DENTINO
JURNAL KEDOKTERAN GIGI
Vol VI. No 1. Maret 2021

PREVALENCE OF HUMAN PAPILLOMA VIRUS IN CANCEROUS AND PRE-CANCEROUS ORAL LESIONS USING SALIVA, SERUM, AND PLASMA ASSAY

Nelly Nainggolan[1)], Wahyu Hidayat[2)]
[1)] Oral Medicine Residency Program, Faculty of Dentistry, Universitas Padjadjaran, Bandung, Indonesia
[2)] Oral Medicine Department, Faculty of Dentistry, Universitas Padjadjaran, Bandung, Indonesia

ABSTRACT
Introduction: *As many as 90%-95% cases of oral cancer are oral squamous cell carcinoma. In South and Southeast Asia, the prevalence of oral cancer is high. Oral cancer is ranked sixth for the most frequent malignancies in Asia with nearly 274,300 new cases occurring every year. Head and Neck Squamous Cell Carcinoma is one of the main public health concerns with 1-2% incident of all cancers worldwide and is also the sixth most common malignant tumor. Human Papillomavirus (HPV) is a DNA virus which is also known as the cause of head and neck cancer. Among all malignancies, oral cancer is one of the conditions where salivary examination can be used to detect oral cancer because it contacts directly with the lesions. The relationship between human papilloma virus (HPV) and oral squamous cell carcinoma has been extensively studied and shows that HPV-16 & 18 can be detected in saliva, serum or plasma samples and patient biopsies.* ***Purpose****: This systematic review aims to reveal the presence of human papilloma virus in cancerous and pre-cancerous lesions of the oral cavity by examining saliva, serum or plasma.* ***Methods****: Systematic Review was carried out using the PRISMA method. Articles with clinical trial types sourced from electronic searches were obtained through the PubMed, Research Gate and Google Scholar portals using keywords: Human Papilloma Virus, Oral Cancer, and Saliva.* ***Conclusion****: Based on the results, it is concluded that HPV is detected in oral cavity cancers, oropharyngeal, oral squamous cell carcinoma and Oral Potentially Malignant Disorders.*

Keywords : Human Papilloma Virus, *Oral cancer,* Saliva
Correspondence: Nelly Nainggolan; Oral Medicine Residency Program, Faculty of Dentistry, Universitas Padjadjaran, Bandung, Indonesia. Email: nellynainggolan.drg@gmail.com

INTRODUCTION

Cancer is a disease resulted from the accumulation of multiple DNA mutation and it is commonly initiated by immortality of cells and loss of control over normal growth mechanisms. Originally, the word cancer is derived from microscopic observations of abnormal 'crab-like' epithelial growth into connective tissues. The formation of malignant tumor involves multi-stage impairment of cell proliferation, differentiation and growth. Tumor initiation and progression may be generated by various physical, chemical and biological agents known as carcinogens, or by mutation due to unknown cause.[1] Meanwhile, pre-cancerous lesion or frequently known as OPMD (oral potentially malignant disorder) is a term for oral lesion that have potential to develop into cancer or malignancy.[2]

The etiology of cancer is varied, in which viruses are concerned in several oral malignancies including: (1) Human papilloma virus (HPV) associated with head-and-neck squamous cell carcinoma, (2) Epstein barr virus (EBV) associated with nasopharyngeal carcinoma and (3) Sarcomas associated with viral herpes associated with oral Kaposi sarcoma. HPV has been found to be associated with cervical, vulvar, vaginal, penile, anal, and head-neck cancers.[3] It is a group of non-envelope tropic epithelial DNA viruses that infect the skin and mucous membranes. High-risk HPV is identified as a causative agent for cancer. Although HPV infection is very common, in some cases the infection will persist and become a risk factor for the development to carcinogenesis.[4–7] Generally, HPV 16 and 18 are detected as types of HPV with high potential for malignancy whereas HPV types 6 and 11 are associated with benign lesions. Epidemiological and molecular studies have found an association between HPV types 16 and 18 with the risk of oral cancer.[8] Oncogenic HPV interferes with cell cycle control and apoptosis through disruption of cyclin-dependent kinases (CDK) pathway.[7,9,10] Retinoblastoma (Rb) and p53 in the cell cycle functions to repair infected or damaged DNA before entering the division phase in cell cycle, stimulating and triggering apoptosis of cells.

In high risk HPV or oncogenic-type HPV, the virus is protected from this cellular defense mechanism because it secretes E6 and E7 which are able to block p53 so that apoptosis will not occur while cell proliferation and differentiation are continuously progressed and cannot be terminated following the mutation of host DNA.[5]

HPV is associated with oropharyngeal cancer as presented by specific data from 2000-2009.[11] The relationship between human papilloma virus (HPV) and squamous cell carcinoma of the head and neck has been widely studied and shows that HPV-16 & 18 can be detected in saliva, serum and cell biopsy samples of patients with PMLs & OSCC.[12] Head and Neck Cancers associated with HPV have different genetic, clinical, and epidemiological characteristics from Head and Neck Cancers that are not associated with HPV. HPV-related Head and Neck Cancers represent approximately 25% of all Head and Neck Cancers in the general population, which arise in the oropharynx and base of the tongue.[13] Head and Neck Squamous Cell Carcinoma is one of the major public health problems with 1-2% incident of all types of cancer worldwide and is ranked sixth of all malignancies.[14] Oral cancer is used to describe cancers of the oral cavity and oropharynx including salivary glands, as well as malignancies in connective tissues. As many as 90% -95% of oral cancer cases are oral squamous cell carcinomas.[1,11,15] Oral cancer is a malignant tumor with a mortality rate of 50% per 5 years. In South and Southeast Asia, the prevalence of oral cancer is high. It ranks sixth as one of the most frequent malignancies in Asia with nearly 274,300 new cases occurring every year.

Among all malignancies, oral cancer is one of the conditions where salivary assay can be applied to detect the presence of viruses that is involved in cancer development because saliva is in direct contact with oral lesions.[16] Salivary markers can observe changes in cellular deoxyribonucleic acid (DNA) including the presence of the Human Papilloma and Epstein Barr viral genome.[14] Salivary biomarkers indicate not only the presence of oral disorders but also any pathology in more distant organs and tissues. Saliva may present as a reservoir for molecules and microbial information which is capable in suggesting the onset or the presence of diseases throughout the human body. Tumors in the oral cavity can release cellular substances directly into the saliva.[17] Saliva contains a protein that is expressed locally which allows the fluid to be the first choice of screening and identification of potential biomarkers for oral cancer. The saliva marker for oral cancer detection can be detected in three different levels; (i) changes in cell DNA, (ii) changes in mRNA transcripts and (iii) changes in protein levels (intracellular, on cell surface or extracellular) .[14,16] As a diagnostic fluid, saliva has advantages over serum because it is easy to obtain and collect, non-invasive, inexpensive and can be very easy to use for patient screening. Several salivary markers were found to be significantly increased in the examination of patients with oral cancer. [16]

This systematic review was performed to identify the presence of HPV in saliva, plasma and serum concerning the occurrence of cancerous and pre-cancerous lesions in the oral cavity.

MATERIALS AND METHODS

A systematic review was conducted to evaluate literatures containing data on the prevalence of HPV in cancerous and pre-cancerous lesions using the salivary examination. This review method had been following the *Preferred Reporting Items for Systematic Reviewed Methods and Meta-Analyzes* (PRISMA) guideline.[18]

Eligibility criteria

Studies presenting the outcome of any Human Papilloma virus presence in oral cancerous and pre-cancerous patients using salivary, serum or plasma assay were considered for inclusion in this study. HPV-positive result was determined by polymerase chain reaction (PCR), in situ hybridization (ISH) or immunohistochemistry P16 (IHC). There were no restrictions on age and gender. Only articles published in English would meet the requirements: clinical trial, human studies. A systematic electronic search was carried out from 2009 to 2019 on Pubmed, Research Gate, and Google Scholar database. A list of identified publication references was included for additional studies. The review was following the PRISMA method with the inclusion criteria that were comprised of articles written in English, full text article and all types of studies reporting the presence of HPV in oral cancer and pre-cancer patients as well as samples that were taken using saliva, serum or plasma.

Search Strategy

Electronic searches on PubMed, ResearchGate, and Google Scholar were undertaken to obtain research on the presence of HPV in cancerous and pre-cancerous lesions in the oral cavity through salivary, serum or plasma assay, from 2009 to 2019. The search strategy included a combination of terms from Medical Subject Heading (MESH): Human Papilloma Virus (MESH provisions) AND Oral Cancer (MESH provisions) AND Saliva (MESH provisions).

Discussion

The articles were screened and assessed throughout the PRISMA Flowchart as shown in Figure 1. After applying the inclusion criteria, thirteen articles were condemned to be eligible for inclusion.

Study Characteristics

All papers adopted for inclusion were published between 2009 and 2019, and it was later obtained a total of thirteen articles.

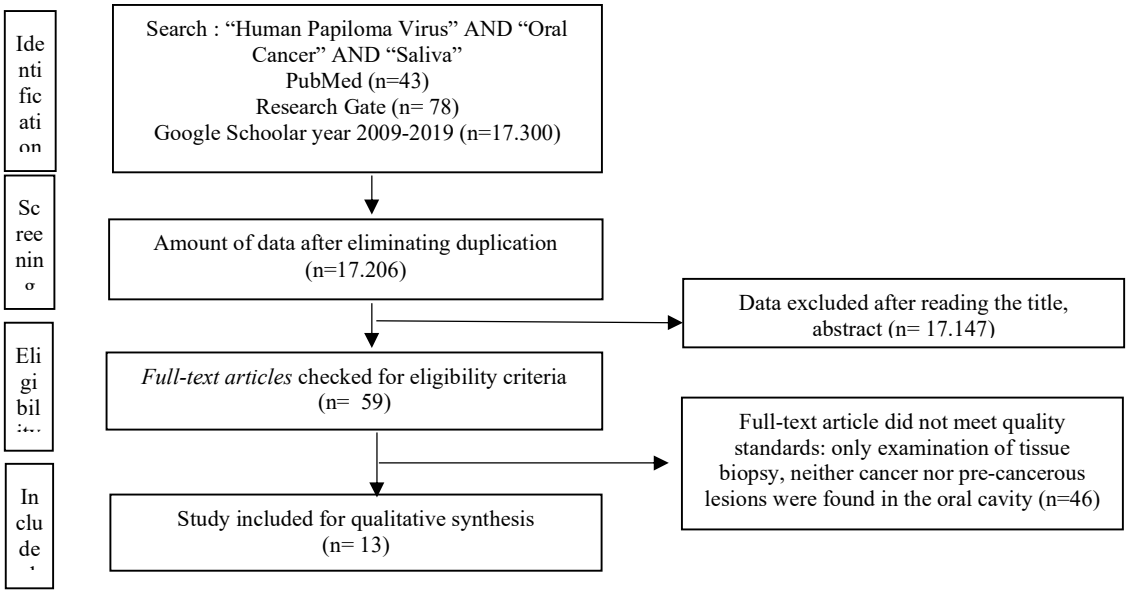

Figure 1. PRISMA flowchart according to the inclusion criteria

Summary of Main Findings

Quality Assessment of the Studies

All articles were selected from Scopus-indexed journals using Scimago. The articles studied were ranked between Q1 to Q3 in Scopus database.

The results of this systematic review presented 13 articles investigating the presence of HPV in saliva, serum and plasma in oral cancer and pre-cancer lesions, which obtained a total of 913 patients with cancer and pre-cancer in the oral cavity and a total of 351 patients with HPV-positive result.[13] Studies examining oral cancers and pre-cancers found significant result for HPV-positive outcomes in 7 oropharyngeal cancer studies, 4 oral cancer studies, 2 OSCC studies, 1 pre-cancer study (oral leukoplakia), 1 oral pre-cancer study of lichen planus and 1 pre-cancer study of OPMD. Meanwhile, the negative results in this study were reported in 2 studies on OSCC and 1 study on pre-cancerous lesions (OPMD).

Table 1. Presence of HPV in cancerous and pre-cancerous oral lesions

No	Type of Lesion		Number of Studies	Authors	Number of Patients	Number of HPV-Positive	Percentage	Details
1	Cancer	Oropharynx	7 studies	Tang, et. al.	54	44	91.7 %	saliva
				Wan, et. al.	54	41	75.9%	saliva
				Yoshida, et. al.	19	9	47.7%	saliva
				Dacewicz, et. al.	78	24	30.8%	saliva, serum
				Lim, et al.	45	39	86.7%	saliva
				Wang, et al.	34	29	85.3%	saliva
				Ahn, et al.	87	75	92.6 %	saliva, plasma
2		Oral Cavity	3 studies	Tang, et al.	9	4	8.3%	saliva
				Wan, et al.	54	5	9.3%	saliva
				Lim, et al.	45	4	8.9%	saliva
				Wang, et al.	46	1	2.2%	saliva
3		OSCC	4 studies	Chen, et al.	40	0	0%	serum
				Zafar, et al.	140	12	8.6%	saliva, serum
				Heah, et al.	14	0	91.7 %	saliva
				Kulkarani, et al.	34	24	70.6%	saliva
4	Pre-cancer	Oral Leukoplakia	1 study	Ferreira, et al.	32	20	62.5%	saliva
						12	50%	serum
5		Oral Lichen Planus	1 study	Sahebjamiee, et al.	40	3	7.5%	saliva
6		OPMD	2 studies	Chen, et al.	40	0	0%	serum
				Heah, et al.	16	1	6.25%	saliva

DISCUSSION

In this systematic review, we investigate the presence of HPV in cancerous and pre-cancerous lesions of the oral cavity by examining saliva, serum and plasma to identify the role of HPV in oral cancer and pre-cancer. The involvement of HPV in oral and oropharyngeal carcinogenesis was first proposed in 1983 by Syrjanen et al. and subsequently also supported by several other authors based on the following evidence: 1) broad epithelial tropism of HPV 2) morphological similarity between oropharynx and genital epithelium 3) immortal ability of human oral keratinocytes in vitro, 4) strong etiological role of High Risk of HPV in cervical squamous cell carcinoma and, finally, 5) detection of high risk HPV genotype in oral squamous cell carcinoma samples.[19]

This systematic review also reveals the presence of HPV in cancerous and pre-cancerous lesions of the oral cavity. Research on cancerous lesions was obtained from 7 oropharyngeal cancer studies, 4 oral cancer studies, and 4 OSCC studies. In 7 studies of oropharyngeal cancer, a total of 371 samples were examined and 261 samples showed a positive result for HPV with the positive presentation up to 70.4%. The data were comprised of Tang's study on 54 patients with 44 HPV-positive outcomes that were determined through salivary examination, Wan's study on 54 cancer patients with 41 HPV-positive outcomes through salivary examination, Yoshida's study on 19 cancer patients with 9 HPV-positive outcomes through salivary examination, Dacewicz's study on 78 cancer patients with 24 HPV-positive outcomes through salivary and serum examination, Lim's study on 45 cancer patients with 39 HPV-positive outcomes through salivary examination, Wang's study on 34 cancer patients with 29 HPV-positive outcomes through salivary examination and Ahn's study on 87 cancer patients with 75 HPV-positive outcomes through salivary and plasma examination. In the oral cancer study, a total of 154 samples were obtained where 14 patients were tested positive for HPV with the presentation up to 9.1% of all subjects. The data were collected from Tang's study in 9 patients with 4 HPV-positive results identified through salivary examination, Wan's study in 54 patients with 5 HPV-positive results from salivary examination, Lim's study in 45 cancer patients with 4 HPV-positive results from salivary examination, and Wang's study in 46 cancer patients with 1 HPV-positive result from salivary examination. Whereas in 4 OSCC studies, a total of 228 patients were reported and it was observed that 36 people were tested positive for

HPV with a presentation of 15.8%. The data was collected from Chen's study on 40 cancer patients where no HPV was detected from salivary examination, Zafar's study on 140 cancer patients where 12 HPV-positive results were determined through salivary and serum examination, Heah's study on 14 cancer patients where no HPV-positive patient was identified through salivary examination and Kulkarani's research on 34 cancer patients with 24 HPV-positive patients detected through salivary examination.

Data for pre-cancerous lesions were obtained from 4 studies, comprising of 1 study on oral leukoplakia, 2 studies on OPMD and 1 study on oral lichen planus. The study on oral leukoplakia patients showed a total of 32 samples where 16 HPV-positive outcomes were determined via serum assay revealing a positive percentage of 50% while 20 HPV-positive outcomes were determined via salivary examination resulting in a percentage of 62.5% thus representing the average of 56.25% in oral leukoplakia lesions. In 1 oral lichen planus study with a total of 40 samples, only 3 patients were tested positive for HPV. Two studies on OPMD reported one study with a single positive result from 16 samples with a percentage of 0.018% and the other study with no positive result for HPV disclosing the prevalence of HPV in OPMD lesions as 0.06%.

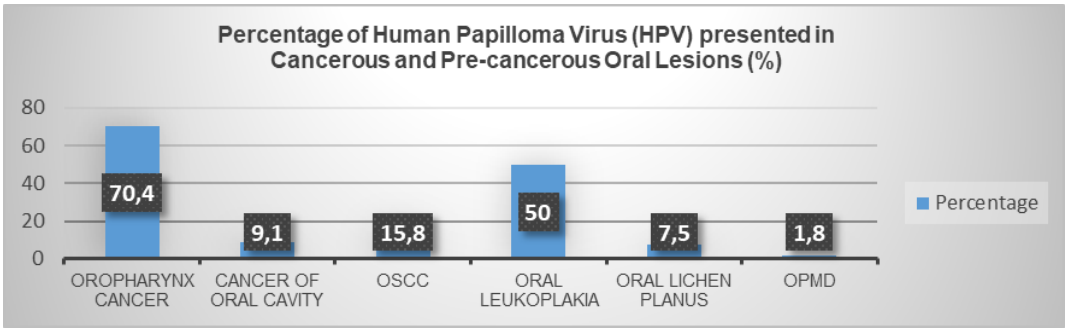

Figure 2. Graph of Human Papilloma Virus Presented in Cancerous and Pre-cancerous Oral Lesions

Syrjanen et al. (2011) pronounced that HPV was found to be significant in OSCC and OPMD compared to controls. The frequency of HPV DNA detected in OPMD was ranging from 1.67% to 95%, while it presented up to 95% in OSCC. Sathish et al. (2012) reported the prevalence of HPV in OSCC was amounted to 23.6%. In this study, HPV-positive results can be observed in 15% of patients with OSCC and 21% of patients with OPMD. The difference in study result can occur due to variations in the percentage of HPV in OSCC and OPMD, along with the number of HPV studies in OSCC and OPMD which are considered to be insufficient. The study of HPV in the oropharynx and oral cancer has been widely conducted. Auguste's study (2017) reported that the prevalence of HPV in the oropharynx was 95%, and Hettmann et al (2018) later found that 50% of patients with cancer of the oropharynx were resulted positive for HPV.[20,21] Meanwhile in this study, the number of HPV-positive reached 70.4% in oropharyngeal cancer and 9.1% in oral cancer. This shows that the role of HPV is not only limited to OSCC and OPMD but also cancer of the oropharynx and oral cavity. Sathish et al (2012) revealed that OSCC patients with HPV-positive result has a better prognosis than HPV-negative patients.[3]

This shows the important role of HPV in OSCC in which the prescribed therapy for HPV will determine the development of OSCC. In this overall systematic review, it can be discovered that patients with oropharynx cancer was presented with the highest prevalence of HPV (70%). It is followed by a prevalence of 50% in oral leukoplakia, 15.8% in OSCC, 9.1% in oral cancer, and 7.5% in oral lichen planus. The lowest prevalence of HPV can be observed in OPMD with a percentage of 1.78%. HPV can be detected in cancerous and pre-cancerous lesions of the oral cavity using saliva, serum, or plasma assay, which shows varied amount of prevalence. The prevalence of HPV presents its tendency as well as its role as a promotor in oral cancer and pre-cancer development by increasing cell proliferation, inhibiting apoptosis and degradation by p53 in cell cycle, therefore resulting in the occurrence of oral cancer and pre-cancer in the oral cavity.

REFERENCES

1. Thomson P. Oral Cancer From Prevention to Intervention. UK: Cambridge Scholars Publishing; 2019.
2. Speight PM, Eng F. Oral potentially malignant disorders: risk of progression to malignancy. Oral Surg Oral Med Oral Pathol Oral Radiol [Internet]. 2018;125(6):612–27. Available from: https://doi.org/10.1016/j.oooo.2017.12.011
3. Sathish N, Wang X, Yuan Y. Human

Papillomavirus (HPV) - associated Oral Cancers and Treatment Strategies. JDR Clin Res Supplemen. 2012;93(7):29–36.
4. Brooks GF, Carroll KC, Butel J, Morse SA, Mietzner T. Medical Microbiology. 26 th. Jawetz M and A, editor. Mc Graw Hill. Mc Graw Hill Lange; 2013. 1 p.
5. Alba A, Cararach M. The Human Papillomavirus (HPV) in Human Pathology : Description , Pathogenesis , Oncogenic Role , Epidemiology and Detection Techniques. Open Dermatol J. 2009;3:90–102.
6. Schupbach J. The Human Retroviruses Human Immunodeficiency Virus and Human T-Lymphotropic Virus. In: Steven Specter, Richard L. Hodinka, Stephen A. Young DLW, editor. Clinical Virology Manual. 4th ed. Washington D.C: ASM Press; 2009. p. 578–629.
7. Wulandari Dwi and Sudiro Mirawati. Pengembangan antivirus human papilloma virus berbasis molekul kecil. MKA. 2014;37.
8. Adamopoulou M, Vairaktaris E, Panis V, Nkenke E, Neukam FW, Yapijakis C. HPV Detection Rate in Saliva May Depend. In Vivo (Brooklyn). 2008;602:599–602.
9. Savira M. Biologi Molekuler. J ilmu Kedokt. 2017;11(1):1–6.
10. Setiawati D. Human Papilloma Virus Dan Kanker Serviks. Al-Sihah Public Heal Sci J. 2014;VI(2):450–9.
11. Glick M. Burket Oral Medicine 12th edition. 12th ed. USA: People'S Medical Publishing House-USA; 2015. 211–281 p.
12. Khyani IAM, Qureshi MA. Salivary detection of human Papilloma virus 16 & 18 in pre-malignant and malignant lesions of oral cavity : Is it feasible in Pakistani context of Socio-Cultural Taboos ? Pak J Med Sci. 2015;31(5).
13. Beachler DC, Gypsyamber D. Oral HPV infection and head and neck cancers in HIV-infected individuals. Curr Opin Oncol. 2013;25(5):503–10.
14. Hegde ND. An Insight on salivary biomarkers for oral Cancer Abstract. J Dent Craniofacial Res. 2018;3(2:8):1–7.
15. Yakin M, Seo B, Hussaini H, Rich A, Hunter K, Sciences H, et al. Human papillomavirus and oral and oropharyngeal carcinoma: the essentials. 2018;0–2.
16. Bano S, David MP, Indira AP. Salivary Biomarkers for Oral Squamous Cell Carcinoma : An Overview. IJSS Case Reports Rev. 2015;1(1):39–45.
17. Maria L, Batista R, Carvalho AC De. Serum , plasma and saliva biomarkers for head and neck cancer. Expert Rev Mol Diagn. 2018;18(1):85–112.
18. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. BioMed Cent. 2015;4(1):1–9.
19. Pannone G, Santoro A, Papagerakis S, Muzio L Lo, Rosa G De. The role of human papillomavirus in the pathogenesis of head & neck squamous cell carcinoma : an overview. Infect Agent Cancer. 2011;6(1):4.
20. Auguste A, Gaëte S, Herrmann C, Leah S. Prevalence of oral HPV infection among healthy individuals and head and neck cancer cases in the French West Indies. Cancer Causes Control. 2017;0(0):0.
21. Hettmann A, Demcsák A, Bach Á, Decsi G, Dencs Á, Pálinkó D, et al. Infection , Genetics and Evolution Prevalence and genotypes of human papillomavirus in saliva and tumor samples of head and neck cancer patients in Hungary. Infect Genet Evol. 2018;59(October 2017):99–106.