SALIVARY HUMAN PAPILLOMA VIRUS DETECTION IN HEAD & NECK CANCERS IN MALAYSIA : A MULTICENTRE STUDY

BY

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Introduction: A steady rise in incidence of oral cavity and oropharyngeal cases especially among young males with no history of tobacco smoking or alcohol consumption have given rise to a new risk factor, namely human papillomavirus (HPV). The role of HPV in these cancers are evidenced in many studies. Local studies on this trend are scarce and HPV prevalence among local population has not been undertaken before.

Objectives: This study was conducted to determine prevalence and association of HPV among oral cavity/oropharyngeal cancer patients and healthy local population. It also aims to identify the association of HPV and risk factors such as smoking, alcohol consumption, betel nut chewing and family history of cancer.

Methodology: This is a case-control study involving a test group (oral cavity and oropharyngeal cancer patients) and a control group (healthy individuals). HPV status is tested via salivary rinse samples collected using Diacarta Quantivirus® HPV salivary rinse collection kit and processed using Diacarta Quantivirus® HPV E6/E7 RNA assay. Data collection and

salivary sample collection were done with informed consent from July 2013 till June 2014 involving patients from 3 different institutions to achieve optimal sample size.

Results: This study involves 58 subjects, consisting of 29 test subjects who are patients with oral cavity cancer and 29 control subjects who are healthy. There were no patients with oropharyngeal cancer in the test group. HPV prevalence was found to be 55.1% among test subjects and 3.4% among control subjects. This was found to be significant (p=0.001) with odds ratio of 33.90 (95% CI 3.88, 295.99). Among the risk factors, smoking habit was seen in 51.2% of test subjects and 13.8% of control subjects. This association was found to be significant (p=0.041) with odds ratio of 4.36 (95% CI 1.06, 17.86). Multicolinearity and interaction term were checked and none found. Alcohol consumption was found to be insignificant (p=0.241) in this study. Family history of cancer was seen in 31.0% of subjects in test group and 6.9% in control group. This was found to be significant (p=0.019) with an odds ratio of 6.08 (95% CI 1.181, 31.244).

Conclusion: HPV prevalence among oral cavity patients was found to be high and this pivotal result demonstrates HPV infection is now an established risk factor in this country. Smoking habit was also found to be a significant risk factor among these patients and did not interact or confound the factor of HPV infection.

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LIST OF ABBREVIATIONS

ADH	Alcohol dehydrogenase
ADLH	Aldehyde dehydrogenase
APC	Annual percentage change
bDNA	Branched deoxyribonucleic acid
CI	Confidence interval
DNA	Deoxyribonucleic acid
HPV	Human papilloma virus
HUSM	Hospital Universiti Sains Malaysia
IARC	International Agency for Research on Cancer
LCR	Long control region
LR	Likelihood ratio
m-RNA	Messenger ribonucleic acid
NNK	4-(methylnitrosoamino)-1-(3-pyridyl)-1 butanone
NNN	N'-nitrosonornicotine
ORL	Otorhinolaryngology
OSCC	Oral Squamous Cell Carcinoma
PAH	Polycyclic aromatic hydrocarbons
PCR	Polymerase chain reaction
RLU	Relative light unit
RNA	Ribonucleic acid

ROC	Receiver Operating Characteristic
SCC	Squamous Cell Carcinoma
SD	Standard deviation
USA	United States of America

ABSTRAK

Pengenalan: Kenaikan bilangan kes kanser rongga mulut dan kerongkong yang berterusan terutama di kalangan lelaki muda yang tidak mempunyai sejarah merokok atau penggunaan alkohol telah membawa kepada penemuan faktor risiko baru, iaitu Human Papillomavirus (HPV). Peranan HPV dalam kanser ini dibuktikan dalam beberapa kajian. Kajian tempatan berkenaan perkara ini adalah terhad dan kelaziman HPV di kalangan penduduk tempatan belum dikenalpasti sehingga kini.

Objektif: Kajian ini dijalankan untuk menentukan kelaziman dan kaitan HPV antara pesakit kanser rongga mulut / kerongkong dan penduduk tempatan sihat. Ia juga bertujuan untuk mengenal pasti kaitan HPV dengan faktor-faktor risiko seperti merokok, pengambilan alkohol, tabiat mengunyah pinang dan sejarah kanser keluarga.

Metodologi: Kajian ini merupakan jenis kes-kawalan yang melibatkan kumpulan ujian (pesakit kanser rongga mulut dan kerongkong) dan kumpulan kawalan (individu yang sihat). Status HPV diuji melalui sampel bilasan mulut yang dikumpul menggunakan kit penggumpulan bilasan mulut Diacarta Quantivirus ® HPV dan diproses menggunakan Diacarta Quantivirus ® HPV E6 / E7 RNA assay. Pengumpulan data dan sampel bilasan mulut telah dilakukan dengan persetujuan pesakit mulai dari Julai 2013 hingga Jun 2014 dan melibatkan pesakit dari 3 institusi yang berbeza untuk menepati saiz sampel kajian yang optimum.

Keputusan: Kajian ini melibatkan 58 peserta, yang terdiri daripada 29 pesakit kumpulan ujian yang terdiri daripada pesakit kanser rongga mulut dan 29 peserta kawalan yang sihat. Tiada pesakit dengan kanser kerongkong dalam kumpulan ujian. HPV didapati positif sebanyak 55.1% di kalangan pesakit kumpulan ujian dan 3.4% di kalangan peserta kawalan. Keputusan ini

didapati signifikan (p = 0.001) dengan nisbah kemungkinan (odds ratio) sebanyak 33.90 (95% CI 3.88, 295,99). Antara faktor-faktor risiko, tabiat merokok dilihat dalam 51.2% daripada pesakit kumpulan ujian dan 13.8% daripada peserta kawalan. Keputusan ini telah didapati signifikan (p = 0.041) dengan nisbah kemungkinan (odds ratio) 4.36 (95% CI 1.06, 17.86). Interaksi antara faktor tabiat merokok dan HPV diperiksa dan tidak dijumpai. Pengambilan alkohol didapati tidak signifikan (p = 0.241) dalam kajian ini. Sejarah kanser dalam keluarga dilihat dalam 31.0% daripada pesakit dalam kumpulan ujian dan 6.9% dalam kumpulan kawalan. Keputusan ini didapati signifikan (p = 0.019) dengan nisbah kemungkinan (odds ratio) 6.08 (95% CI 1,181, 31,244).

Kesimpulan: Kelaziman HPV di kalangan pesakit rongga mulut telah didapati tinggi dan ini menunjukkan jangkitan HPV kini merupakan faktor risiko yang penting di negara ini. Tabiat merokok juga didapati menjadi faktor risiko yang ketara di kalangan pesakit-pesakit ini dan tidak berinteraksi dengan faktor jangkitan HPV.

ABSTRACT

Introduction: A steady rise in incidence of oral cavity and oropharyngeal cases especially among young males with no history of tobacco smoking or alcohol consumption have given rise to a new risk factor, namely human papillomavirus (HPV). The role of HPV in these cancers are evidenced in many studies. Local studies on this trend are scarce and HPV prevalence among local population has not been undertaken before.

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Conclusion: HPV prevalence among oral cavity patients was found to be high and this pivotal result demonstrates HPV infection is now an established risk factor in this country. Smoking habit was also found to be a significant risk factor among these patients and did not interact or confound the factor of HPV infection.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1 ORAL CAVITY ANATOMY

Oral cavity, or better known as the mouth, is an important structure on the face and is one of the first structures noted when looking at a face of an individual (Lewis, 1918). Its primary function is to serve as the entrance of the alimentary tract that initiates the digestive process by salivation, bolus formation and propulsion of the bolus into the pharynx. It also functions as a secondary respiratory conduit after the nose, a site of sound modification & pronunciation for speech and chemosensory organ. Lips, in particular, play a role in speech production, whistling, singing, playing of certain musical instruments and human behavioural communication such as smiling & kissing.

Oral cavity is oval shaped and it extends from the lips and cheeks externally to the anterior pillars of the fauces (palatoglossal arches) internally as seen in Figure 1.1. Hard palate forms the roof of the oral cavity and separates it from the nasal cavity. Floor of the mouth is formed by the mylohyoid muscles and is occupied by the tongue. Lateral wall are defined by the cheeks and retromolar regions.

It is divided into oral vestibule and oral cavity proper. Oral vestibule is the anterior most portion of the oral cavity and consists of a slit-like space between the lips or cheeks on one side and the teeth on the other. It is limited, both superiorly and inferiorly, by the reflection of the mucous membrane from the lips and cheeks to the gum covering the upper and lower jaw alveolar arch respectively. When the teeth occlude, the oral vestibule becomes a closed space that only communicates with the

oral cavity proper in the retromolar regions behind the last molar tooth on each side. Oral cavity is bounded laterally and anteriorly by the alveolar arches & teeth and communicates posteriorly to the oropharynx via the oropharyngeal isthmus. It is roofed by the hard and soft palates, while the greater part of the floor is formed by the tongue with the remainder by the reflection of the mucous membrane from the sides. Mucosa of the oral cavity is generally made up of non-keratinized stratified squamous epithelium except anterior 2/3 of the dorsal surface of the tongue which consists of keratinized stratified squamous epithelium. Three pairs of major salivary glands (parotid, submandibular and sublingual) open into the mouth together with numerous minor salivary glands (labial, buccal, palatal, lingual).

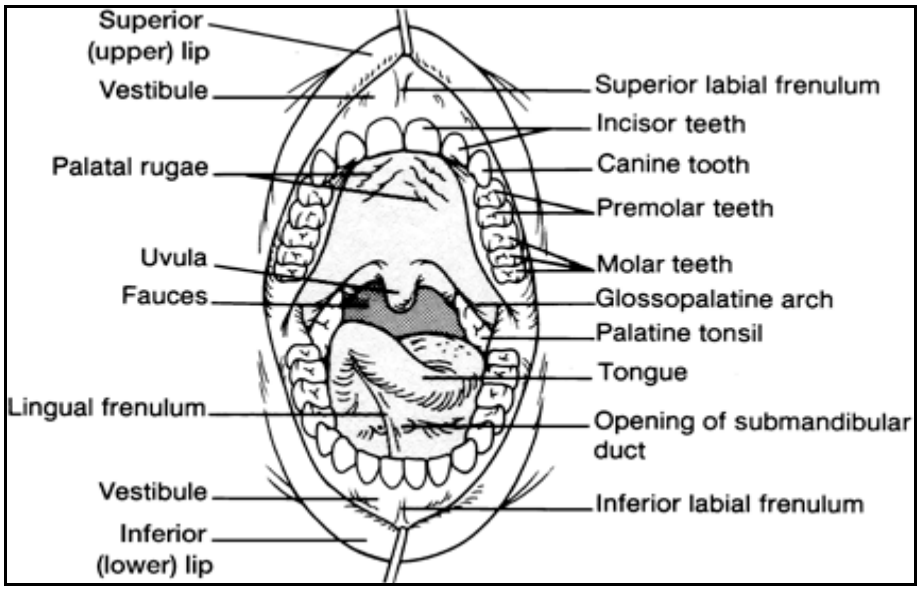

Figure 1.1: Anatomy of the oral cavity *(Source: card 234.gif, Applied Biology – Study cards, http://www.mhhe.com/biosci/abio/images/card234.gif)*

The gums (or gingiva) are composed of dense fibrous tissues which is closely attached to the periosteum of the alveolar processes and surrounding the necks of the teeth. They are covered by the mucous membrane which is smooth and vascular.

The palate is divisible into 2 regions, hard palate anteriorly and soft palate posteriorly. The hard palate is formed by the palatine processes of the maxilla and horizontal plates of the palatine bones. Oral mucosa is tightly bound to the underlying periosteum while in posterior and lateral regions it possesses a submucosa where neurovascular bundles lie. The periphery of the hard palate is covered by gingiva and a zone running anteroposteriorly in the midline as a narrow, low ridge is the palatine raphe. This zone ends anteriorly in a small papilla corresponding with the incisive canal. This surface is covered with stratified squamous epithelium and numerous palatal mucous salivary glands between the mucous membrane and the bone surface especially in the posterior half.

The soft palate is a mobile flap suspended from the posterior border of the hard palate which forms an incomplete septum between mouth and pharynx. It consists of a fold of mucous membrane enclosing muscular fibers, an aponeurosis, vessels, nerves, adenoid tissue, mucous glands and some taste buds on its oral surface. When occupying its usual relaxed position, its anterior surface is concave, continuous with the roof of the mouth, and marked by a median raphé. Its posterior surface is convex, and continuous with the mucous membrane covering the floor of the nasal cavities. Its upper border is attached to the posterior margin of the hard palate, and its sides are blended with the pharynx. Its lower border is free and its lower portion, which hangs like a curtain between the mouth and pharynx is termed the palatine velum. Hanging from the middle of its lower border is a small, conical, pendulous process, the palatine uvula; and arching lateralward and downward from the base of the uvula on either side are two curved folds of mucous membrane, containing muscular fibers, called the arches or pillars of the fauces.

The floor of mouth comprises a small horseshoe shaped region beneath the tongue as seen in the Figure 1.1. A fold of tissue called the lingual frenulum is seen to extend onto the inferior surface of the tongue in the midline. Sublingual papilla is also located at the base of tongue and the submandibular salivary ducts open into this papilla. Sublingual folds are noted at either side of sublingual papilla and the muscle forming the floor of mouth is the mylohyoid muscle.

Tongue is situated in the floor of the mouth within the curve of the mandibular body. It functions as the organ of the sense of taste and plays an important role in speech. It also assists in mastication and deglutition of food. Its root is directed backward, and connected with the hyoid bone by the hyoglossus and genioglossus muscles and the hypoglossal membrane; with the soft palate by the glossopalatine arches; and with the pharynx by the superior pharyngeal constrictor and the mucous membrane. The apex is thin, narrow and directed forward against the lingual surfaces of the lower incisor teeth. It is a muscular structure invested by mucous membrane, submucous fibrous layer and is provided with both mucous and serous glands. Dorsum of the tongue is convex and marked by median sulcus which divides it in symmetrical halves. This sulcus ends 2.5cm from the root of the tongue in a depression called foramen cecum. A shallow grove called sulcus terminalis runs lateralward and anteriorly on either side to the margin of tongue.

The anterior two-thirds of dorsum of tongue is anterior to this sulcus and its surface is rough and covered with papilla. The posterior third is smoother and contains mucous glands and lymph follicles (lingual tonsil). There are 3 types of papillae, that are filiform, fungiform and circumvallate papillae. All papillae except filiform

papillae bear taste buds. The tongue is divided into lateral halves by a median fibrous septa which extends throughout its entire length and it’s fixed below to the hyoid bone. Its movement is controlled by 2 sets of muscles on either half of the tongue, which are the extrinsic and intrinsic muscles. The extrinsic muscles consist of genioglossus, chondroglossus, hyoglossus and styloglossus. It originates from structures outside the tongue and insert into the tongue. It plays a role in the overall position of the tongue which includes protrusion, retraction, depression and elevation of the tongue. The intrinsic muscles consist of superior longitudinal muscle, inferior longitudinal muscle, verticalis muscle and transverses muscle. These four paired muscles originate and insert within the tongue, running along its length. It alters the shape of tongue by lengthening and shortening it, curling and uncurling its apex and edges, & flattening and rounding its surface.

1.2 ORAL CAVITY CANCERS

Oral cavity cancers accounts to 2.64% of all cases worldwide in the year 2000 according to Parkin (2001). Incidence of oral cavity cancer worldwide in the year 2000 was 267,000 cases while prevalence of this cancer was 707,000 cases.

There is a wide geographical variation in the incidence of oral cancers. Warnakulasuriya (2009) outlined the areas characterized by high incidence rates are found in South and Southeast Asia (eg Sri Lanka, India, Pakistan and Taiwan), parts of Western (eg. France) and Eastern Europe (eg. Hungary, Slovakia, Slovenia), parts of Latin America and the Caribbean (eg. Brazil, Uruguay, Puerto Rico) and in Pacific regions (eg. Papua New Guinea).

Local epidemiology data also reveals high number of oral cancers in Malaysia. According to National Cancer Registry Report in 2007 (Omar and Ibrahim, 2008), there were a total of 353 cases of oral cancer cases consisting of lip, tongue and mouth cancers, in which 171 cases were male and 182 cases were female. It was to be the 21st most common cancer in general population, 17th most common in males and 16th most common in females. Further analysis revealed it was predominant among the Indian ethnic group as tongue and mouth cancers were among 10 most common cancers in both male and female. Table 1.1 and Table 1.2 show gender-specific distribution of oral cancer incidence by ethnic group and site of cancer in 2007 followed by Figure 1.2 illustrating age-specific distribution of oral cancers by gender in 2007.

Table 1.1: Gender-specific distribution of oral cancer incidence by ethnic group in 2007

Ethnic group	Gender	
	Male	Female
Malay	52	57
Chinese	62	32
Indian	36	70

Table 1.2: Gender-specific distribution of oral cancer incidence by site of cancer in 2007

Site of cancer	Gender	
	Male	Female
Lip	9	8
Tongue	93	78
Mouth	69	96

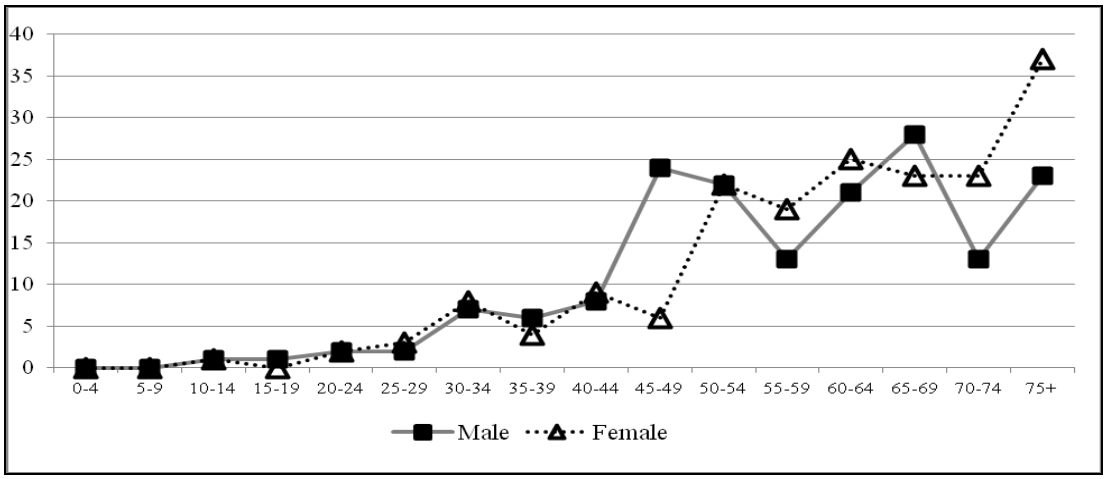

Figure 1.2: Age-specific oral cancer incidence by gender in 2007

Although there were 353 cases, only 205 cases had recorded the disease stage in which 12.1% presented in stage I, 23.3% in stage II, 20.0% in stage III and 44.6% in stage IV.

In general, oral cavity cancers are mostly squamous cell carcinoma and have certain related risk factors. Table 1.3 illustrates its histopathology subtypes and associated risk factors.

Table 1.3: Histopathological subtypes and associated risk factors of oral cancers (Webster, 2008; Bradley, 2008; Birchall and Pope, 2008)

Site	Histopathology Subtype	Risk factors
Oral Cavity Cancer -Tongue -Mouth -Hard Palate -Buccal -Floor of mouth	-Squamous cell (90%) -Tumours arising from minor salivary glands (5%) -Melanoma, Lymphoma, Sarcoma (1-2%)	-Tobacco smoking -Excessive consumption of alcohol -HPV infection -Diet & nutrition deficiencies (namely vitamin A, C, E, iron, selenium, folate) -Pre-malignant conditions (leukoplakia, erythroplakia) -Betel quid chewing -Poor oral hygiene

1.3 OROPHARYNGEAL ANATOMY

Oropharynx is one of 3 segments of the pharynx which forms a conduit for respiration, food passage and speech as shown in Figure 1.3 & Figure 1.4 (Lewis, 1918). Pharynx is a fibromuscular tube attached to base of skull and continuous below with the esophagus. It is 12cm in length. Superiorly, it is attached to the base of skull till the lower border of cricoid cartilage inferiorly at level C6 vertebra. Posterior to it is the prevertebral fascia while the anterior wall is largely deficient as it communicates with nose and mouth.

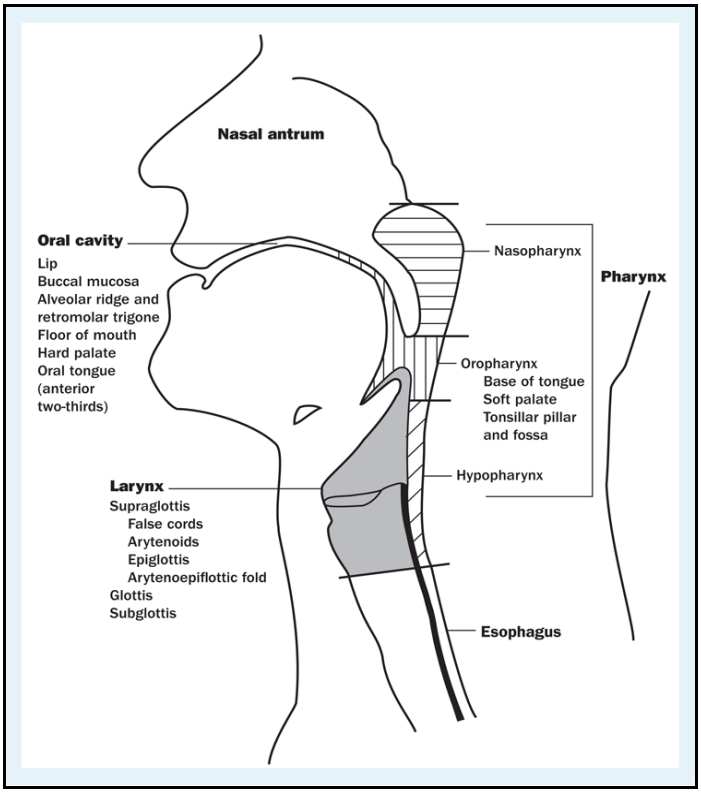

Figure 1.3: Anatomy of pharynx and its segments *(Source: Figure 1, Head and Neck Tumours, http:/www.surgery.gr/index.php/news/408-head-and-neck-tumors)*

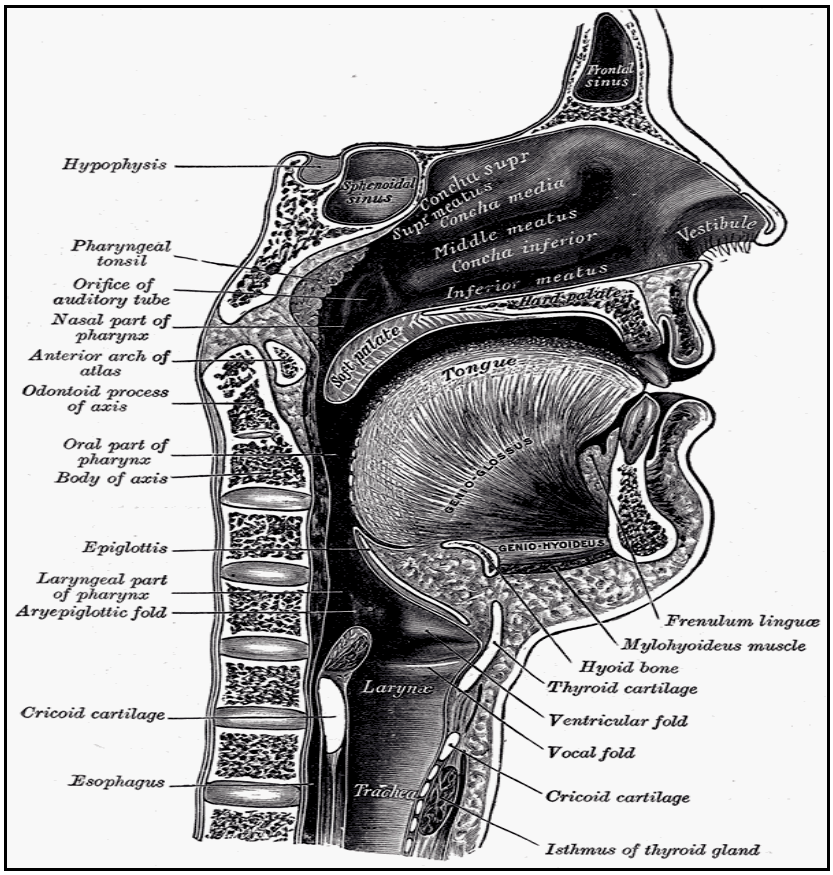

Figure 1.4: Sagittal section of the nose, mouth and larynx *(Source: Figure 994, Chapter XI- Splanchnology, Gray's Anatomy of the Human Body 20th Edition. https://s.yimg.com/lq/i/edu/ref/ga/l/994.gif*)

The oropharynx segment extends from lower surface of soft palate to upper border of epiglottis (halfway down C3 vertebral body). It opens anteriorly into the mouth via isthmus of the fauces while its lateral wall is the palatine tonsil between the two palatine arches. Structures within it include palatine tonsil & its surrounding arches, lingual tonsil and valleculla as illustrated in Figure 1.5.

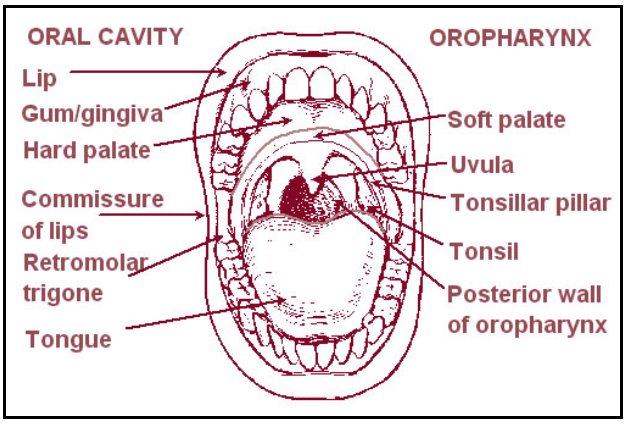

Figure 1.5: Illustration of the oral cavity and oropharynx (*Source:- mouth.jpg, SEER training modules, National Cancer Institute. http://training.seer.cancer.gov/images/head-neck/mouth.jpg*)

There are 2 pairs of arches on both sides of the lateral wall of the oropharynx, which are palatopharyngeal and palatoglossal arches. These are projecting ridges occurring bilaterally at the sides of the posterior part of tongue forming the pillars of the fauces. The palatoglossal arch, also called the anterior pillar of fauces, runs downward, lateralward and forward to the side of base of tongue from the soft palate and is formed by the projection of the palatoglossus muscle covered by mucous membrane. The palatopharygeal arch, also called the posterior pillar of fauces, is larger and projects downward, lateralward and backward to the side of the pharynx. It is formed by the projection of palatopharyngeus muscle covered by mucous membrane. These 2 arches are separated by a triangular interval on either side called tonsillar fossa in which the palatine tonsil is situated.

Palatine tonsil is a large collection of lymphoid tissue which projects into oropharynx from tonsillar fossa. It extends up to soft palate superiorly and down to dorsum of the tongue and can be divided to upper pole, body & lower pole. Palatoglossal fold is found anterior to tonsil while the palatopharyngeal fold is found posterior to it as shown in Figure1.6. The medial surface is covered by pharyngeal mucosa with

epithelial downgrowths (tonsilar crypts) on its surface. Its floor (lateral wall) consists of lower part of the superior constrictor muscle and is covered by fibrous tissue with forms the tonsillar hemicapsule.

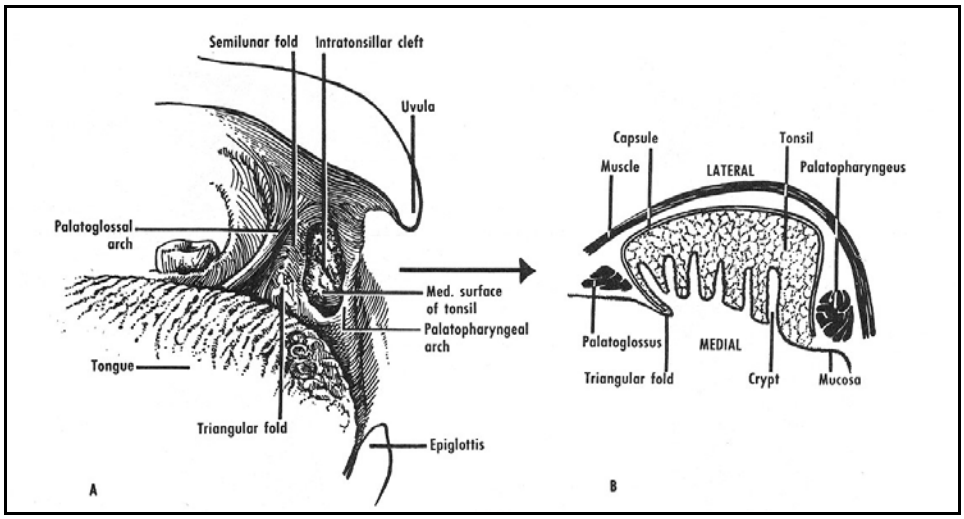

Figure 1.6: A, Right palatine tonsil and its surroundings, medial aspect. B, Horizontal section throught the tonsil (*Source: Figure 53-6, Basic Human Anatomy, Dartmouth Medical School. http://www.dartmouth.edu/~humananatomy/figures/chapter_53/53-6_files/image002.jpg)*

Palatine tonsil is supplied mainly by tonsillar branch of facial artery with contributions from lingual, ascending pharyngeal, and ascending & greater palatine vessels. Its lymphatics drain to deep cervical nodes especially jugulodigastric nodes below angle of mandible. The mucous membrane overlying the tonsil is supplied by tonsillar branch of glossopharyngeal nerve.

Lingual tonsil is a rounded mass of dense and nodular lymphatic tissue on dorsal surface of base of tongue. Its surface consists of stratified squamous epithelium which invaginates as a single crypt into each lingual tonsil. Valleculla is the area between epiglottis and posterior surface of tongue and consists of shallow fossa separated by median glossoepiglottic fold as shown in Figure 1.7. It is limited

inferolaterally by lateral glossoepiglottic folds and sensory innervation is by internal laryngeal nerve.

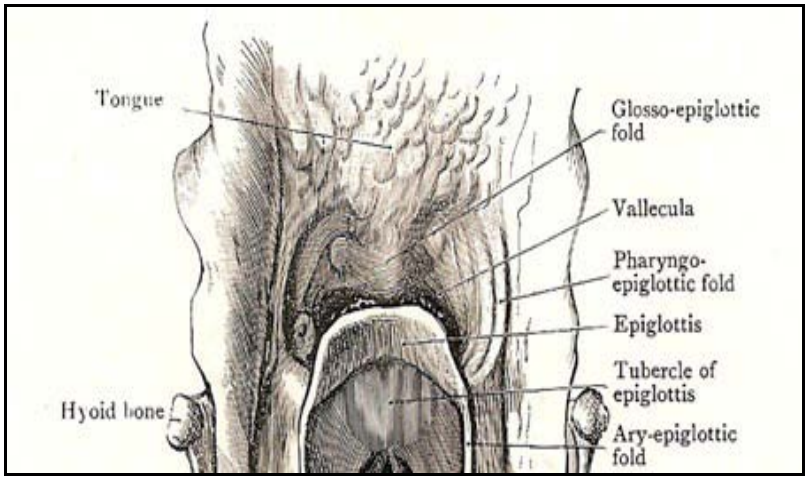

Figure 1.7: Anterior wall of laryngopharynx *(Source: Anatomy of pharynx, Encyclopedia of Science. http://www.daviddarling.info/images/laryngopharynx.jpg)*

The wall of the oropharynx is made up of 4 layers, which are mucous membrane lining, pharyngobasilar fascia, muscle layers and buccopharyngeal fascia. The mucous membrane is lined by non-keratinizing stratified squamous epithelium followed by connective tissue lamina propria immediately beneath the epithelium. The pharyngobasilar fascia is a fibrous layer attached superiorly to the basilar region of occipital bone and petrous part of temporal bone medial to carotid canal. It bridges below Eustachian tube and extends forward to posterior border of medial pterygoid plate and pterygomandibular raphe. It also bridges the gap between superior constrictor and base of skull. It is strengthened posteriorly by a fibrous band attached above to pharyngeal tubercle and passes down as median raphe which serves as an attachment to the constrictor muscles. The muscular layer is further divided to inner longitudinal and outer circular layers. The inner longitudinal layer is formed by 3 paired muscles, that are stylopharyngeus, palatopharyngeus and salpingopharyngeus.

The outer circular layer also consists of 3 paired muscles, which are superior, middle and inferior constrictors. Each constrictor muscle is shaped like a fan which arise from lateral wall and sweep around to be inserted into median raphe posteriorly. These muscles overlap each other posteriorly, being telescoped into each other like 3 stacked cups (Figure 1.8). During swallowing, the longitudinal muscles elevate and shorten larynx while the constrictors contract in a coordinated way to propel the bolus into the esophagus.

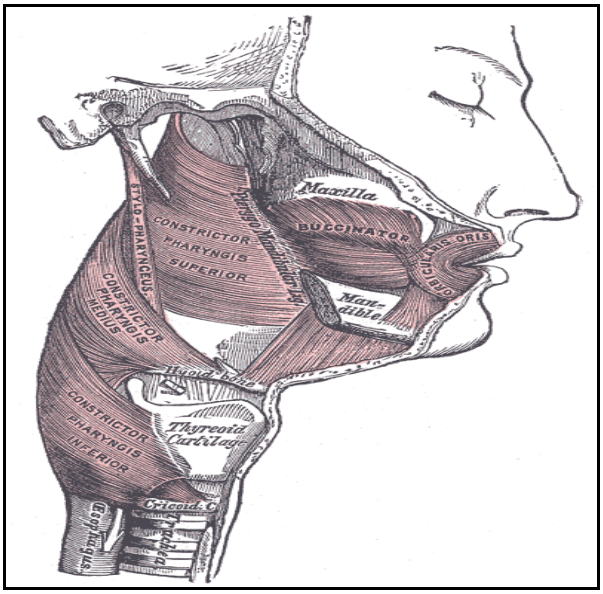

Figure 1.8: Muscles of pharynx and cheek *(Source: Figure 1030, Chapter XI-Splanchnology, Gray's Anatomy of the Human Body 20th Edition. https://s.yimg.com/lq/i/edu/ref/ga/l/1030.gif*)

The buccopharyngeal fascia is a thin coat of areolar tissue covering the pharyngeal constrictor muscles and contains the pharyngeal plexus of nerves and veins. It is loosely attached to the prevertebral fascia posteriorly and styloid process laterally. The oropharynx is supplied via the pharyngeal plexus which is formed by the pharyngeal branches of glossopharyngeal & vagus nerve and sympathetic fibres from superior cervical ganglion. All muscles of pharynx are supplied by vagus nerve except stylopharyngeus which is supplied by glossopharyngeal nerve. Sensory

innervation is by glossopharyngeal nerve and internal laryngeal nerve (branch of vagus). Blood supply is mainly from ascending pharyngeal artery and venous supply drains into internal jugular and anterior facial veins. Lymphatic drainage is to the upper deep cervical nodes.

1.4 OROPHARYNGEAL CANCERS

Incidence of oropharygeal cancers in the international scene has shown a gradual increase over recent years. Statistics in the United States shows incidence had increased 2-3% annually from 1973 till 2001 (Andrews, 2009). However, from 2000 to 2004, the incidence oropharyngeal malignancy increased annually by 5.22%.

Local epidemiology data shows oropharyngeal cancers are also rampant in Malaysia. According to National Cancer Registry Report in 2007 (Omar and Ibrahim, 2008), there were a total of 66 cases of oropharyngeal cancer cases consisting of tonsil and other sites, in which 42 cases were male and 24 cases were female. Table 1.4 and Table 1.5 show gender-specific distribution of oropharyngeal cancer incidence by ethnic group and site of cancer in 2007 followed by Figure 1.9 illustrating age-specific distribution of oropharyngeal cancers by gender in 2007.

Table 1.4: Gender-specific distribution of oropharyngeal cancer incidence by ethnic group in 2007

Ethnic group	Gender	
	Male	Female
Malay	6	6
Chinese	11	7
Indian	21	6

Table 1.5: Gender-specific distribution of oropharyngeal cancer incidence by site of cancer in 2007

Site of cancer	Gender	
	Male	Female
Tonsil	20	18
Other oropharyngeal sites	22	6

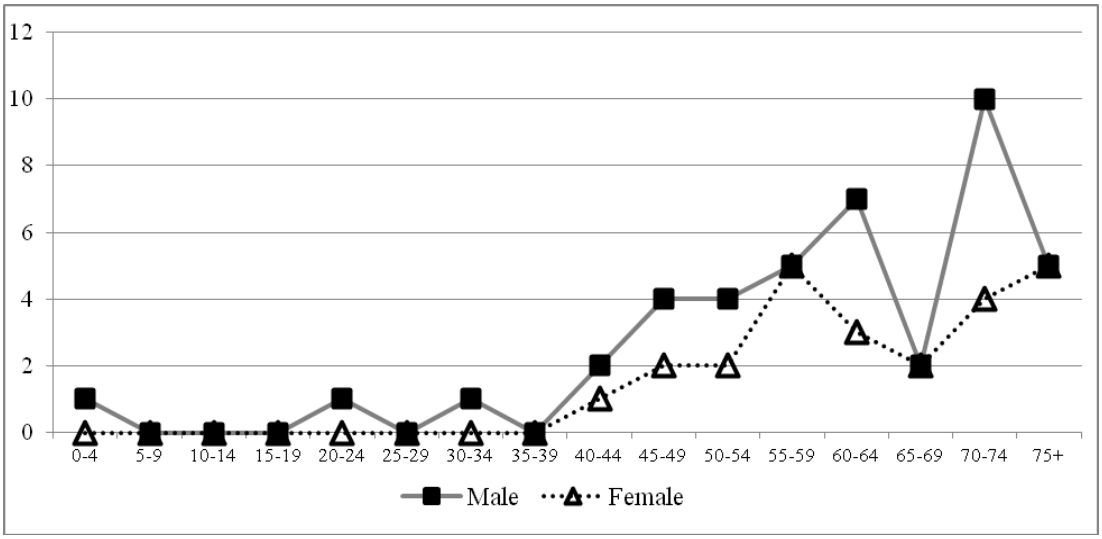

Figure 1.9: Age-specific oropharyngeal cancer incidence by gender in 2007

Table 1.6 highlights histopathological subtypes seen in oropharyngeal cancers and the risk factors involved which predispose to higher chance of acquiring this cancer.

Table 1.6: Histopathological subtypes and associated risk factors of oropharyngeal cancers. (Webster, 2008; Bradley, 2008; Birchall and Pope, 2008)

HPE Subtype		**Risk Factors**
<u>Benign</u>	**<u>Malignant</u>**	-Smoking -Alcohol consumption
-Squamous papilloma -Adenoma -Fibroma -Hemangioma -Leiomyoma -Lipoma -Lymphangioma -Schwanoma -Neurofibroma	-Squamous cell (70%) -Lymphoma (25%) -Salivary gland malignancy (<5%)	-Dietary deficiencies of vitamin A -Chronic irritants -Poor dental hygiene -Syphilis -Marijuana smoking -HPV 2, 11, 16 -HIV

1.5 OVERVIEW OF HUMAN PAPILLOMAVIRUS (HPV)

HPV belongs to Papillomaviridae virus family in which HPV 16 belongs to Alfa-papillomavirus genus as discussed by Salgado (2011). They are icosahedral, non-enveloped particles, 55nm in diameter, double-stranded DNA viruses containing approximately 7900 base pairs (Torrente, 2007). The genome organization includes 8 open reading frames and a non-coding region. The six early open reading frames (E1-E6) encode proteins involved in DNA replication, transcription and cellular transformation; the capsid proteins (L1 & L2) are encoded by late open reading frames as shown in Figure 1.10 (Salgado, 2011; Torrente, 2007).

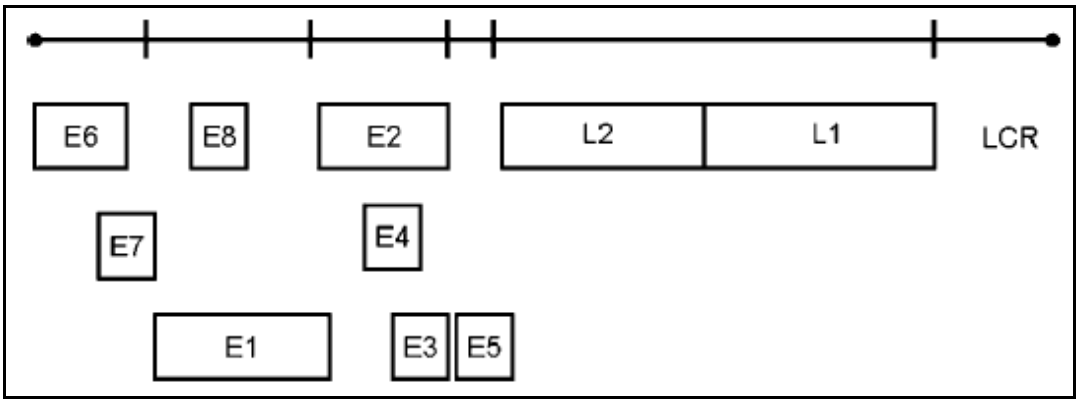

Figure 1.10: HPV genome; early genes (E1-E6), late genes (L1 & L2), long control region (LCR). *(Reproduced with permission: Figure 1, Torrente MC, Ojeda JM. Exploring the relation between human papilloma virus and larynx cancer. Acta Oto-Laryngologica 2007; 127: 900-906)*

Torrente (2007) and Boulet (2007) explain that early genes (E1 & E2) are expressed as proteins that bind to DNA and act as transcriptional activators or repressors, thus regulating virus transcription and genome replication. The E4 gene is expressed relatively late in virus replication and is involved in maturation and release of papillomavirus particles. The E6 and E7 gene are viral oncogenes and are responsible in producing viral oncogenic proteins. The E6 gene encodes a protein that binds to

tumour suppressor protein p53 which induces its degradation. The E7 gene encodes a protein that binds to retinoblastoma protein (Rb). The non-coding region or long control region (LCR) contain regulatory sequences that respond to steroid receptor hormones. The summary of these genes are shown in Table 1.7.

Table 1.7: Summary of HPV gene functions (Kajitani et al, 2012)

Gene Product	Function	Activities
E1	Replication of viral genome	DNA-binding activity, helicase activity, ATPase
E2	1. Transcription of viral genes 2. Replication and maintenance of viral genome	Transactivation/transrepression, DNA-binding activity, DNA segregation in mitotic cell
E4	Unknown	Destruction of keratin network, induction of G_2M arrest of cell cycle
E5	Possibly involved in proliferation and/or inhibition of apoptosis	Affection of cellular signaling pathway
E6	1. Reactivation of cellular replication mechanisms 2. Proliferation, immortalization, inhibition of apoptosis 3. Maintenance of viral genome	Interactions with various cellular proteins
E7	1. Reactivation of cellular replication mechanisms 2. Proliferation, genomic instability, inhibition of apoptosis 3. Maintenance of viral genome	Interactions with various cellular proteins
L1	Major capsid protein	
L2	Minor capsid protein	

These papilloma viruses infect epithelial cells and initial infection involves exposure of infectious particles to basal layer. Expression of viral gene product occurs as infected basal cell migrates to epithelial surface. Expression of E6 & E7 in epithelial layers drives cells into S-phase, conducive for viral genome replication and cell proliferation. In progression towards carcinogenesis, viral genome integrates into host genome and this disrupts E2. As a result, E6 & E7 expression is accelerated and bind to p53 and retinoblastoma tumour suppressors as mentioned earlier.

HPV can be classified as low-risk and high risk types based on established cervical oncogenicity (Baseman, 2005). Low risk HPV types (eg. type 6 and 11) induce benign lesions with minimum risk of progression to malignancy such as in laryngeal papilloma (Boulet, 2007; You, 2010; Almadori, 1996; Milian, 1998). On the other hand, high risk HPV have higher oncogenic potential with HPV 16 being the most prevalent followed by types 18, 31 & 33 (Boulet, 2007; You, 2010). Table 1.8 lists down the various types of HPV based on risk classification.

Table 1.8: Classification of HPV types (Baseman, 2005)

Risk Classification	**HPV types**
High risk	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82
Probable high risk	26, 53, 66
Low risk	6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108
Undetermined risk	34, 57, 83

1.6 LINK BETWEEN ORAL AND OROPHARYNGEAL CANCERS WITH HPV

Since the 1990's, a steady rise in incidence in oral cavity cancers especially among young males with no history of tobacco or alcohol use (Warnakulasuriya, 2009; Chatuwad, 2008) has given rise to a new risk factor, namely human papillomavirus (HPV). The role of human papillomavirus (HPV) in oral squamous cell carcinomas (OSCC) are evidenced in a few studies (Chatuwad, 2008; Ryerson, 2008; Jayaprakash, 2011) and need to be taken in consideration in battling these cancers.

Although overall incidence of oral cavity cancers has decreased in past decades in certain countries such as United States of America (USA) and France with annual percentage change (APC) of 1.5% and 1.0% respectively (Warnakulasuriya, 2009), however HPV-related oral cavity cancers have shown an increase especially among younger individuals without typical risk factors such as tobacco or alcohol use as observed by Chatuwad (2008). Incidences of HPV-related OSCC in the USA increased significantly from 1973 to 2004 with APC of 0.80 ($p<0.001$) especially among younger age group while HPV-unrelated OSCC decreased from 1983 to 2004 with APC of -1.85 ($p<0.001$).

Jayaprakash (2011) highlighted the prevalence of HPV at 24.5%, particularly HPV 16 & 18, among dysplastic and invasive cancers of oral cavity & oropharynx which supports the assumption that HPV infection occurs during early phase of these cancers. Another study (Hansson, 2005) estimated proportion of HPV infection among oral and oropharygeal SCC at 35%.

In more recent decades, rising incidence of oropharyngeal cancers also have been linked to this virus (Warnakulasuriya, 2009; Timbrell 2011). Andrews (2009) and Marur (2010) have shown detection of HPV, especially HPV 16, in tonsils and base of tongue cancers up to 85% in young, non-smoking, non-drinking patients. Transmission of HPV (although sexual transmission is established in HPV-induced cervical cancer) in these cancers point to oro-genital contact and an increase in oral sex as mode of HPV transmission to oral mucosa as suggested by Timbrell (2011). Increased number of sex partners was noted to be a consistent risk factor as studies have shown strong association between lifetime number of sex partners and genital HPV acquisition both in women and men (Baseman, 2005; Timbrell, 2011, Gabriela, 2008).

Clinically, HPV-positive tumours present at an earlier tumour (T) stage but more advanced nodal (N) stage and have better treatment outcomes than HPV-negative tumours (Chatuwad, 2008; Timbrell, 2011). When treated with radiation, HPV-positive OSCC have significantly higher survival rate than HPV-negative OSCC at all stages (Chatuwad, 2008; Marur 2010; O'Rorke, 2012). Amin Kotb and Petersen (2012) discuss that significant differences in HPV-positive and HPV-negative head & neck squamous cell carcinoma supports the fact that HPV-positive cancers represent a tumour entity of its own that is biologically and clinically different from HPV-negative cancers. HPV carcinogenesis is less frequently linked to polyploidy and have smaller tumour nuclei than cancers caused by smoking and alcohol.

HPV have also been persistently detected in post treatment HPV-positive patients irrespective of tumour site, time interval, type of treatment and presence of

recurrence (Morbini, 2013). This suggests that the patients can still be tested post treatment whether it is post surgery, post radiotherapy or post chemoradiotherapy.

International Agency for Research on Cancer (IARC), in its monograph released 2007, have concluded there is sufficient evidence in humans for the carcinogenicity of HPV 16 in oral cavity and oropharynx but limited evidence in humans for the carcinogenicity of HPV 18 in oral cavity.