High risk - Human Papillomavirus (HR-HPV) Infection and Associated Risk Factors among Oropharyngeal Cancer Patients Attending Yangon General Hospital

Mu Mu Shwe[1], Myo Myint Maw[2], Aye Myat Mon[2], Lynn Pa Pa Aye[1], Kham Mo Aung[1], Nu Nu Lwin[1], Ssu Wynn Mon[1] & Win Maw Tun[1]*

[1]Department of Medical Research
[2]Medical Oncology Department, Yangon General Hospital

Oropharyngeal cancer (OPC) is a significant component of the global burden of cancer, worldwide being the sixth most common cancer and the eighth most common cause of cancer death. Nowadays, Human Papillomavirus (HPV) infection becomes the main risk factor for head and neck cancer development. In Myanmar, the related role of this viral infection to OPC is still uncertain. The objective of this study was to determine the proportion of high risk Human Papillomavirus infection and to detect the associated risk factors among oropharyngeal cancer patients by a cross-sectional descriptive method. OPC patients from Oncology Department, Yangon General Hospital were investigated during 2018-2019. Oral swabs were obtained from the tumor by citoswab and kept in DNA fixative medium. Firstly, DNA extraction from the oral swabs was performed. HPV-DNA testing was done using polymerase chain reaction method. Consensus sequence primer pairs within the E6 and E7 open reading frames were used to amplify HR-HPV (HPV-16, -18, -31, -33, -35, -52, -58). In total 60 patients (medium age 56 years; range 33-85) were studied and most patients were 51-60 years (38.3%) of age group, followed by 61-70 years (30%), 41-50 years (18.3%), 31-40 years (6.7%) and >70 years (6.6%). Most of them (81.7%) were male and 18.3% was female. Anatomical types of OPC were cancers of soft palate (33.3%), tonsil (30%), posterior or lateral pharyngeal wall (16.7%), base of tongue (13.3%), posterior 1/3 of tongue (5%) and mixed soft palate, tonsil and uvula (1.7%). The most common histological type was squamous cell cancer (SCC) (95%) and the rest were mucoepidermoid cancer, large anaplastic cancer and adenoidcystic cancer (1.7% each). Associated risk factors among OPC patients were smoking (80.0%), betel quid chewing (71.7%) and alcohol drinking (65%). HR-HPV was detected in only two cases of OPC (3.3%) which were SCC of tonsil and base of tongue. The percentage of HPV-related OPC is considerably low when compared to the Western countries. This may be due to the cultural habit but other HPV types that not included in the consensus sequence primers may be. The findings highlighted the more need for education on smoking-, betel quid chewing- and alcohol-related oropharyngeal cancers.

Keywords: HPV, Associated risk factors, Oropharyngeal cancer (OPC)

INTRODUCTION

The International Agency for Research on Cancer (IARC) published that the global cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2018. One in 5 men and one in 6 women worldwide develop cancer during their lifetime, and one in 8 men and one in 11 women die from the disease.[1] Worldwide,

*To whom correspondence should be addressed.
Tel: +95-95193958
E-mail: drmumushwe@gmail.com
DOI: https://doi.org/10.34299/mhsrj.009111

oropharyngeal cancer (OPC) is a significant component of the global burden of cancer. Incidence and mortality rates are higher in men than women. ICO/IARC HPV Information Centre (2019) reported that age standardized incidence rate of OPC among male patients were 1.8% in the world, 1% in south-east Asia and 2.3% in Myanmar. Age standardized mortality rate of OPC among male patients were 1% in the world, 0.5% in South-east Asia and 1.5% in Myanmar.

In 2018, Annual number of new OPC cancer cases among male and female in the world, SEA and Myanmar were 74, 472/18, 415, 3, 130/932, 562/70, respectively. Annual number of death from OPC cancer cases among males and females in the world, SEA and Myanmar were 42, 116/8, 889, 1, 686/406, 347/38, respectively.[1, 2]

In South East Asia regions (Globocan 2018), lip and oral cavity cancers is the first most common cancer in male. But it ranks as the fourth most common cancer and the fourth leading cause of cancer death in both sexes. Oropharyngeal cancers ranks as 22nd most common cancer and the 18th leading cause of cancer death in both sexes in WHO SEARO regions.[3]

In Myanmar (Globocan 2018), about 632 new oropharyngeal cancers are diagnosed and 385 cases died from it annually.[4] In 2017, 575 cases of oral cancer and 110 cases of oropharyngeal cancer were admitted at the Medical Oncology Department and Radiation Oncology Department of Yangon General Hospital.[5]Oropharyngeal cancers include malignancies that occur where the oral cavity and pharynx merge, including in the palatine and lingual tonsils, the posterior 1/3 (base) of the tongue, the soft palate, and the posterior pharyngeal wall. Most of the Oropharyngeal Cancers are classified histologically as squamous cell carcinoma (OPSCC).[6]

The risk of oral and oropharyngeal cancer is greatly increased by 2 factors: (1) tobacco use including cigarettes, cigars, pipes, chewing tobacco, and snuff, is the single largest risk factor for head and neck cancer. Eighty-five percent (85%) of head and neck cancer is linked to tobacco use. Chewing tobacco or snuff is associated with a 50% increase in the risk of developing cancer in the cheeks, gums, and inner surface of the lips, where the tobacco has the most contact. Second hand smoke may also increase a person's risk of head and neck cancer and (2) frequent and heavy consumption of alcohol increases the risk of head and neck cancer.[6]

Other factors that can raise a person's risk of developing oral and oropharyngeal cancer include (1) prolonged sun exposure: excessive and unprotected exposure to the sun is linked with cancer in the lip area (2) Human papillomavirus (HPV) is a risk factor for oropharyngeal cancer. In recent years, HPV-related oropharyngeal cancer in the tonsils and the base of the tongue has become more common. Sexual activity, including oral sex, with someone who has HPV is the most common way someone gets HPV.[6]

When monitoring the population-level effect of HPV vaccination on oropharyngeal cancer occurrence in the United States, data on the incidence and type-specific prevalence of this disease are essential. Previously, the prevalence of cases attributable to viral infection and the consequent effects of vaccine programs were approximated from some published studies, which estimated HPV to be detected in 37%-60% of OPSCC in North America.[7-11]

Global prevalence of HPV among oro-pharyngeal cancer varies and more frequent in Western countries than Asia.[12] In Myanmar, the related role of this viral infection to OPC is still unclear. Few researches based on Myanmar population were documented.

This study aimed to determine the proportion of high risk Human Papilloma-virus (HR-HPV) infection and associated risk factors among oropharyngeal cancer patients attending Oncology Department, Yangon General Hospital.

MATERIALS AND METHODS

Study population and design

This study was a cross-sectional descriptive study. In total, 60 OPC patients (medium age 56 years; range 33-85) from Oncology Department, Yangon General Hospital were investigated during 2018-2019.

After obtaining the written informed consent, a thorough history was taken using structured-proforma. Then, clinical and oral examination was performed under good light source. Oral swabs were obtained from the tumor by citoswab and kept in DNA fixative medium. Those samples were transported to Technology Development Division, Department of Medical Research where the samples were stored in -20°C freezer.

DNA extraction

DNA extraction was performed using QIAamp DNA mini kit (Qiagen) comprising Proteinase K, Buffer AL, Buffer ATL, Buffer AW-1, Buffer AW-2, and Buffer AE according to the manufacturer's instruction.

Polymerase chain reaction

HPV-DNA testing was performed using polymerase chain reaction (PCR) method. Consensus sequence primer pairs within the E6 and E7 open reading frames i.e., forward primer (*pU-1M*): 5'-TGTCAAAAACCGTT GTGTCC-3' and reverse primer (*pU-2R*): 5'-GAGCTGTCGCTTAATTGCTC-3') (oligo @sigma genosys-PCR, Japan) were used to amplify pooled HR-HPV (HPV-16, -18, -31, -33, -35, -52b, -58).[8]

Reaction mixture was done using 0.15µL of taq polymerase (Applied Biosystems, Roche, USA), 2 µL 10Xbuffer, 3.2 µL dNTPs, 0.4 µL of forward and reverse primers, 12.85 µL distilled water and 1 µL DNA. They were subjected to 35 cycles of amplification using ASTEC thermal cycler. The reaction mixtures will be subjected to 35 cycles of amplification using a thermal cycler (ASTEC, Japan). Each cycle included a denaturation at 95°C for 1min, annealing at 55°C for 1 minute and extension at 72°C for 1min.

Detection of the PCR product

This was performed by the electrophoresis on a 2% agarose gel in 1XTBE (Tris- Boric acid, Ethylene diamine tetraacetic acid) using 100 bp molecular weight marker, 100V, 30 minutes and ethidium bromide staining. HR-HPV detection will be performed using Gel documentation (Bio-Red).

Statistical analysis

Data analysis was done by using Microsoft Office Excel 2007 and the Statistical Package for Social Sciences (SPSS-16).

Ethical consideration

This study was approved by Institutional Review Board (IRB), Department of Medical Research, Yangon (ERC No- 2018-65, Approval No- Ethics/DMR/2018/094.

RESULTS

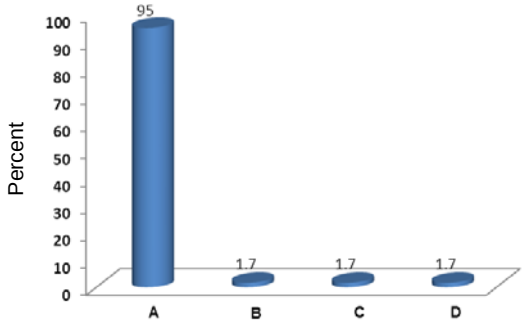
In total, 60 OPC patients (medium age 56 years; range 33-85) from Oncology Department, Yangon General Hospital were investigated during 2018-2019. In this study, most OPC patients were 51-60 years (38.3%) of age group, followed by 61-70 years (30%), 41-50 years (18.3%), 31-40 years (6.7%) and >70 years (6.6%). Most of them (81.7%) were male and 18.3% was female.

Anatomical types of OPC were cancers of soft palate (33.3%), tonsil (30%), posterior or lateral pharyngeal wall (16.7%), base of tongue (13.3%), posterior 1/3 of tongue (5%) and mixed soft palate, tonsil and uvula (1.7%) (Table 1).

Table 1. Proportion of types of Oropharyngeal Cancer Patients attending Yangon General Hospital

Types of OPC	Frequency	Percent
Soft palate Ca	20	33.3
Tonsil Ca	18	30.0
Post or lat pharyngeal wall Ca	10	16.7
Base of tongue Ca	8	13.3
Posterior 1/3 of tongue Ca	3	5.0
Mixed type Ca	1	1.7
Total	60	100

The most common histological type was squamous cell cancer (SCC) (95%) and the rest were mucoepidermoid cancer, large anaplastic cancer and adenoidcystic cancer (1.7% each) (Fig. 1).



A= Squamous Cell Ca, B= Mucoepidermoid Ca
C= Large anaplastic Ca, D= Adenoidcystic Ca

Fig. 1. Proportion of histological types of Oropharyngeal Cancer Patients

Regarding risk factors for OPC in this study, 48/60 (80%) of OPC patients had smoking, 43/60 (71.7%) had betel quid chewing and 39/60 (65%) had alcohol drinking (Table 2). Regarding the duration of smoking in OPC patients, 77.1% of patients had history of smoking for >10 years and 22.9% of patients had history of smoking for ≤10 years. Regarding the duration of Betel Quid Chewing (BQC) in OPC patients, 60.5% had history of BQC for >10 years and 39.5% had history of BQC for ≤10 years. Regarding the duration of alcohol drinking in OPC patients, 51.3% had history of alcohol drinking for >10 years, and 48.7% of patients had history of alcohol drinking for ≤10 years (Table 2).

Table 2. Proportion of associated risk factors and duration of risk factors among Oropharyngeal Cancer Patients

Risk factors	Smoking		Betel quid chewing		Alcohol drinking	
	F	Percent	F	Percent	F	Percent
Yes	48	80	43	71.1	39	65
No	12	20	17	28.3	21	35
Total	60	100	60	100	60	100
Duration						
≤10 yrs	11	22.9	17	39.5	19	48.7
≥10 yrs	37	77.1	26	60.5	20	51.3
Total	48	100	43	100	39	100

F=Frequency

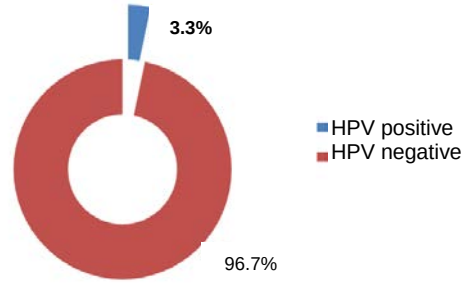


Fig. 2. Proportion of HPV infection among Oropharyngeal Cancer Patients

In this study, HR-HPV was detected in only two cases of OPC (3.3%) (Fig. 2). Histologically, one case had SCC of tonsil and another had SCC of base of tongue. HR-HPV positive OPC cases were male gender and age older than 45 years. All HR-HPV positive cases had history of BQC, alcohol drinking and smoking for more than 10 years.

DISCUSSION

The last evaluation of the International Agency for Research in Cancer (IARC) on the carcinogenicity of HPV in humans concluded that (a) there is enough evidence for the carcinogenicity of HPV type 16 in the oral cavity, oropharynx (including tonsil cancer, base of tongue cancer and other oropharyngeal cancer sites), and (b) limited evidence for laryngeal cancer. There is increasing evidence that HPV-related oropharyngeal cancers constitute an epidemiological, molecular and clinical distinct form as compared to non HPV-related ones. Some studies indicate that the most likely explanation for the origin of this distinct form of head and neck cancers associated with HPV is a sexually acquired oral HPV infection that is not cleared, persists and evolves into a neoplastic lesion. The most recent figures estimate that 25.6% of all oropharyngeal cancers are attributable to HPV infection with HPV16 being the most frequent type.[12]

According to a systematic review and meta-analysis of articles (2019 ICO HPV and related diseases), the global estimate of oropharyn-

geal cancer (OPSCC) attributable to high-risk HPV varies from 0% to 95.5%. In Lohavanichbutr study (USA), HPV prevalence among cases of oropharyngeal cancer among men and women were 82.1% and 0%, respectively. Näsman (Sweden) reported that HPV prevalence among oropharyngeal cancer among men and women were 81.6% and 95.5%, respectively. Li (China) reported that HPV prevalence among oropharyngeal cancer among men and women were 14.3% and 60%, respectively.[12]

With regard to risk factors, tobacco and alcohol consumption account for more than 75% of oral squamous cell carcinoma (OSCC) whereas high-risk HPV types account for a very small proportion (1-3%).[13-17] Betel quid use, with or without tobacco, is also an established risk factor for OSCC and OPSCC, particularly in the Indian subcontinent.[18, 19] There is a global increase in the prevalence of human papillomavirus (HPV)-driven oropharyngeal squamous cell carcinoma (OPSCC) in Australia and New Zealand. Risk factors for HPV-positive OPSCC are male gender, white race, age older than 40 but younger than 59 years old, having multiple lifetime sex partners, having oro-genital and oro-anal sex. High-risk HPV subtypes play a major role in the patho-genesis of OPSCC.[20]

In this study, using PCR method, HR-HPV was detected only in 3.3% of OPC in Myanmar. In Myanmar, HPV type 16 and 18 was detected in 2 out of 40 oral cancer cases in a study done by Thar Htet San, et. al in 2010.[21] In Moe Thida Htwe's study (2012), HPV was negative in all oral potentially malignant disorders (OPMD) and only 3/62 (4.8%) of oral squamous cell carcinomas (OSCC) were positive and did not find any significant association HPV infection and gender, age, site of the lesions, histological differentiation of OSCC and grade of dysplasia for OPMD.[22]

Some studies have shown that HPV positivity rates in OPSCC were lower in Asian countries when compared to the Western world.[7, 23] A cohort study in Japan showed that HPV infection plays a minor role in oral oncogenesis in the Eastern part of the world.[24] In Myanmar, the percentage of HPV related OC and OPC in those studies were considerably low which was very similar to the above studies.

The major risk factors for HNSCC include tobacco cigarette/beedi smoking, betel nut chewing and alcohol.[25, 26] In developing nations, tobacco is the main etiological factor for cause of head-and-neck cancers, whereas in developed countries with decreased tobacco use, increased awareness, and early detection of cancer with a higher infusion of medical awareness and treatment facilities, there is still increased incidence of oro-pharyngeal carcinoma (especially in non-smokers and nondrinkers), which tends to show the spread of high-risk HPV (16 and 18) and propose HPV infection as an independent etiological risk factor for OPSCC and OSCC, similar to alcohol and tobacco.[27]

In 2018, Satheesh K, Bandhary1, *et al* (India) published that HPV was detected only in 2.6% of OPC. HPV does not play a major role in the carcinogenesis of HNSCC but betel nut chewing, tobacco exposure and alcohol consumption remain major risk factors for HNSCC.[28]

In this study, eighty percent of OPC patients were smokers, 71.7% were betel quid chewers and 65% were alcohol drinkers. Long time duration especially more than 10 years duration of smoking, betel quid chewing and alcohol drinking were associated with OPC. This study was very similar to above studies. Therefore, smoking, betel quid chewing and alcohol were the most important risk factors in OPC. HPV infection interaction with tobacco and alcohol acts in a synergistic mechanism in the oral and oropharyngeal regions, and the pathogenesis is yet unclear.

Conclusion

The percentage of HPV-related OPC in this study was considerably low when compared

to the Western countries. This may be due to the cultural habit but other HPV types that not included in the consensus sequence primers may be. Although HPV may be one of the etiological factors in carcinogenesis of OPC, low positivity of HPV raises some questions over the role of HPV in this group of patients.

To get the concrete conclusion regarding the role of HPV in OPC, future studies using different technologies with larger sample size should be studied. The findings highlighted the more need for health education on tobacco smoking-, betel quid chewing- and alcohol- related oropharyngeal cancers.

Competing interests

The authors declare that they have no competing interests.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to Director General and Board of Directors for their permission to conduct this study. We are much grateful to doctors from Medical Oncology Department, Yangon General Hospital and all the patients participating in this research.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA & Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians* 2018; 68(6): 394-424. http:// dx.doi:10.3322/caac.21492. Epub 2018 Sep 12.
2. Bruni L, Albero G, Serrano B, Mena M, Gómez D, Muñoz J, *et al.* ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in Myanmar. *Summary Report* 17 June 2019. 27-213.
3. World Health Organization. South-east Asia region (SEARO) 2018. Available from: http:// gco.iarc.fr/today/ data/ factsheets/popu -lations/995-who-south-east-asia-region-searo -fact-sheets.pdf
4. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, *et al.* Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today, [Accessed 05 October 2018].
5. Hospital Stastics of Oncology Department Yangon General Hospital, Myanmar 2017.
6. Oral and Oropharyngeal Cancer. Available from: https://www.cancer.net/ cancer-types/ oral-and-oropharyngeal -ncer/2019.
7. Toman J, Von Larson S, Umeno H, Kurita T, Furusaka T, Hasegawa H, *et al.* HPV-Positive Oropharyngeal Cancer Via p16 Immunohistochemistry in Japan. *Annals of Otology, Rhinology & Laryngology* 2017 Feb; 126(2):152-158. http://dx.doi: 10.1177/ 0003489416681582. PMID: 27913709.
8. Kouketsu A, Sato I, Abe S, Oikawa M, Shimizu Y, Takahashi T, *et al.* Detection of human papillomavirus infection in oral squamous cell carcinoma: a cohort study of Japanese patients. *Journal of Oral Pathology & Medicine* 2016 Sep; 45(8): 565-572. http://dx.doi: 10.1111/jop.12416. Epub 2015 Dec 29. PMID: 26711722.
9. Migaldi M, Pecorari M, Forbicini G, Nanni N, Grottola A, Grandi T, *et al.* Low prevalence of human papillomavirus infection in the healthy oral mucosa of a Northern Italian population. *Journal of Oral Pathology & Medicine* 2012; 41(1): 16-20. http://dx. doi.org/10.1111/ j.1600- 0714.2011. 01062.x
10. Martin-Hernan F, Sánchez-Hernández JG, Cano J, Campo J & del Romero J. Oral cancer, HPV infection and evidence of sexual transmission. *Medicina Oral Patologia Oraly Cirugia Bucal* 2013; 18(3): e439-444.
11. Dhanapal R, Ranganathan K, Kondaiah P, Devi RU, Joshua E & Saraswathi TR. High-risk human papilloma virus in archival tissues of oral pathosis and normal oral mucosa. *Contemporary Clinical Dentistry* 2015 Apr-Jun; 6(2): 148-152. http://dx. doi: 10.4103/ 0976- 237X.156033.
12. Bruni L, Albero G, Serrano B, Mena M, Gómez D, Muñoz J, *et al.* ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). HPV and Related Diseases in the World. *Summary Report* 17 June 2019. 213-230
13. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oral Oncology* 2009; 45(4-5): 309-316.

14. Shaikh MH, McMillan NAJ & Johnson NW. HPV-associated head and neck cancers in the Asia Pacific: A critical literature review & meta-analysis. *Cancer Epidemiology* 2015; 39(6): 923-938.
15. 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 Oncology* 2013 Jan; 49(1): 1-8. http://dx.doi: 10.1016/j.oraloncology. 2012.07.002. Epub
16. Shield KD, Ferlay J, Jemal A, Sankaranarayanan R, Chaturvedi AK, Bray F, *et al*. The Global Incidence of Lip, Oral Cavity, and Pharyngeal Cancers by Subsite in 2012. *CA: A Cancer Journal for Clinicians* 2017 Jan; 67(1): 51-64. http://dx.doi: 10.3322/caac.21384. Epub 2016 Oct 19. PMID: 28076666.
17. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F & Franceschi S. Global burden of cancers attributable to infections in 2012: A synthetic analysis. The *Lancet Global Health* 2016; 4(9): e609-e616.
18. Guha N, Warnakulasuriya S, Vlaanderen J & Straif K. Betel quid chewing and the risk of oral and oropharyngeal cancers: A meta-analysis with implications for cancer control. *International Journal of Cancer* 2014; 135(6): 1433-1443.
19. Gupta B & Johnson NW. Systematic Review and Meta-Analysis of Association of Smokeless Tobacco and of Betel Quid without Tobacco with Incidence of Oral Cancer in South Asia and the Pacific. *PLOS ONE* 2014; 9(11): e113385.
20. M Yakin, B. Seo, H Hussaini, A Rich & K Hunter. Human papillomavirus and oral and oropharyngeal carcinoma: The essentials. *Australian Dental Journal* 2019; 64(1): 11-18. http://dx.doi: 10.1111/adj.12652
21. Thar Htet San, Yi Yi Myint, Wai Wai Khaing, Sann Sanda Khin, Phyu Synn Oo, Myo Htet Htet Khaing, *et al. Myanmar Health Sciences Research Journal* 2010; 22(1): 46-50.
22. Moe Thida Htwe. The role of human papillomavirus (HPV) in oral leukoplakia, oral submucous fibrosis and oral squamous-cell carcinomas. [DrDSc *thesis*]. University of Dental Medicine: Yangon; 2012.
23. Adilbay D, Adilbayev G, Kidirbayeva G, Shipilova V, Sadyk Z, Koyanbekova G, *et al*. HPV infection and P16 expression in oral and oropharyngeal cancer in Kazakhstan. *Infectious Agents and Can*cer 2018; 13(2) http://dx.doi.org/ 10.1186/s 13027-018-0175-8.
24. Kouketsu A, Sato I, Abe S, Oikawa M, Shimizu Y, Takahashi T, *et al*. Detection of human papillomavirus infection in oral squamous cell carcinoma: a cohort study of Japanese patients. *Journal of Oral Pathology & Medicine* 2016 Sep; 45(8): 565-572.
25. Sturgis EM, Wei Q & Spitz MR. Descriptive epidemiology and risk factors for head and neck cancer. *Seminars in Oncology* 2004; 31(6): 726-733.
26. Sturgis EM & Cincirpini PM. Trends in head and neck cancer incidence in relation to smoking prevalence: An emerging epidemic of human papillomavirus-associated cancers? *Cancer* 2007; 110(7): 1429-1435.
27. Chaturvedi AK, Engels EA, Pfeiffer RM, Brenda Y. Hernandez BY, Weihong Xiao, Esther Kim, *et al*. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *Journal of Clinical Oncology* 2011; 29(32): 4294-4301. http://dx.doi.org/ 10.1200/JCO.2011.36.4596
28. Satheesh K Bandhary, Veena Shetty, Marina Saldanha, Priya Gatti, Devananda Devegowda, Pushkal SR, *et al*. Detection of Human Papilloma virus and risk factors among patients with head and neck squamous cell carcinoma attending a tertiary referral centre in South India. *Asian Pacific Journal of Cancer Prevention* 2018; 19(5): 1325-1330. http:// dx.doi: 10.22034/ APJCP. 2018. 19.5. 1325.