in 24% of oral cancer cases, although the presence of HPV DNA is not sufficient to establish a causal relation from the molecular perspective. The etiologic fraction of high-risk DNA that induces oral cancer is only 5.9%, which is categorized as a low level.[21]

The smoking habit is more closely associated with HPV persistence than with HPV transmission.[22] Nevertheless, the association between smoking and HPV prevalence could not be evaluated in this study. The single incidence of oral HPV detected in this study complicates the risk factor analysis. A future prospective study concerning the association between tobacco use and oral HPV infection could investigate the viral persistence seen in smokers.

Another limitation of this study is the consecutive, hospital-based sampling method, which meant that the samples did not fully describe the real population. This could have led to selective bias. The smoking habit is more closely associated with HPV persistence than with HPV transmission.[22] Nevertheless, the association between smoking and HPV prevalence could not be evaluated in this study.

The single incidence of oral HPV detected in this study complicates the risk factor analysis. A future prospective study concerning the association between tobacco use and oral HPV infection could investigate the viral persistence seen in smokers. Another limitation of this study is the consecutive, hospital-based sampling method, which meant that the samples did not fully describe the real population. This could have led to selective bias.

The relatively high tobacco consumption and risky sexual behavior pattern seen in Indonesian youngsters nowadays should be taken seriously. The tendency toward poor personal hygiene reflected in this study should also be taken into consideration. As professional health care workers, dentists should recognize that there are lots of possible transmission factors for oral HPV in every patient.

Dentists should be able to perform a screening for high-risk habits and participate in the early detection of HPV infection; hence, they should ensure the appropriate referral of every suspicious oral finding.

Conclusion

This study found a very low prevalence of oral HPV (1.08%) among the male smokers, while the total prevalence (all male subjects) was found to be 0.54%. The non-smokers tended to have a previous history of oral surgery as a non-sexual risk factor. The smokers usually had an earlier age of sexual debut than the non-smokers, and they were more likely to have had more than one sexual partner. The association between the smoking habit and the increase in the prevalence of high-risk oral HPV could not be clarified in this study

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Declaration of Interest

The authors report no conflict of interest.

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