Human Papillomavirus Associated Oropharyngeal Carcinomas

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Abstract

Advances in laboratory molecular detection method facilitate the detection of infectious agents in various types of cancer. Human papillomavirus (HPV) is found to be one of the most common infectious agents implicated in causing cervical cancers worldwide. With the advent of HPV vaccines developed for prevention of cervical carcinoma in females, there has been an increasing interest in HPV associated cancers in other anatomic locations. Apart from carcinomas of the cervix, that arising from the oropharyngeal region is now the second most common type of cancer found to be caused by HPV 16 and 18. They are commonly known as "high-risk" HPV. In contrast to tumours that are unrelated to HPV, HPV associated cancers of oropharyngeal origin are said to be more responsive to radiation and chemotherapy, and have an improved prognosis and overall survival. Infection by HPV has been found to be closely linked with sexual behaviour. An increase in the number of lifetime sex partners, active oral sex, and an early age of sexual experience has been suggested. Even though smoking and alcohol consumption are well-known risk factors for development of cancers, their association with HPV in carcinogenesis of the oropharynx remains unclear. Theoretically, HPV vaccination should confer protection for HPV infections and any cancers related to them. However, unlike the case of cervical cancer, there has been no mechanism analogous to the Pap smear in the screening of oropharyngeal carcinogenicity at an early stage. Thus the monitoring of the effect of HPV vaccination on the prevention of associated diseases would be difficult. The current review discusses the recent concept in the area of HPV associated oropharyngeal cancers.

Key words: human papillomavirus (HPV), oropharyngeal carcinomas, HPV vaccination

Introduction

The development of most cancers is known to be induced by multiple factors. One of these, such as cancers induced by infectious agents, has received much attention in recent

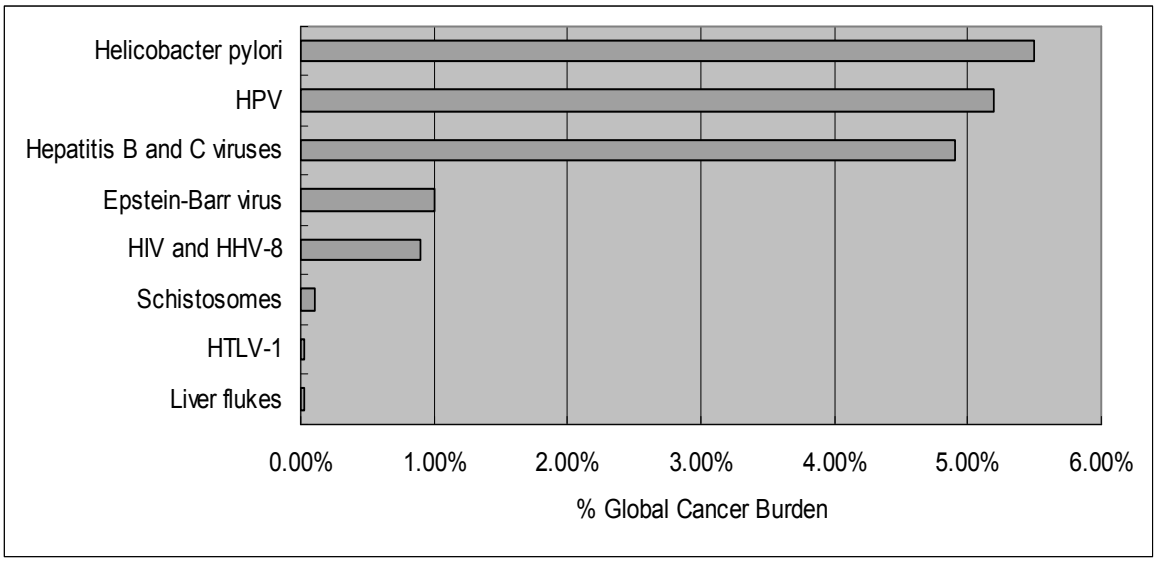

Figure 1. **World-wide prevalence of different infectious agents causing cancer in human. Data from Parkin[1] (HPV: Human papillomavirus; HIV: Human immunodeficiency virus; HHV-8: Human herpes virus 8; HTLV-1: Human T-cell lymphotropic virus type I)**

years. The most common cancer-associated infectious agent worldwide is Helicobacter pylori, constituting 5.5% of all cancers; and the second most prevalent, being human papillomavirus, accounting for 5.2% of all cancers, as indicated in the global health study by Parkin.[1] (Figure 1)

The majority of HPV infections is asymptomatic and usually resolves without any treatment, e.g. common warts which grow on hands and feet. However, a minority of HPV infections may lead to the development of cancers, those commonly found in the female genital tract, and more specifically at the uterine cervix. Cancers from other sites have also been recognized as HPV related, such as those arising in the head and neck region, and in the anogenital area.[2,3]

Given the well established causal link between HPV infection and cancers, HPV vaccines are currently administered to young females with the goal of protecting them against HPV virus-associated cervical cancers. The advent of such vaccines has also heightened our awareness to the fact that HPV is associated not only with cervical cancer and genital warts, but also with other non-cervical tumors, such as those of the head and neck and anogenital regions.[1,4,5]

HPV infection, HPV16 in particular is now acknowledged by the International Agency for Research against Cancer as a risk factor for the development of oropharyngeal squamous cell carcinomas.[6-8]

Human Papillomavirus

HPV is a DNA virus. They are named 'papillomavirus' as the initial serotypes were

found to cause warts, or papillomas. HPV is a ubiquitous virus which can infect skin and mucous membranes, stratified squamous epithelium of the anogenital region as well as upper aerodigestive tract.[2,3] There are now more than 150 serotypes found[3], and identified by their numbers in order of their discovery. Some serotypes such as 16, 18, 31 and 45, are regarded by some, but not all, to be 'sexually transmitted', and are known to associated with the development of cervical, vulvovaginal, anal and penile cancers.[1,5] These HPV serotypes are often referred to as "high-risk" types in contrast to those "low-risk" HPVs which do not cause cancers, like HPV subtypes 6 and 11 which commonly cause genital warts.[3,9]

Since the application of PAP smear in the 1940s as a cancer screening tool, it resulted in a significant reduction in the incidence of cervical cancers in women. With the advent of molecular pathology, our knowledge of the link between HPV and cancers has accrued over the ensuring decades. Besides cervical cancers, it is now known that the second most frequent HPV associated tumor is oropharyngeal cancers.[1,5] This may be due to the morphological similarity between the oropharyngeal and cervical mucosa. Specifically, they are both covered by stratified squamous epithelium, which are now known to be the cell types preferentially infected by HPV.

HPV Genome And Its Role In Oncogenesis

The HPV-16 genome is a double strand circular DNA of about 8 kb, and is enclosed in a 52-55 nm viral capsid. The DNA sequence codes for two late genes namely the L1 and L2, which codes for the viral capsid proteins; and six early genes namely the E1–E2 and E4–E7, which control gene transcription and replication.[8,10] These proteins are also known as oncoproteins, implying their ability to induce cancers, and are known to play a crucial role in gene regulation, replication, pathogenesis, and transformation.[10] The E6 and E7 oncoproteins are key factors that lead to HPV-induced carcinogenesis.[11-13]

Under normal circumstances, should cells be infected by the HPV virus, our body's immune system would clear the virus rapidly and would not progress to cancer development. However, in those with persistent infection, the virus interacts with the host DNA and may lead to development of cancers. This process of viral trans-formation is so slow that in many people who are infected, it takes many years before any symptoms would appear.

HPV E6 and E7 oncoproteins cause impairment in our body's immune system, which is a complex interaction between these oncoproteins to our body's normal cell cycle including the p53, Rb, cylin-CDK complex, p21 and p27.[6] These factors are believed to be the key points behind the development of neoplasia. With persistent infection, the viral load becomes high and the viral DNA

becomes integrated into the cellular DNA, which led to the upregulation of the HPV E6 and E7 oncoproteins. The E6 oncoprotein mainly causes the degradation of the tumor suppressor gene p53 and leads to the delay in cell apoptosis. While the E7 oncoprotein mainly inhibits the function of retinoblastoma protein (Rb), and stimulates the cellular DNA synthesis and pathological cell growth.[10,14] With the recent findings of research on HPV positive oropharyngeal cancer cell line, it leads to a better understanding of E6/E7 biological pathways and the association with TP53 and p16.[15]

As mentioned earlier, the cause of cancer is multifactorial, and it is logical to assume that HPV viral agent on its own is not sufficient to cause cancer, like those in cervix; there should be some co-factors that lead to the development of cancer.[16-18] These additional factors include tobacco use, alcohol consumption, malnutrition, immuno-compromised states, which are often implicated in most cancer studies.[4,6]

A recent study by Furniss *et al*. showed that the pathogenesis of human papillomavirus in head and neck squamous cell carcinoma involves complex interactions with tobacco and alcohol exposure.[19] Other authors suggested that smoking and drinking led to higher susceptibility to HPV infection, as a result of local immunosuppression.[20]

HPV Detection

There are wide range of methods in detecting HPV infection in head and neck squamous cell carcinoma (HNSCC), the choice depends on the availability of tissue and resources. Formalin fixed paraffin tissue, fresh or frozen tissue and cytologic preparation are commonly used, and current methods mainly target on protein, DNA and mRNA.

With the advance in molecular detection methods, low DNA content in samples can be detected with amplification systems. It was described as the gold standard for identification of HPV as recently published by Zaravios *et al*.[21] and classified them into three categories, including those non-amplified hybridization assays (eg. Southern Transfer Hybridization (STH) and *In Situ* Hybridization (ISH)), and those signal amplified hybridization assay (e.g. Polymerase Chain Reaction (PCR) and *In Situ* PCR). PCR based detection was highly recommended due to its highly sensitivity and specificity,[21,22] and are considered as the major powerful techniques employed in recent studies.[10] Apparently, this may be accounted for one of the reasons more HPV positive cases were reported in the past decade.

With the use of generic mixture of HPV primers or HPV type specific primers in PCR, identification of HPV type can be achieved and the results can be confirmed by the Southern blot hybridization or DNA sequencing. Besides the detection of E6 and E7 expression using PCR or Real-time PCR,

L1 region primers were also used for detecting HPV viral genome.[11,12,23] In laboratories where molecular techniques are not available, p16 immunohistochemical analysis may also be used as a surrogate marker for HPV infection.[10,24] The E6/E7 expression, together with the p16 upregulation are commonly measured in current studies of HPV association on HNSCC and oropharyngeal squamous cell carcinomas (OSCC),[15,25-27] including some used known biomarker for HPV related cancer, like CDKN2A.[25]

Evidence Of Association Between HPV And Oropharyngeal Cancers

More focus is now on the etiologic role of HPV in the increase incidence of oropharyngeal squamous cell carcinomas, which is a subset of head and neck squamous cell carcinoma. HNSCC is regarded as the sixth most common type of cancer, and within the group of HNSCC, about 10% are OSCC.[1,10]

Gillison *et al.* demonstrated a strong association between HPV and HNSCC. These investigators used a combination of L1 and E7 region primers for PCR amplification and sequencing with Southern blot hybridization, and showed that 25% of 253 HNSCC samples in their study had HPV genomic DNA (Table 1), 90% of which were HPV-16 associated.[6] Similar finding was reported by D'Souza *et al*. and Ernster *et al*., revealed that 72% and 69% of the oropharyngeal cancer specimens were HPV 16 positive in their respective studies.[4,28] (Table 2)

In addition to demonstrating HPV association with HNSCC, Gillison *et al*. also revealed that HPV DNA was predominantly found in oropharynx samples, which account for 57%, as compared to 19% found in

Table 1. **Reported HPV prevalence in Head and Neck Squamous Cell Carcinomas.**

Sources	No. of cases	HPV positivity (%)	HPV detection method
Gillison *et al*.[6]	253	25	PCR for L1 and HPV16 + HPV18 E7
Fakhry *et al*.[29] *	96	40	Wide spectrum PCR and HPV16 ISH
Jung *et al*.[25]	231	13	Wide spectrum PCR

* Only looked at oropharyngeal and laryngeal sites.

Table2. **Reported HPV prevalence in Oro-pharyngeal Squamous Cell Carcinomas.**

Sources	No. of cases	HPV positivity (%)	HPV detection method
Weinberger *et al*.[30]	79	61	HPV16 specific PCR
Mellin *et al*.[31]	60	43	PCR for L1
D'Souza *et al*.[4]	100	72	HPV16 ISH
Ernster *et al*.[28]	72	69	HPV16 + HPV18 specific PCR
Sedaghat *et al*.[32]	49	53	HPV16 ISH

larynx, 12% in oral cavity. Furthermore, within the HPV positive oropharyngeal tumors, it was revealed that 94% were palatine or lingual tonsils tumor and 62% were tonsil or base of tongue tumor.[6] These become the important subsites of HNSCC that recently studies on HPV related HNSCC are focused on, and account for the overall high prevalence of HPV related HNSCC or OSCC.[11,12,17,23,33]

These phenomena were proven in systemic reviews conducted by Kreimer *et al*.[34] and the meta-analysis by Termine *et al*.[22] In the former, 5046 HNSCC from 60 studies showed that the overall HPV prevalence was 25.9%, and significantly higher in oropharyngeal tumors with 35.6% as compared with those of the laryngeal and oral sites (24% and 23.5% respectively). In the later study, the overall HPV prevalence was found to be 34.5%, and also found higher in OSCC (38.1%) as compared to non site-specific HNSCC (24.1%) in subgroups analysis.

In addition to HPV 16, HPV 18 was found in significantly high percentage in head and neck or oropharyngeal tumors, Anaya-Saavedra *et al*. revealed that 55.6% of HPV 16 and 18.5% of HPV 18 were detected in the OSCC samples, suggesting that HPV 16 and HPV 18 are the most common subtypes for oral squamous cell carcinoma.[6,35,36]

HPV 33 and HPV 31 were also reported in some studies, although they were less common as compared with HPV 16 or 18.[6] Attner *et al*.[11] and Jung *et al*.[25] both found up to 10% of HPV 33 in their studies; while Mellin *et al*. found a single case of HPV 33 out of 22 samples.[23] Additionally, low risk HPV 6 and HPV 11 were notably reported in cases of HNSCC.[6,19]

Nowadays, wide-spectrum HPV detection was achievable by using consensus primers set (like GP5/6 and MY09/11), 20 to 30 HPV types could be identified by type-specific PCR, and or by sequencing.[37-39] Obviously, more HPV serotypes related to HNSCC or OSCC can be identified.

Global Prevalence and Consequences of HPV Related OSCC

During the past decades, several studies showed a significant increase in HPV related OSCC, tonsillar cancers and base of tongue cancers.[10,40]

Hammarstedt *et al*. reported a 2.8 fold increase in HPV positive tonsillar cancers in Stockholm during the period 1970-2002.[17] This observation was in agreement with a similar study conducted by Näsman *et al*. The HPV positive tonsillar cancers were almost double for each decade between 1970-2007 (Table 3) suggesting an epidemic of HPV induced carcinoma in the country.[13] In contrast, there was a parallel decrease in HPV negative cases.

In Finland, Syrjänen reported an overall increase in the HPV positive oral cancers

Table 3. **Prevalence of HPV + Tonsillar or Base of Tongue Cancer in Sweden.**

Sources	Site	Year	HPV positivity (%)
Hammar-stedt[17]	Tonsil	1970 - 1979	23.3
		1980 - 1989	29
		1990 - 1999	57
		2000 - 2002	68
Näsman[13]	Tonsil	2000 - 2002	68
		2003 - 2005	77
		2006 - 2007	93
Attner[11]	Base of tongue	1998 - 2001	58
		2004 - 2007	84

which increased from 22% to 51% within 2002, and that tonsillar cancers seem to be the highest prevalence ones among all non-genital cancers.[7]

Another study performed by Tachezy *et al*. in Czech Republic demonstrated a strong association of HPV with oropharyngeal tumor as revealed by a 51.5% of HPV DNA detected in the samples; and that 80% of them was HPV 16 positive.[41] Interestingly, this study also found out that HPV cancer is higher in non-smoker and non-drinker, which were believed to be the traditional etiologic factors of cancers.[42]

It has been reported that there was a rising incidence in HPV positive oropharyngeal cancer in males in United States of America.[28] Chaturvedi *et al*.[43] found a significantly increase of HPV related OSCC from 1973 to 2004 particularly in white men and at younger ages while Shiboski *et al*.[40] addressing the increasing trends in tonsil and tongue squamous cell carcinoma in younger population of ages 20-44 in similar period (1973-2001). More recently Hong *et al*. reported that there was increase from 19% to 47% during the year 1987 to 2005 for HPV related oropharynx cancer in Austarlia.[36]

The worldwide increase in the incidence in HPV related oropharyngeal cancers and an increasing trend of these cancers being diagnosed at a younger age urged the governments and health organizations to be more aware of these epidemiological facts.[43] Further studies and research should focus more on prevention, early diagnosis, and better treatment.[6,31,33]

Clinical Diagnosis, Treatment And Prevention Of OSCC

Cancer of Oropharynx

Oropharynx is a tube like structure from behind the nose down to the neck, and is the middle part of the throat (also call pharynx), that forms part of the esophagus. It includes the soft palate, the base of the tongue, and the tonsils. The symptom of oropharyngeal cancer may include sore throat, odynophagia, bleeding, and ear-ache. Sometimes the presentation may be late and the disease may not been detected until it has spread to the regional lymph nodes. If patient is suspected of oropharyngeal cancer, the clinician will usually perform pharyngo-laryngoscopy and may take a tissue biopsy under direct visualization for confirmative diagnosis. Once diagnosis is confirmed the cancer staging can usually determined by clinical

examination and radiological investigations for planning of the treatment.[42]

Aetiology of OSCC

Even though smoking and drinking are the traditional etiologic factors implicated for oropharyngeal cancers development,[35,42] not all studies support this hypothesis.[4,41,44] For example, Nguyen *et al*. found an increasing trend of OSCC in both non-drinkers or non-smokers.[44] The trend of increasing of oropharyngeal cancers among both non-smoker and non-drinkers has been observed worldwide[44,45] which implied that HPV infection is the key causative agents of the oropharyngeal tumor.[18,41,44]

With the ever increasing reports of HPV infection associated with cancers of the oropharynx, it has been suggested that this infection is strongly associated with high-risk sexual behavior, analogous to cervical cancer.[43,44,46] Chaturvedi *et al*. suggested that the increased in HPV related OSCC in United State from 1973 to 2004 was due to the changing of sexual behavior.[43] Other studies revealed that the increase in lifetime sex partners, practicing of oral sex, even open mouth kissing, a young age of sexual experience were associated with the development of HPV related OSCC.[35,46] This may partly be the reason as to why patients are found to be younger in some studies.[43,44]

Treatments and Prognosis

Currently, the main stream of treatment of oropharyngeal cancers is usually surgical. If the lesion is small, it may be removed by local resection with or without the use of laser. If the lesion is more extensive, removing the entire anatomical organ, such as pharyngo-laryngectomy may be indicated. Apart from excision of the lesion, regional lymph nodes are usually resected. These lymph nodes are usually located in the neck. Depending on the stage of the disease, patients with stage I/II disease usually receive radiation therapy postoperatively. With advanced stage diseases, i.e. stage III/IV, the patients would normally receive a combination of chemotherapy and radiation therapy postoperatively.[42] There are many factors in determining the prognosis of oropharyngeal carcinomas. Apart from age of patient, completeness of resection, presence or absence of lymphovascular space or perineural invasion, the stage of the cancer remains to be the most important factor. Studies have revealed that HPV positive oropharyngeal cancers consist of distinct molecular, clinical, and pathologic disease entity, which is likely associated with poor tumor grade, basaloid morphology, and wild-type TP53,[6,27] but a markedly improved prognosis and better survival rate.

Gillison *et al*. found that there was a significant reduction of 60% in the risk of death from cancer, if that lesion were HPV positive.[6] This finding was supported by other studies in which the disease specific survival rate was better in HPV positive patients.[28,32,44] Nguyen *et al*. also suggested that HPV positive cancers may show more

responsiveness to radiation and chemo-therapy agents.[44] Fakhry *et al*. found that patients with HPV positive tumors had a higher chemotherapy response rate (82% vs 55%) and chemoradiation response (84% vs 57%) as compared to patient with HPV negative tumors.[29]

Mellin *et al*. found that survival rate of HPV positive tonsillar patients were significantly better, regardless of the treatment received and TNM stage. The 3-year survival rate in HPV positive and negative patients were found to be 65.3% and 31.5% respectively, while the 5-year survival rate in HPV positive and negative patients were found to be 53.3% and 31.5% respectively.[31] Similarly Ang *et al*. revealed that patients with HPV positive oropharyngeal tumors had a better 3-year overall survival rate and a 58% reduction in risk of death after adjustment, regardless of the type of radiotherapy received.[47] (Table 4, Figure 2) However, Ang *et al*. also found out that the risk of death will increase with the number of pack-year of smoking in HPV positive tumor patients, suggesting a smoking induced genetic alteration in these HPV tumors.

In Weinberger *et al*.'s study, OSCC that are HPV positive and had p16 over expression (namely class III in the study) have a more favorable prognosis as compared to the other two class models (HPV-/p16 low and HPV+/p16 low), which may define a narrative classification scheme to clinical trials on HPV targeted therapies. The overall 5-year survival rate of class III was up to 79%.[30] Similarly, Lassen *et al*. assessed p16(NK4A) as a prognostic marker of treatment response and survival, and found that p16 expression has significant impact on locoregional tumor control (58%), improved disease-specific survival (72%), and overall survival (62%).[26]

Table 4. Overall survival compare between HPV+ and HPV- patients with HNSCC or OSCC.

	3 yrs Overall Survival		5 yrs Overall Survival		
First author and reference no.	**HPV+ patients**	**HPV- patients**	**HPV+ patients**	**HPV- patients**	**Treatment**
Mellin *et al*.[31]	65.3%	31.5%	53.5%	31.5%	Radiotherapy (55% in combination of surgery)
Lassen *et al*.[26]			62%	26%	Radiotherapy (conventional)
Ang *et al*.[47]	82.4%	57.1%			Radiotherapy (accelerated/ standard fraction)

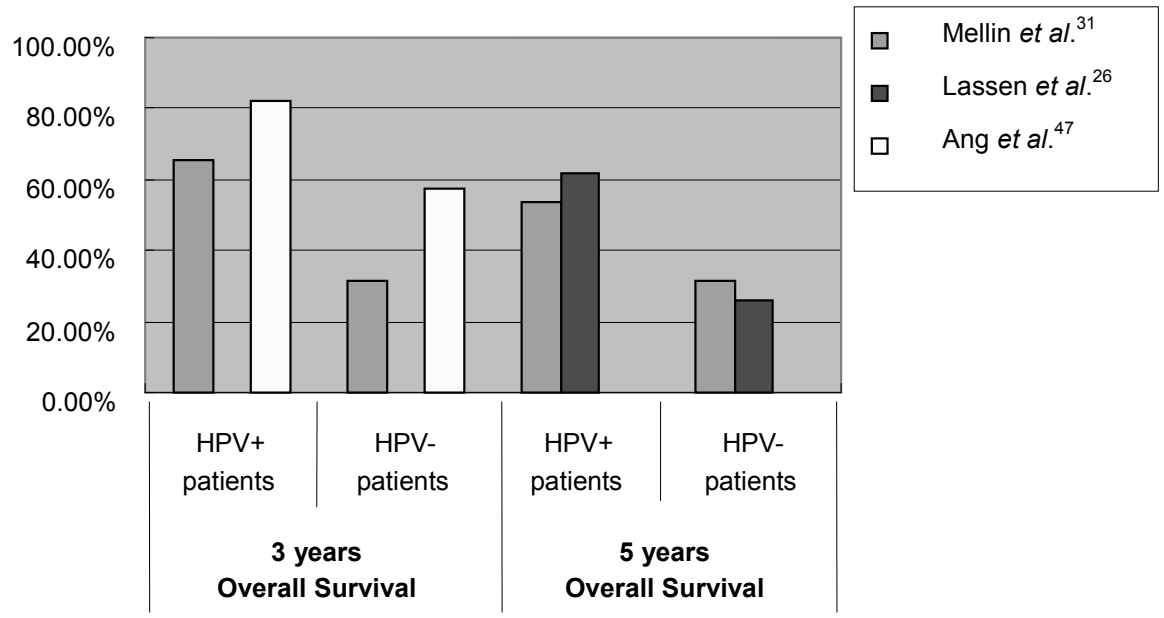

Figure 2. 3 yr and 5 yr overall survival rate in HPV+ vs HPV- patients with HNSCC or OSCC.

These observations may be explained in part by studies that have taken into account of these factors associated with immune status. Spanos *et al*. compared the *in-vitro* cell line with *in-vivo* HPV positive tumors, and found that there was an active antiviral immune response.[48] This finding also concurred with those of Dahlstrand and Dalianis in which HPV positive patient were more frequently found to have an impaired immune system.[33]

As all these studies revealed that there was a better prognosis and survival rate on HPV positive OSCC patient, there should be better treatment and therapy profile optimized for them in future. This also relies on the development in predictive markers and clinical diagnosis to locate these HPV positive OSCC patients.[10,31]

Prevention and Vaccination

Prevention can be achieved by educating public on the harmfulness of smoking and cigarette control, as suggest in the study of Sturgis which may help to reduce the prevalence of head and neck cancer.[18] In addition, safe-sex education to young people and a reduction of lifetime sex partners should somehow decrease the incidence of oropharyngeal cancers if sexual behavior is concerned. But more importantly and to a greater extent is the vaccination program in the protection of individual against HPV infection is highly recommended.

Gardasil® and Cervarix® are two Food and Drug Administration (FDA) approved vaccines. They are targeted against HPV types 6, 11, 16 and 18 in the former, and

types 16 and 18 in the latter. Currently they are approved for protection for women against cervical cancer; theoretically, they can also be used to protect the public against HPV related cancers elsewhere in the body. As HPV 16 and 18 are the commonest HPV serotypes found in oropharyngeal cancers, the currently available vaccines should be effective against both of these, irrespective of the site of infection.[5,49]

The effectiveness of the prophylactic vaccines on cervical cancer already showed great promise as observed in many studies.[5,9,50] Munoz *et al*. found that vaccination was up to 100% effective in HPV negative population in reducing high grade cervical, vulva, vaginal lesions and genital warts.[50] Currently, the effectiveness of vaccines on cancers that are non-cervical origin is unknown. It has been suggested that the HPV vaccination program should be offered to men and adolescent boys and girls, as men are known to be silent HPV carriers, and that the HPV cancers, such as those of anal, oropharyngeal and penile origin, are more likely found in men.[5,18] The HPV vaccination program should eventually reduce the burden of most HPV associated cancers, in addition to cervical cancers.[5]

The effectiveness of HPV vaccination for cervical cancer prevention may be monitored by a well-established cervical cancer screening program. However, such screening program, including the use of an effective tool like the Pap smear is lacking in screening for cancers of the upper aerodigestive tract. Currently, there is no universally accepted guideline for screening for cancers at these sites.

Although detection of HPV infection may possibly achieved by obtaining samples from oropharyngeal secretions, the main mode of investigation still involves endoscopy and biopsy in suspected cases. On the other hand, in a recent study by Furniss suggested the advantage of detecting HPV DNA in patient serum, which cause relatively less burden to patient and could even detected lower levels of HPV DNA than in biopsy samples, may become more feasible in the future HPV studies in HNSCC.[19] Until a simpler, more acceptable and cost-effective method is developed it would be extremely difficult to assess and monitor the impact of HPV vaccination in prevention of HPV associated oropharyngeal cancers.[5,49]

Conclusion

Human papillomavirus is found to be an important etiological agent for the development of cancer in the oropharyngeal region. The most frequent serotypes include HPV 16 and 18. In contrast to tumours that are unrelated to HPV, HPV associated cancers of oropharyngeal origin are said to be more responsive to radiation and chemotherapy, and have an improved prognosis and overall survival. Infection by HPV has been found to be closely linked with sexual behaviour. Theoretically, HPV

vaccination should confer protection for HPV infections and any cancers related to them. However, unlike the case of cervical cancer, there is no well-established screening tool for detecting cancers at an early stage or for monitoring of the impact of HPV vaccination. Future research should focus on development of screening method, refining laboratory diagnosis and therapeutic strategies.

References

1. Parkin DM. The global health burden of infection-associated cancers in the year 2002. Int J Cancer 2006; 118: 3030–3044.
2. Division of STD Prevention. Prevention of genital HPV infection and sequelae: Report of an external consultants' meeting. Atlanta, GA: Centers for Disease Control and Prevention, 1999.
3. National Cancer Institute Fact Sheet, 12/13/2010. "Human Papillomaviruses and Cancer" [http://www.cancer.gov/cancertopics/factsheet/Risk/HPV] (accessed 10.5.2011 16:34)
4. D'Souza G, Kreimer AR, Viscidi R, *et al*. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007; 356: 1944–56.
5. Gillison ML, Chaturvedi AK, Lowy DR. HPV prophylactic vaccines and the potential prevention of noncervical cancers in both men and women. Cancer 2008; 113(Suppl): 3036–46.
6. Gillison ML, Koch WM, Capone RB, *et al*. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 2000; **92**: 709–20.
7. Syrjänen S. Human papillomavirus (HPV) in head and neck cancer. J Clin Virol 2005; 32(Suppl 1): S59–66.
8. World Health Organization. IARC monographs on the evaluation of carcinogenic risk to humans. Vol. 90. Human papillomaviruses. Lyon (France): International Agency for Research on Cancer 2007; 90: 47-177.
9. Villa LL, Costa RL, Petta CA, *et al*. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomized double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol 2005; 6: 271–8.
10. Ramqvist T, Dalianis T. Oropharyngeal cancer epidemic and human papillomavirus. Emerg Infect Dis 2010; 16: (11).
11. Attner P, Du J, Näsman A, *et al*. The role of human papillomavirus in the increased incidence of base of tongue cancer. Int J Cancer 2010; 126: 2879–84.
12. Lindquist D, Romanitan M, Hammarstedt L, *et al*. Human papillomavirus is a favourable prognostic factor in tonsillar cancer and its oncogenic role is supported by the expression of E6 and E7. Mol Oncol 2007; 1: 350–5.

13. Näsman A, Attner P, Hammarstedt L, *et al*. Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: an epidemic of viral-induced carcinoma? Int J Cancer 2009; 125: 362–6.
14. Feller L, Wood NH, Khammissa RA, *et al*. Human papillomavirus-mediated carcinogenesis and HPV-associated oral and oropharyngeal squamous cell carcinoma. Part 1: human papilloma-virus-mediated carcinogenesis. Head Face Med 2010; 6:
15. Rampias T, Sasaki C, Weinberger P, *et al*. E6 and E7 gene silencing and transformed phenotype of human papillomavirus 16-positive oro-pharyngeal cancer cells. J Natl Cancer Inst 2009; 101: 412–23.
16. Feller L, Wood NH, Khammissa RA, *et al*. Human papillomavirus-mediated carcinogenesis and HPV-associated oral and oro-pharyngeal squamous cell carcinoma. Part 2: Human papillomavirus associated oral and oropharyngeal squamous cell carcinoma. Head Face Med 2010; 6:
17. Hammarstedt L, Lindquist D, Dahlstrand H, *et al*. Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. Int J Cancer 2006; 119: 2620–3.
18. Sturgis EM, Cinciripini PM. Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus–associated cancers? Cancer 2007; 110: 1429–35.
19. Furniss CS, McClean MD, Smith JF, *et al*. Human papillomavirus 6 sero-positivity is associated with risk of head and neck squamous cell carcinoma, independent of tobacco and alcohol use. Ann Onco 2009; 20: 534–41.
20. Poppe WA, Ide PS, Drijkoningen MP *et al*. Tobacco smoking impairs the local immunosurveillance in the uterine cervix. An immunohistochemical study. Gynecol Obstet Invest 1995; 39: 34–8.
21. Zaravinos A, Mammas IN, Sourvinos G, *et al*. Molecular detection methods of human papillomavirus (HPV). Int J Biol Markers 2009; 24: 215–22.
22. Termine N, Panzarella V, Falaschini S, *et al*. HPV in oral squamous cell carcinoma vs head and neck squamous cell carcinoma biopsies: a meta-analysis (1988 to 2007). Ann Oncol 2008; 19: 1681–90.
23. Mellin H, Dahlgren L, Munck-Wikland E, *et al*. Human papillomavirus type 16 is episomal and a high viral load may be correlated to better prognosis in tonsillar cancer. Int J Cancer 2002; 102: 152–8.
24. Marur S, D'Souza G, Westra WH, *et al*. HPV-associated head and neck cancer: a virus-related cancer epidemic. Lancet Oncol 2010; 11: 781–9.
25. Jung AC, Briolat J, Millon R, *et al*. Biological and clinical relevance of transcriptionally active human papillo-mavirus (HPV) infection in oropharynx squamous cell carcinoma. Int J Cancer

2010; 126: 1882–94.

26. Lassen P, Eriksen JG, Hamilton-Dutoit S, *et al*. Effect of HPV associated p16INK4A expression on response to radiotherapy and survival in squamous cell carcinoma of the head and neck. J Clin Oncol 2009; 27: 1992–8.
27. Licitra L, Perrone F, Bossi P, *et al*. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. J Clin Oncol 2006; 24: 5630–36.
28. Ernster JA, Sciotto CG, O'Brien MM, *et al*. Rising incidence of oropharyngeal cancer and the role of oncogenic human papilloma virus. Laryngoscope 2007; 117: 2115–28.
29. Fakhry C, Westra WH, Li S, *et al*. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst 2008; 100: 261–9.
30. Weinberger PM, Yu Z, Haffty BG, *et al*. Molecular classification identifies a subset of human papillomavirus–associated oropharyngeal cancers with favorable prognosis. J Clin Oncol 2006; 24: 736–47.
31. Mellin H, Friesland S, Lewensohn R, *et al*. Human papillomavirus (HPV) DNA in tonsillar cancer: clinical correlates, risk of relapse, and survival. Int J Cancer 2000; 89: 300–4.
32. Sedaghat AR, Zhang Z, Begum S, *et al*. Prognostic significance of human papillomavirus in oropharyngeal squamous cell carcinomas. Laryngoscope 2009; 119: 1542–9.
33. Dahlstrand HM, Dalianis T. Presence and influence of human papillomaviruses (HPV) in tonsillar cancer. Adv Cancer Res 2005; 93: 59–89.
34. Kreimer AR, Clifford GM, Boyle P, *et al*. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005; 14: 467–475.
35. Anaya-Saavedra G, Ramirez-Amador V, Irigoyen-Camacho ME, *et al*. High association of human papillomavirus infection with oral cancer: a case–control study. Arch Med Res 2008; 39: 189–97.
36. Hong, A.M., Grulich, A.E., Jones, D., *et al*. Squamous cell carcinoma of the oropharynx in Australian males induced by human papillomavirus vaccine targets. Vaccine 2010; 28(19): 3269-72.
37. de Roda Husman AM, Walboomers JM, van den Brule AJ, *et al*. The use of general primers GP5 and GP6 elongated at their 30 ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. J Gen Virol 1995; 76: 1057–62.
38. Morshed K, Polz-Dacewicz M, Szyman´ ski M, *et al*. Shortfragment PCR assay for highly sensitive broad-spectrum detection of human papillomaviruses in laryngeal squamous

cell carcinoma and normal mucosa: clinico-pathological evaluation. Eur Arch Otorhinolaryngol 2008; 265: S89–S96.

39. Williamson AL, Rybicki EP. Detection of genital human papillomaviruses by polymerase chain reaction amplification with degenerate nested primers. J Med Virol 1991; 33: 165–71.
40. Shiboski CH, Schmidt BL, Jordan RC. Tongue and tonsil carcinoma: increasing trends in the US population ages 20 to 44 years. Cancer 2005; 103: 1843–49.
41. Tachezy R, Klozar J, Salakova M, *et al*. HPV and other risk factors of oral cavity/oropharyngeal cancer in the Czech Republic. Oral Dis 2005; 11(3): 181-85.
42. Licitra L, Bernier J, Grandi C, *et al*. Cancer of the oropharynx. Crit Rev Oncol Hematol 2002; 41: 107–22.
43. Chaturvedi AK, Engels EA, Anderson WF, *et al*. Incidence trends for human papillomavirus–related and –unrelated oral squamous cell carcinomas in the United States. J Clin Oncol 2008; 26: 612–9.
44. Nguyen, N.P., Chi, A., Nguyen, L.M., *et al*. Human papillomavirus-associated oropharyngeal cancer: a new clinical entity. QJM 2009; 103(4): 229–36.
45. Lee WT, Tubbs RR, Teker AM, *et al*. Use of in situ hybridization to detect human papillomavirus in head and neck squamous cell carcinoma patients without a history of alcohol or tobacco use. Arch Pathol Lab Med 2008; 132: 1653–6.
46. D'Souza G, Agrawal Y, Halpern J, *et al*. Oral sexual behaviors associated with prevalent oral human papillomavirus infection. J Infect Dis 2009; 199: 1263–9.
47. Ang KK, Harris J, Wheeler R, *et al*. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010; 363: 24–35.
48. Spanos WC, Nowicki P, Lee DW, *et al*. Immune response during therapy with cisplatin or radiation for human papillomavirus–related head and neck cancer. Arch Otolaryngol Head Neck Surg 2009; 135: 1137–46.
49. Chesson HW, Ekwueme DU, Saraiya M, *et al*. Cost-effectiveness of human papillomavirus vaccination in the United States. Emerg Infect Dis 2008; 14: 244–51.
50. Muñoz N, Kjaer SK, Sigurdsson K, *et al*. Impact of human papillomavirus (HPV)-6/11/16/18 vaccine on all HPV-associated genital diseases in young women. J Natl Cancer Inst 2010; 102: 325–39.